

IAFP 2025 ABSTRACT BOOK

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GENERAL SESSIONS ABSTRACT



IVAN PARKIN LECTURE

ALEJANDRO MAZZOTTA

**SENIOR VICE PRESIDENT OF QUALITY,
FOOD SAFETY & REGULATORY AFFAIRS**

CHOBANI | NEW YORK, NEW YORK

ABSTRACT:

Less is More: Ditching Distractions and Focusing on Value

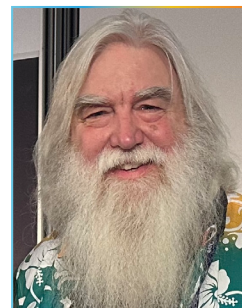
Dr. Alejandro Mazzotta is Senior Vice President of Global Quality, Food Safety and Regulatory Affairs for Chobani, LLC in New York, New York. Before joining Chobani, Dr. Mazzotta held positions in Food Safety, Quality, and Microbiology at Campbell Soup Company, McDonald's Corporation, Pillsbury/General Mills, and The National Food Processors Association.

Prior to his career in the food industry, Dr. Mazzotta was appointed as Scientific Investigator by the Argentine Antarctic Scientific Institute where he served for four years, two of which were based in the Antarctic Peninsula conducting research on the ecology of Antarctic fish under a national fisheries program.

Dr. Mazzotta joined IAFP in 1999. In 2013, he was elected to serve on the IAFP Executive Board for a five-year term of successive positions, assuming the presidency in 2016. He served on the *Journal of Food Protection (JFP)* Management Committee from 2000–2002 and the JFP Editorial Board from 2001–2003. He is a member of several IAFP Professional Development Groups (PDGs) and served on IAFP's Program Committee from 2006–2011. In addition, he has served on numerous IAFP Award Selection Committees.

From 2004–2009, Dr. Mazzotta was appointed to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF). He served on the Editorial Board of *Applied and Environmental Microbiology* from the American Society for Microbiology. He currently serves on both the Center for Food Safety at the University of Georgia (CFS) and the bioMérieux Industry Advisory Boards. Dr. Mazzotta is a Professional Member of the Institute of Food Technologists. He has published more than 25 publications in peer-reviewed scientific journals in both English and Spanish and has spoken at numerous international meetings and symposia.

Dr. Mazzotta participates in the Cornell Food Science Advisory Council and was appointed as Adjunct Professor at Cornell University's Department of Food Science. A native of Argentina, he earned his B.S. and M.S. in Biological Sciences from the University of Buenos Aires and his Ph.D. in Food Science from Rutgers University.



JOHN H. SILLIKER LECTURE

JULIAN COX

**ASSOCIATE PROFESSOR –FOOD MICROBIOLOGY,
AND ASSOCIATE DEAN – INTERNATIONAL**

FACULTY OF ENGINEERING |
UNSW SYDNEY, NSW, AUSTRALIA

ABSTRACT:

Of Poultry, Pathogens and People: Perennial Passions

Dr. Julian Cox is Associate Professor – Food Microbiology, and Associate Dean – International, Faculty of Engineering, at UNSW Sydney, New South Wales, Australia. Though officially semi-retired, Dr. Cox maintains a very active program of activities.

Dr. Cox received his B.Sc. and Ph.D. in Microbiology and Food Microbiology from the University of Queensland. Upon graduation, he spent time in the egg industry before returning to his alma mater as a postdoctoral Fellow researcher of *Salmonella* and eggs.

Dr. Cox then joined The University of South Wales as a researcher before becoming a faculty member and has maintained his affiliation to date. During this time, he served as Associate Dean of Education on the Faculty of Science and as International Associate Dean on the Faculty of Engineering.

While supported by an ongoing research program involving pathogens and poultry and educating within his framework and methods, Dr. Cox's focus shifted increasingly toward teaching success skills and public service. He continues to teach food science and technology as well as education for Ph.D. candidates. He is a regular speaker on food microbiology and food safety at both national and international academic and industry meetings. He undertakes a range of volunteer 'passion projects,' including as a laboratory auditor for more than 30 years; as an auditor training framework with GFSI; and as Editor-in-Chief of a major reference text, including journal articles, conference papers and abstracts, and book chapters.

Perhaps most notable in Dr. Cox's work is serving as the Scientific Director of the Food Safety Information Council (FSIC), a health promotion charity in Australia that helps reduce the number of people who fall ill from foodborne illnesses. Through this charity, Dr. Cox communicates food safety messages to the public, including regular appearances on radio and television.

Symposium Abstracts

S1: MYTH BUSTING - SAFETY OF FOOD ADDITIVES AND INGREDIENTS

Primary Author:

Neal Saab, IAFNS

Persons:

Neal Saab, IAFNS

Jason Hlywka, Kraft Heinz

Craig Llewellyn, Exponent

Dominik Alexander, MetaMethod

Norbert Kaminski, Michigan State University

Session Type: Short Symposium - 1.5 hours

Session Summary: Attention often focuses on the safety of food additives and ingredients that are already in market or subject to restrictions in countries outside the U.S. Reports can be found in the literature and popular press linking a food additive or ingredient to toxicity or to disease. The session will present on standards of evidence and differences between purported association and causation and indicate how the weight of evidence approach is used in a post-market evaluation of safety of existing food additives and ingredients. This session will also provide review and insights into how concerns that challenge the prevailing conclusion of safety food additives and ingredients have come about and how they are addressed through a weight-of-evidence approach and post-market surveillance programs. We will also address how regulators can set levels for potentially deleterious substances in foods, taking into consideration unavoidability, feasibility, remediation technology, and new data. The session will address myths associated with food additive and ingredient safety assessment, how food safety decisions must be made with available information while ensuring a plentiful and safe food supply, and provide an understanding into post-market surveillance for the safety of food additives and ingredients. Last but not least, the session will highlight strategies for effective communications to combat these myths.

Primary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

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

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Food Safety Assessment, Audit and Inspection

Keywords: Public Health, Risk Assessment, Risk Management, Toxicology

S2: FUTURE DIRECTIONS OF SALMONELLA CONTROL FOR POULTRY PRODUCTS - WHAT'S NEXT?

Primary Author:

Garth Hoffmann, FREMONTA Corp.

Persons:

Garth Hoffmann, FREMONTA Corp.

Ashley Peterson, National Chicken Council

Dr. Terry Arthur, FREMONTA Corp

Alexandra Calle, Texas Tech University

William K. Shaw, Jr., USDA FSIS

Session Type: Short Symposium - 1.5 hours

Session Summary: In August 2024, USDA-FSIS published a new policy proposal to reduce Salmonella in raw poultry products. The proposed Salmonella Framework was supported by a risk assessment and reflects feedback from extensive stakeholder engagement. It directs attention from farm to fork including preharvest, process control, and finished poultry products. This proposal would establish a standard for Salmonella as an adulterant at a certain level of Salmonella coupled with certain Salmonella serotypes. To implement the framework, if finalized, would require increased levels of testing, process control measures, and finished product disposition. Integral to the testing programs will be the development of lotting strategies to microbiologically divide the product into manageable units for disposition and aid in maintaining control of product pending test results. The implementation of these practices would require cooperation and communication between the regulators, industry, academics, and technical experts. Though USDA-FSIS withdrew this proposal in April 2025, this symposium will present three perspectives on the future of Salmonella control for poultry including from USDA-FSIS, the poultry industry, and the research community. A USDA-FSIS representative will discuss testing and diagnostic needs of the Agency as well as thoughts on potential future Salmonella strategies across FSIS-regulated industries. A poultry industry representative will review the implementation challenges of the Salmonella Framework and alternative approaches to meaningfully impact public health. The industry representative will also discuss research and information needs of the poultry sector. Lastly, a research scientist will present data collected from process control, raw material and finished product testing projects designed to address the needs of the industry.

Primary PDG Sponsor: Meat and Poultry Safety and Quality PDG

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

Primary Topic Area: Meat and Poultry Safety and Quality

Secondary Topic Area: Food Safety Assessment, Audit and Inspection

Keywords: Meat and Poultry, Pathogens, Sampling

S3: FUR REAL AND NOT JUST A BONE TO PICK: PATHOGEN CONTROL IN MANUFACTURING RAW PET FOOD DIETS

Primary Author:

Alvin Lee, Institute for Food Safety and Health

Persons:

Alvin Lee, Institute for Food Safety and Health

Mary-Grace Danao, University of Nebraska-Lincoln

Lloyd Parks, Cuddon Freeze Dry

Session Type: Short Symposium - 1.5 hours

Session Summary: Pet ownership is at all time high globally and 70% of U.S. households are pet owners compared to 56% of UA households in 1988. In Asia, pet ownership is on the rise too. Therefore expenditures on pet food and treats is growing at a steady pace with raw diet pet food as one of the fastest growing categories because owners believe that raw feeding can improve pet health. Raw diet pet food is biochemically distinct and complex and usually not thermally treated compared to conventional extruded or canned pet food. Therefore, raw diet pet food can pose health risks to pets, owners and manufacturers if pathogens are not properly controlled. This symposium will discuss the efficacy of various strategies used by raw pet food manufacturers to control pathogens in their products. These strategies include (a) the use of high pressure processing (HPP) and leverage its synergy with some natural antimicrobials in the production of high-water activity diets; (b) the use of HPP or steam treatments with freeze drying in the production of low-water activity diets; and (c) a certification scheme developed by UK Pet Food for raw pet food manufacturers to support the safe production of raw diets. The scheme is based on a comprehensive standard that encompasses all aspects of hygiene and feed safety and is applied to the entire process, from the selection of raw materials to the finished products.

Primary PDG Sponsor: Animal and Pet Food Safety PDG

Secondary PDG Sponsor: Low Water Activity Foods PDG

Primary Topic Area: Animal and Pet Food Safety

Secondary Topic Area: Low Water Activity Foods

Keywords: Animal and Pet Food Safety, Low-water Activity Foods, Pathogens

S4: FUTURE-PROOFING RETAIL SUCCESS: THE POWER OF FOOD SAFETY CULTURE IN A RAPIDLY EVOLVING INDUSTRY

Primary Author:

Lone Jespersen, Cultivate

Persons:

Lone Jespersen, Cultivate

Larry Kohl, Ahold Delhaize USA

Andrew Clarke, Loblaw Companies Limited

Shingai Nyarugwe, University of Central Lancashire

Bobby Krishna, Dubai Municipality

Session Type: Short Symposium - 1.5 hours

Session Summary: The food retail industry's critical role in ensuring consumer trust and protecting brand reputation has driven the integration of food safety culture within broader organizational risk management strategies. With annual sales reaching an estimated \$880.3 billion in 2021 and employing millions of people worldwide (García-Herrero et al., 2022), a strong food safety culture is key to sustaining success in this sector. This symposium explores the importance of assessing and transforming organizational culture to improve food safety performance in retail. Drawing from the experiences of industry leaders, discussions will revolve around the impact of these changes, challenges encountered during implementation, and strategies for measuring food and product safety culture in a science-based and practical manner. A pilot assessment conducted in 2024 with retailers in the USA, Romania, and Belgium will serve as a prominent example. Over the past decade, food safety culture has become crucial in ensuring consumer well-being (Griffith et al., 2020). Speakers will offer insights on successfully navigating this evolving landscape, including effective assessment methods, key learnings, and communication strategies within organizations. Participants will gain practical knowledge on evaluating and strengthening food safety culture in their retail operations while addressing sector-specific challenges such as high frontline employee turnover—which averages around 60% (McKinsey), organization size and multi-location management, rapidly changing customer demands, and the potential scale of supply chain-related recalls. By attending, participants will be better equipped to secure long-term success and maintain consumer trust in this essential industry.

Primary PDG Sponsor: Food Safety Culture PDG

Secondary PDG Sponsor: Retail and Foodservice PDG

Primary Topic Area: Food Safety Culture

Secondary Topic Area: Retail and Foodservice

Keywords: Data, Food Safety Culture, Retail

S5: FOOD TRACEABILITY RULE UPDATES, INDUSTRY COMPLIANCE PREPARATION AND INDUSTRY TRAINING CURRICULUM

Primary Author:

Jason Wan, Institute for Food Safety and Health

Persons:

Jason Wan, Institute for Food Safety and Health

Melinda Hayman, U.S. Food and Drug Administration

Jennifer McEntire, Food Safety Strategy LLC

Lisa Weddig, National Fisheries Institute

Michael Roberson, Publix Super Markets, Inc.

Session Type: Short Symposium - 1.5 hours

Session Summary: Published in November 2022, the FDA's Food Traceability Rule (FTR) represents the final regulation of the Food Safety Modernization Act (FSMA). The FDA recently announced its intention to extend the compliance date to July 2028. This rule covers the entire food supply chain and applies to both domestic and foreign entities that manufacture, process, pack or hold foods that are on the Food Traceability List (FTL). The FTR requires covered entities to maintain key data elements (KDEs) for foods on the FTL for specific events that occur in the supply chain (Critical Tracking Events or CTEs) in order to facilitate faster identification and rapid removal of potentially contaminated food from the market. With the compliance deadline approaching, this session aims to provide additional insight into what preparation for this rule might mean for industry. Food Traceability Rule resource and implementation updates, industry perspectives (including from the produce, seafood, manufacturing, and retail sectors) on working towards compliance with rule requirements, and key components of a core FTR training curriculum that is currently being developed for covered industry by the Food Safety Preventive Controls Alliance (FSPCA) will be discussed. The session will also include time for Q&A, so please come with any questions you may have about preparing for compliance with the FTR. **Intended Audience:** Growers, food manufacturers, retailers and other food supply-chain sectors, food safety personnel, government regulatory staff, food inspectors, auditors, and students majoring in food science and food safety. **Session Benefits to IAFP Members:** This session proposal addresses the following key objectives of the IAFP: • Food traceability • Supply chain management • Retail, produce and seafood food safety • Food safety education and training

Primary PDG Sponsor: Food Safety Education PDG

Secondary PDG Sponsor: Food Law PDG

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

Primary Topic Area: Food Safety Education

Secondary Topic Area: Food Law

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

Keywords: Education, Regulations, Traceability

S6: PREVENTIVE CONTROLS, HACCP AND BEYOND: EFFECTIVENESS OF CURRENT RISK REDUCTION STRATEGIES IN THE GLOBAL FOOD SUPPLY CHAIN

Primary Author:

Carol Wallace, University of Central Lancashire

Persons:

Carol Wallace, University of Central Lancashire

Andrew Wilson, Cultivate SA

Benjamin Warren, U.S. FDA, Cntr for Food Safety & Applied Nutrition, Office of Food Safety

Sara Mortimore,

Session Type: Short Symposium - 1.5 hours

Session Summary: Foodborne illness continues to rise globally while use of food safety management systems has also increased, considered by many a food safety paradox. Nevertheless, food safety risk management through identifying and analyzing food safety hazards and implementing preventative control systems in the food supply chain remains the most logical approach to food protection, if properly applied. While the concepts of HACCP and risk-based preventive controls go back much further into food safety history, the year 2025 marks 10 years since publication of the FSMA Final Rule for Preventive Controls for Human Food in the USA, and 5 years since the latest revision of Codex HACCP principles. Their anniversaries make 2025 a good time point to reflect on effectiveness of these systematic risk reduction strategies and discuss challenges and opportunities. Both documents provided a strengthened framework for applying food safety risk management principles and have further influenced legislative and private standards around the world. Thus, the tools needed to manage food safety hazards effectively are available, but gaps exist in the systems that support businesses to understand and implement them in a practical and effective manner. Against a backdrop of significant change in the global food supply chain, this symposium will consider data on food safety management in practice, discuss the role of systems, people, culture, language and competency and reveal some of the food safety management system application challenges. Attendees will hear about learnings from 10 years of formal risk-based preventive controls; global considerations for interpretation and implementation of food safety risk management requirements; and more effective HACCP through food safety competency building. The symposium will highlight key considerations and opportunities for food businesses to strengthen their approaches for effective use of food safety risk management throughout the global supply chain.

Primary PDG Sponsor: HACCP Utilization and Food Safety Systems PDG

Secondary PDG Sponsor: Food Safety Education PDG

Primary Topic Area: HACCP Utilization and Food Safety Systems

Secondary Topic Area: Food Safety Education

Additional Topic Area (Optional): Food Law

Keywords: Education, Food Safety Culture, HACCP, Regulations, Risk Management

S7: RISK ASSESSMENTS FOR PRECAUTIONARY LABELING ALLERGEN THRESHOLDS

Primary Author:

Tracie Sheehan, Mérieux NutriSciences

Persons:

Tracie Sheehan, Mérieux NutriSciences

Steve Taylor, University of Nebraska

Joseph Baumert, University of Nebraska Lincoln

Jasmine Lacis-Lee, BVAQ and The Allergen Bureau

Angèle van den Heuvel, Allergenen Consultancy B.V.

Session Type: Short Symposium - 1.5 hours

Session Summary: Research conducted using the internationally recognized Double Blinded Placebo Control (DBPCFC) protocols for clinical trials on food allergic patients has enhanced the scientific knowledge on the dose required to elicit an allergic reaction in food allergic patients. The clinical trial data can be statistically analyzed to establish reference doses. The Australian Allergen Bureau leverages reference doses in their VITAL (Voluntary Incidental Trace Allergen Labelling) program to protect allergic consumers bringing scientific risk assessment into precautionary labeling. The Netherlands has recently implemented a new policy for allergens that includes threshold-based regulations for precautionary labelling of pre-packaged food based on the reference doses established based on clinical trials. This symposium is a hot topic in the allergen research and regulatory community. We cover how to conduct scientific risk assessments, how to establish precautionary labeling limits, and how to leverage the science for practical use in the industry for precautionary allergen statements.

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

Secondary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Food Safety Assessment, Audit and Inspection

Additional Topic Area (Optional): International Food Protection Issues

Keywords: Allergens, Labeling, Risk Assessment

S8: AVIAN INFLUENZA AND VIRUS CONFIRMATIONS - BE CAREFUL WHAT YOU WISH FOR

Primary Author:

Daniel DeMarco, Self

Persons:

Daniel DeMarco, Self

Jennifer DeMarco, Eurofins DiscoverX

Keith Poulsen, University of Wisconsin

Session Type: Short Symposium - 1.5 hours

Session Summary: Reports of avian influenza (H5:N1) positive samples of pasteurized milk at retail in the United States generated much angst among the general public and dairy industry stakeholders. The initial positive results were from a PCR method, and confirmatory testing ultimately showed no infective virus present in any of the PCR positive samples. Nonetheless the damage to the reputation of the dairy industry had been done. The incident resulted in an informal agreement between public health officials and others involved in avian influenza testing to no longer report PCR test results without accompanying confirmation results. At the same time there were calls to introduce the term/concept "presumptive" positive into virus testing generally in order to prevent such misunderstandings from happening again in the future. While it may be helpful in that respect, the term/concept "presumptive" positive also brings a host of complications that those who have been using it for decades in reporting results of bacterial pathogen tests understand very well. Most impactful have been the difficulties associated with cultural confirmations from PCR and other rapid bacterial pathogen screening methods, resulting in very high rates of non confirming presumptives (NCPs) with some pathogen targets. This symposia will provide a forum for deeper exploration of the issues associated with bacterial pathogen test method confirmations, and, using avian influenza as the example case, show how viral confirmation methods will face similar or greater challenges. Presenters will share the state of the art today for confirmation of avian influenza and pathogenic foodborne viruses generally, and describe possible future approaches, including options for avian influenza not requiring growth in chicken eggs. Finally, the current state of high throughput testing for avian influenza and other foodborne viruses will be described along with the unique challenges it poses for virus confirmations.

Primary PDG Sponsor: Applied Laboratory Methods PDG

Secondary PDG Sponsor: Viral and Parasitic Foodborne Disease PDG

Primary Topic Area: Applied Laboratory Methods

Secondary Topic Area: Viral and Parasitic Foodborne Disease

Keywords: Dairy,Laboratory and Detection Methods,Viruses

S9: IMPLICATIONS OF EXTREME WEATHER ON FOOD SAFETY FROM FARM-TO-FORK AND BEYOND

Primary Author:

Claire Murphy, Washington State University

Persons:

Claire Murphy, Washington State University

Laura Strawn, Virginia Tech

Camila Rodrigues, Auburn University

Geetika Joshi, California Department of Food and Agriculture

Erika Austhof, University of Arizona Mel and Enid Zuckerman College of Public Health

Session Type: Short Symposium - 1.5 hours

Session Summary: As extreme weather events become increasingly prevalent, their impact on food safety across the global food system has emerged as a critical concern. This symposium aims to unravel the intricate connections between climate extremes and food safety, focusing on the entire supply chain from farm-to-fork. We will begin with Dr. Erika Austhof, from the University of Arizona's College of Public Health, who will present compelling epidemiological evidence linking weather events to public health outcomes. This foundational overview will highlight the urgency of addressing food safety risks posed by climate change and weather events, establishing a clear connection between human health and environmental factors. Next, California, a key agricultural state in the U.S. grappling with the dual threats of flooding and drought. Dr. Geetika Joshi from the California Department of Food and Agriculture (CDFA) will share case studies that illustrate the pre-harvest challenges posed by these extreme weather events. Case studies emphasize the need for adaptive strategies to mitigate contamination risks and ensure the safety of produce before it even reaches processing. Finally, Dr. Laura Strawn will discuss management and mitigation strategies that span both pre- and post-harvest stages. Her talk will focus on innovative practices and collaborative efforts within the industry to safeguard food safety in the face of climate volatility. By integrating perspectives from public health, industry, and environmental science, this session will foster a comprehensive understanding of the implications of extreme weather on food safety. This session seeks to bridge the gap between epidemiological research and industry practice, ensuring a resilient and secure food system in the era of climate change.

Primary PDG Sponsor: Water Safety and Quality PDG

Primary Topic Area: Pre Harvest Food Safety

Secondary Topic Area: Water Safety and Quality

S10: A DECADE OF FOOD SAFETY CULTURE: ADVANCING FOOD SAFETY THROUGH ORGANIZATIONAL CULTURE AND HUMAN BEHAVIORS

Primary Author:

Lone Jespersen, Cultivate

Persons:

Lone Jespersen, Cultivate

Conrad Choiniere, US FDA

Lawrence Goodridge, University of Guelph

Andrew Wilson, Cultivate

John Boyce, Boyce Food Safety Consulting Ltd.

Session Type: Short Symposium - 1.5 hours

Session Summary: Over the past decade, fostering a robust food safety culture has become a priority across regulatory agencies, industry, and academia. Despite progress, foodborne illnesses continue to have severe consequences on individuals and communities worldwide, with an estimated 420,000 deaths and 600 million cases of illnesses annually, disproportionately affecting vulnerable populations. The economic burden on food-producing companies is also substantial, with an estimated US\$15 billion in annual losses due to food recalls, legal liabilities, and potential market devaluation. This symposium brings together experts from various sectors to share their unique perspectives on key learnings from the past ten years and how these insights were gained, with a particular emphasis on the importance of organizational culture and human behaviors in preventing foodborne illnesses. Speakers will discuss the evolution of food safety regulations and industry practices, highlighting the role of research in shaping policy and promoting best practices that focus on improving organizational culture and human behaviors. They will also explore the importance of cross-sector collaboration in identifying emerging trends and challenges, as well as developing innovative solutions to enhance food safety culture across the supply chain. Through real-world examples and case studies, participants will gain valuable insights into the progress made in strengthening food safety culture by addressing organizational and behavioral factors. Additionally, speakers will identify areas where further improvement is needed to drive future advancements in food safety. Attendees will leave with a deeper understanding of the lessons learned over the past decade and the strategies that can be employed to drive future advancements in food safety through a strong focus on organizational culture and human behaviors.

Primary PDG Sponsor: Food Safety Culture PDG

Secondary PDG Sponsor: International Food Protection Issues PDG

Primary Topic Area: Food Safety Culture

Secondary Topic Area: International Food Protection Issues

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

Keywords: Food Safety Culture, Intervention, Risk Management

S11: WHEN IS A “NEGATIVE” TRULY “NEGATIVE?”

Primary Author:

Joshua Gurtler, USDA-ARS

Persons:

Joshua Gurtler, USDA-ARS

Jeffrey Kornacki, Kornacki Microbiology Solutions, Inc.

Bradley Stawick, AOAC INTERNATIONAL

Brian Eyink, Hogan Lovells

Session Type: Short Symposium - 1.5 hours

Session Summary: Product and environmental testing are an important part of microbiological food safety assurance. Decisions related to such test results can have profoundly serious public health consequences, as well as legal and financial implications for the food industry. Thus, the accuracy and reliability of both sampling and testing are extremely important. But how many samples and tests are enough? Can we ever have sufficient statistical confidence? In addition, there are questions regarding the reliability of a negative pathogen test. Examples will be provided of approaches to take when (1) highly sensitive screening assays are “confirmed” as culturally “negative” by officially recognized approaches, while (2) more rigorous cultural and genetic based assays yield a definitive “positive” result. What is the right answer? Explanations concerning these critical questions, as well as hypotheses related to these pertinent issues, will be provided.

Primary PDG Sponsor: Applied Laboratory Methods PDG

Primary Topic Area: Applied Laboratory Methods

Secondary Topic Area: Data Management and Analytics

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

Keywords: Data, Laboratory and Detection Methods, Risk Analysis, Sampling

S12: RISKY BUSINESS: UNDERSTANDING AND COMMUNICATING THE COSTS AND BENEFITS OF RISK MITIGATION PROGRAMS FOR FOOD SAFETY

Primary Author:

Timothy Jackson, FDA CFSAN

Persons:

Timothy Jackson, FDA CFSAN

Felicia Wu, Michigan State University

Bernhard Steves, Steves Risk Strategies LLC

Sara Mortimore,

Gregory Pritchard, Nestle USA, Inc.

Gary Van Breda, McDonald's Corporation

Gregory Astill, USDA Economic Research Service

Mark Moorman, Food and Drug Administration

Hilary Whitham, Centers for Disease Control and Prevention

Lone Jespersen, Cultivate

Session Type: Full Symposium - 3.0 hours

Session Summary: Food companies and government authorities are responsible for ensuring the safety of food for humans and animals. Strong food safety cultures, and the “management commitment” identified in food safety standards (ISSO 22000) prioritize investment in food safety, including human capital (people and time), prioritization of capital expenses, sufficient investment in relevant operational expenses (e.g., cleaning and sanitation, design, monitoring and verification of control measures, supply-chain programs, defect reduction). This symposium will balance the perspectives of food companies and regulators, exploring the following questions: - How can food operators and regulators determine the appropriate investment in food safety program cost accounting for organization size, product type and complexity? - What are the economic and financial benefits and incentives of programs and infrastructure important for food safety? What are the costs of not having sufficient investment in food safety? - How can the benefits of food safety programs and justification of cost be communicated to senior leadership of food companies, government authorities, consumers and taxpayers? What are the other organizational and business of programs that also support food safety?

The PDG(s) selected have been notified and approve of this submission:

No

Non PDG Sponsor (Organization, Association, etc.): FDA, CDC, USDA ERS

Primary Topic Area: Food Safety Culture

Secondary Topic Area: HACCP Utilization and Food Safety Systems

Additional Topic Area (Optional): Retail and Foodservice

Keywords: Production/Plant Design,Recalls,Risk Management

S13: OUTBREAKS LINKED TO CANTALOUPE: IMPROVING FOOD SAFETY AND PROTECTING PUBLIC HEALTH

Primary Author:

Katherine Marshall, CDC

Persons:

Katherine Marshall, CDC

Sonia Salas, Western Growers Association

Scott Monroe, Purdue University

Colin Schwehsohn, Centers for Disease Control and Prevention

Michelle Danyluk, University of Florida

Kristin Esch, Food and Drug Administration

Session Type: Short Symposium - 1.5 hours

Session Summary: The occurrence of large outbreaks of *Listeria* and *Salmonella* infections linked to cantaloupe warrants further exploration of trends and themes among outbreaks. Understanding these patterns can help identify prevention strategies to reduce outbreaks and illnesses. During this session, we will explore findings from a decade of melon-associated outbreaks, describe efforts to detect and respond to outbreaks sooner by tracking REP (reoccurring, emerging, and persisting) strains historically associated with melons, share findings from select on-farm investigations, show survey results on consumer perceptions and handling of cantaloupe, hear an update on the revision of the U.S. national cantaloupe and netted melons guidelines, and discuss additional work underway to prevent future illness.

Primary PDG Sponsor: Fruit and Vegetable Safety and Quality PDG **Secondary**

PDG Sponsor: Committee on Control of Foodborne Illness

Primary Topic Area: Fruit and Vegetable Safety and Quality

Secondary Topic Area: Pre Harvest Food Safety

Additional Topic Area (Optional): Epidemiology

Keywords: Outbreak, Pre-harvest, Produce

S14: INNOVATIVE PRODUCT DESIGN FOR QUALITY AND FOOD SAFETY - CREATIVE APPROACHES TO NOVEL PLANT-BASED PRODUCTS DEVELOPMENT

Primary Author:

Aaron Pleitner, Impossible Foods

Persons:

Aaron Pleitner, Impossible Foods

Deepa Thiagarajan, Mars

Lily Yang, The Acheson Group

Olivia Arends, The Kraft Heinz Company

Alejandra Ramirez-Hernandez, Impossible Foods

Nicolette Hall, Kerry

Session Type: Short Symposium - 1.5 hours

Session Summary: Market screening by the Plant-Based Foods Association (PBFA) in 2023 identified that retail sales in the category had reached \$8.1 billion annually with 62% market penetration of households. E-commerce continues to boom with a three-year growth rate of 16.4% and a total market space of \$394 million, while 41% of foodservice customers were recorded as choosing plant-based menu items (PBFA 2023 Report). To fulfill the demand for plant-based products and to sustain this continued growth, innovative product development is necessary to offer novel alternative products with increased culinary and sensory performance. However, the evolution in these areas should not and cannot come at the expense of consistent quality and food safety performance. Formulation, process and finished good design need to be accounted for to prevent and control microbiological and chemical hazards. Such preventive measures do not stop at the time of manufacturing; quality and food safety have to be maintained throughout the entire product shelf life, considering potential handling and customer use cases. The ability of QFS professionals to work collaboratively and bring adaptable solutions to R&D partners is necessary to include considerations early in innovation and commercialization cycles. In this symposium, approaches and management of ingredients, processes, formulation and packaging will be presented by leading industry representatives for novel products. Insight into function-driven formulation efforts and the validation of these efforts on the finished product will be presented. Target areas will include extending product shelf life, controlling in-process performance at manufacturing, antimicrobial applications for matrix-specific efficacy and monitoring microbiological presence and behavior from farm to fork. Enhancing a more thorough understanding of these target areas and applying benchmarked approaches will ensure consumer health, safety, product preference, and category growth.

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

Secondary PDG Sponsor: HACCP Utilization and Food Safety Systems PDG

Primary Topic Area: Plant-Based Alternative Products

Secondary Topic Area: HACCP Utilization and Food Safety Systems **Additional**

Topic Area (Optional): Microbial Modelling and Risk Analysis

Keywords: Antimicrobials, Genomics, Microbiome, Pathogens, Plant-based foods, Shelf-life, Spoilage

S15: CUTTING THROUGH THE HYPE: REAL-WORLD BENEFITS OF AI IN FOOD SAFETY

Primary Author:

James Doyle, Creme Global

Persons:

James Doyle, Creme Global

Brendan Ring, Creme Global

Carrie Rigdon, Association of Food & Drug Officials

David Monk,

Amani Babekir, Ecolab

Mark Moorman, Food and Drug Administration

Sarah Murphy, FDA

Session Type: Short Symposium - 1.5 hours

Session Summary: Artificial Intelligence (AI) is poised to transform the global food sector by enhancing regulatory compliance, industry operations, and food safety outcomes. This session will highlight the transformative potential of AI technologies—including Machine Learning (ML), Generative AI (GenAI), and Open AI systems—across the supply chain. Participants will explore how these tools will revolutionize food safety by addressing critical challenges, optimizing processes, and fostering collaboration between industry and regulators. The session will showcase how regulators plan to leverage data-driven AI models like ML and rule-based systems to target compliance, predict risks, and identify high-risk products with greater precision. It will also examine the expanding role of GenAI in simulating complex scenarios and generating innovative solutions for food safety management. AI systems, enabled by cross-functional collaboration and data sharing, will be presented as key drivers in mitigating global food safety risks. Real-world case studies will illustrate how AI plays a pivotal role in environmental monitoring, inspections, and outbreak investigations, helping organizations refine safety protocols and anticipate emerging threats. Applications of AI will demonstrate how these technologies support predictive modeling to address challenges such as PFAS contamination, fraud prevention, and supply chain disruptions, ultimately improving food safety and resilience. Attendees will learn how AI tools—ranging from ML to GenAI—will be applied at various supply chain stages to enhance decision-making, achieve compliance, and safeguard food integrity. The session will provide actionable guidance on overcoming implementation challenges and fostering collaboration between regulators and industry. Participants will leave with strategies to adopt cutting-edge AI solutions that will strengthen food safety systems and drive global food protection efforts.

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: Data, Modeling, Outbreak, Public Health, Risk Assessment, Risk Management

S16: DAILY LUNCH MEAT SAFETY: LISTERIA OUTBREAKS AND RECALLS LINKED TO LUNCHEON MEAT

Primary Author:

Ravirajsinh Jadeja, Oklahoma State University

Persons:

Ravirajsinh Jadeja, Oklahoma State University

Rigo Soler, Texas Tech University

Surabhi Wason, Kerry Ingredients and Flavors

Saurabh Kumar, KERRY

Marcos Sanchez, Texas Tech University

Mindy Brashears, Texas Tech University

Joyjit Saha, Kerry

Daniele Sohler, Hygiena

Session Type: Short Symposium - 1.5 hours

Session Summary: Although the prevalence of *Listeria monocytogenes* in ready-to-eat meat and poultry products has steadily decreased in the past two decades until the recent outbreak and recall concerning Boars Head (July 2024) that rocked the nation. A recall of roughly 7.2 million pounds of product with at least 57 people hospitalized and 9 deaths across 18 states in the United States followed by multi-agency investigation, several lawsuits and closing of plant. *Listeria monocytogenes* can grow at refrigeration temperature and is widely distributed in food processing plants and can survive on processing equipment such as meat slicers which serve as a potential contamination source. Ensuring the safety and quality of meat products involves managing multiple systems across meat processing industry from sanitation to product safety. Environmental monitoring programs with systematic sampling, testing production environment for potential sources of contamination of pathogens could help understand the operation and validate the sanitation programs. Likewise for product safety, tools like predictive models such as FSSP, Combase, PMP, FDA iRISK, QMRA, and @Risk, etc. could be key for helping processors access their preservation system or change in formulations or any deviations in the storage parameters such as temperature etc. The symposium will explore the current outbreak and empower stakeholders in food industry with the knowledge and strategies needed to navigate the integration of multiple independent strategies ranging from processing hygiene, regulatory frameworks, preservation technologies and modeling science to promote the meat safety. Symposium attendees will learn about: 1. Understanding the outbreak and recalls related to luncheon meat 2. Merits of effective environmental monitoring and sanitation programs; daily intervention as well as deep cleans 3. Tools available for developing effective sampling plans 4. Use of predictive modeling to validate changes

Primary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Secondary PDG Sponsor: Applied Laboratory Methods PDG

Primary Topic Area: Meat and Poultry Safety and Quality

Secondary Topic Area: Microbial Modelling and Risk Analysis

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

S17: THE TORTUOUS TANGLE OF WATER REGULATIONS FOR FRESH PRODUCE: STRATEGIES TO NAVIGATE

Primary Author:

Don Stoeckel, Produce Safety Alliance

Persons:

Don Stoeckel, Produce Safety Alliance

Phyllis Posy, PosyGlobal

Donna Clements, Produce Safety Alliance

Steve Strub, Wegmans

Michelle Danyluk, University of Florida

Sonia Salas, Western Growers Association

Session Type: Short Symposium - 1.5 hours

Session Summary: The intersection of the Food Safety Modernization Act Produce Safety Rule (FSMA PSR) and various U.S. Environmental Protection Agency (USEPA) regulations present navigational challenges to produce growers and packers in the United States, and those who export to the US. These challenges impact the security of the year-round food supply for U.S. consumers as well as international trade. At stake is the ability of produce growers to supply food with certainty of regulatory compliance, and use developing science to promote produce safety across the food supply chain. Pre-existing USEPA regulations and guidance are the foundation of some FSMA PSR requirements for agricultural water quality: drinking water in 21 CFR 112.43(c) and antimicrobial pesticides in 112.46(a). Specific exemptions to FSMA PSR agricultural water requirements rely on USEPA microbial standards for drinking water or use of EPA-registered antimicrobial pesticides (sanitizers) for water treatment. The same FSMA PSR requirements require documentation of data supporting comparable water quality or treatment practices in lieu of a formal certification process. Availability of scientific data is particularly important to those exporting to the U.S. from countries that do not have equivalent regulatory structures for drinking water or for sanitizer chemicals. Scientific research on efficacy and safety can have limited utility if not designed to accommodate on-farm regulatory requirements (e.g., labels, certifications, technical literature). Speakers in this symposium will bring industry, scientific, and cross-sector perspectives to explain what is known and what is unclear when navigating the interplay between water related requirements from different authorities. IAFP members will hear perspectives on shaping policies and developing best practices that drive solutions through mechanisms such as development of actionable guidance and coordination of efforts across traditional silos. Overall, this session aims to help navigate the labyrinth that leads to a safe, wholesome food supply and promotes success of produce-growing operations.

Primary PDG Sponsor: Water Safety and Quality PDG

Secondary PDG Sponsor: Food Law PDG

Primary Topic Area: Food Law

Secondary Topic Area: Food Safety Assessment, Audit and Inspection

Keywords: Produce, Regulations, Water

S18: INNOVATIONS IN THE DAIRY CLEANING AND SANITATION: SAFETY, EFFICIENCY AND SUSTAINABILITY IMPACTS

Primary Author:

Arshpreet Kaur Khattri, Michigan State University

Persons:

Arshpreet Kaur Khattri, Michigan State University

Pratibha Chaudhary, Darigold

Nathan Mirdamadi, Kerry Ingredients

Aakash Sharma, Dairy Farmers of America

Shalini Sehgal, Bhaskaracharya College of Applied Sciences University of Delhi

Surabhi Wason, Kerry Ingredients and Flavors

Amrit pal Singh, Tennessee State University

Vijay Juneja, USDA-ARS-ERRC

Session Type: Short Symposium - 1.5 hours

Session Summary: Cleaning and sanitation of dairy industry processing lines, machines, and storage silos are primarily conducted through the Cleaning-in-Place (CIP) procedure. This method was developed to overcome the limitations of traditional hand cleaning (Cleaning on Place) such as higher cleaning time and lower cleaning efficiency and to improve product safety and quality. CIP techniques often involve heat treatments and non-biodegradable chemicals like acids and alkalis. Despite stringent controls over time, temperature, flow, and chemical concentrations, traditional CIP systems can still result in dairy products with compromised shelf life, as they are often ineffective in fully eliminating biofilms. Additionally, proper neutralization of chemicals before disposal is essential, raising sustainability concerns, such as higher biological oxygen demand (BOD) and increased waste generation. To address these challenges, research has explored alternatives like enzymes, bacteriophages, ozone, and chlorine substitutes like EDTAs. These alternatives not only help prevent biofilm formation but also reduce water and energy consumption. Additionally, introduction of jet cleaners, innovative strainers, optimizing the time for each cleaning cycle, and monitoring processing parameters have further enhanced the effectiveness of CIP of dairy equipment and lines. This symposium delves into the historical background of cleaning practices and sanitation in the dairy industry, essential factors influencing cleaning, and a comparison of traditional and innovative methods in terms of effectiveness, safety, and environmental impact. Symposium attendees will learn: 1. Deeper understanding of the evolution of cleaning and sanitation practices in the dairy industry 2. Better understanding of factors affecting the CIP of dairy processing unit 3. Regulatory prospective of cleaning and sanitation procedures in the dairy industry 4. Sustainability prospective of traditional CIP systems in dairy area

Primary PDG Sponsor: Dairy Quality and Safety PDG

Secondary PDG Sponsor: Food Hygiene and Sanitation PDG

Primary Topic Area: Dairy Quality and Safety

Secondary Topic Area: Sanitary Equipment and Facility Design

Keywords: Dairy,Environmental monitoring,Sanitation

S19: RISK BUSINESS IN LOW- AND MIDDLE-INCOME COUNTRIES

Primary Author:

Kang Zhou, Food and Agriculture Organization of the United Nations

Persons:

Kang Zhou, Food and Agriculture Organization of the United Nations

Myoengsin Choi, Codex

Mirian Bueno, SENASA

Lise Korsten, University of Pretoria

Session Type: Short Symposium - 1.5 hours

Session Summary: Risk analysis is not simply a theoretical concept nor an approach limited to addressing food safety challenges in well-resourced settings. The application and integration risk assessment, risk management and risk communication are mechanisms that can (and should) be widely and effectively leveraged to improve the lives and the livelihoods of individuals and communities in low- and middle-income countries (L&MICs) - among those living with a disproportionate burden of foodborne illness. However, to achieve this goal, awareness of the benefits of applying risk-based frameworks to food safety must be first be raised and stakeholders must be empowered with the knowledge, skills, tools and motivation to put these principles into practice. Though highlighting recent workshops in Africa, Asia, and Latin America, the objective of this symposium is to provide participants examples of how food safety risk analysis capacity can be tailored, encouraged, supported and strengthened in resource-limited settings. Lessons and experiences learned will be discussed and participants will be invited to apply their skills and experience to assist in paving the way for better global food safety across different countries and cultures.

Primary PDG Sponsor: International Food Protection Issues PDG

Primary Topic Area: International Food Protection Issues

Secondary Topic Area: Microbial Modelling and Risk Analysis

Keywords: One Health, Regulations, Risk Analysis

S20: AI FOR PREDICTIVE MICROBIAL RISK ASSESSMENT IN FOOD PROCESSING

Primary Author:

Luyao Ma, Oregon State University

Persons:

Luyao Ma, Oregon State University

Vijay Juneja, USDA-ARS-ERRC

Subrata Kumar Bag, West Bengal University of Animal & Fishery Sciences

Abhinav Mishra, University of Georgia

Session Type: Short Symposium - 1.5 hours

Session Summary: A revolution in the way we predict microbial risks in food processing environments came with the integration of machine learning (ML), a subfield of artificial intelligence (AI), into food safety management. This symposium will focus on the application of advanced ML algorithms in enhancing the predictive capability of microbial risk assessments that can improve food safety. Traditional methods, which rely on historical data and static models, fail to capture the dynamic nature of food processing environments. In contrast, ML algorithms can analyze large amounts of environmental and processing data in real-time, identify patterns, and accurately predict potential microbial hazards within those environments. This symposium will explore the development and validation of robust ML models tailored for food processing environments. Key topics include strategic data collection on environmental and processing parameters, such as temperature, humidity, and time, in correlation with microbial counts. Discussions will cover the selection and optimization of ML algorithms, including Decision Trees, Random Forests, Support Vector Machines, and Neural Networks. Quantifying target pathogens in the presence of food matrices and natural microflora in food processing environment will be highlighted to ensure reliability and real-world deployment for data collection. The symposium will also address challenges like data quality, model interpretability, and integration with existing food safety systems. Case studies showcasing successful ML applications in microbial risk prediction will demonstrate the practical feasibility and potential for widespread use. By fostering interdisciplinary collaboration among food technologists, data scientists, and industry stakeholders, the event aims to drive innovative solutions that enhance food safety and protect public health.

Primary PDG Sponsor: Modelling and Risk Analysis PDG

Secondary PDG Sponsor: Advanced Molecular Analytics PDG

Primary Topic Area: Microbial Modelling and Risk Analysis

Secondary Topic Area: Advanced Molecular Analytics

Additional Topic Area (Optional): Data Management and Analytics

Keywords: Modeling, Pathogens, Risk Assessment

S21: UNCHARTED TERRITORY: THE IMPORTANCE OF GENOMIC SURVEYS OF FOODBORNE PATHOGENS FROM “UNCOMMON” ENVIRONMENTS

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

Abigail Snyder, Cornell University

Tim Stubbs, Dairy Management Inc

Andrea Moreno Switt, Catholic University of Chile

Martin Wiedmann, Cornell University

Session Type: Short Symposium - 1.5 hours

Session Summary: Pathogen genomic databases have become essential tools for ensuring food safety. The data primarily come from clinical, food, and food production environment samples. However, national genomic surveys of foodborne pathogens from diverse environments, such as consumer households or pristine environments (ie., undeveloped natural settings) provide important insights supporting outbreak investigations, comprehensive assessments of contamination risk, and prioritization of surveillance efforts. National genomic surveys can reveal differences in regional distribution of pathogen subtypes across the U.S. These surveys help identify the prevalence of pathogens in non-food production environments, and genomic data from these pathogens shed light on possible routes of pathogen transmission. For example, a clinical isolate that is closely related to an isolate found in a pristine environment suggests a complex route of pathogen transmission, which can aid our understanding of produce safety. Large surveys of pathogens in household settings provide much needed data on the role of cross-contamination that occurs in the home. For example, genomic surveys of *Cronobacter* spp. from households reveals the need for risk mitigation strategies from consumers to compliment the strategies implemented by dry dairy and infant formula companies during product manufacturing. In this session, we will discuss these databases, and data gaps where national genomic surveys of foodborne pathogens can support identification of matches between clinical specimens and non-clinical isolates. Then we will share examples from two national genomic surveys of foodborne pathogens. The next talk will focus on a longitudinal sampling effort in 120 households across the U.S. Finally, we will address outcomes from a large genomic survey of foodborne pathogens collected from natural environments.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: Dairy Quality and Safety PDG

Non PDG Sponsor (Organization, Association, etc.): Institute for the

Advancement of Food and Nutrition Sciences

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Dairy Quality and Safety

S22: OUTBREAK SYMPOSIUM

Primary Author:

Laura Gieraltowski, Centers for Disease Control and Prevention

Persons:

Laura Gieraltowski, Centers for Disease Control and Prevention

Ewen Todd, Ewen Todd Consulting LLC

Kari Irvin, Food and Drug Administration

Amanda Conrad, Centers for Disease Control and Prevention

Katherine Marshall, CDC

Kailey Lewis, FDA

Dan Gerlach, Minnesota Dept. of Health

Sheryl Shaw, USDA, FSIS

Session Type: Full Symposium - 3.0 hours

Session Summary: This symposium will gather experts from public health and regulatory agencies to discuss five different topics surrounding specific outbreak investigations and outbreak investigation processes. Additionally, a presentation time slot will be held open for a late-breaking outbreak investigation highlighting a recent illness outbreak investigation. In 2024, contaminated cucumbers were linked to a large outbreak of 551 infections of multiple strains of *Salmonella*. Testing identified outbreak strains in untreated canal water used by the growers. Speakers will describe the epidemiology of the outbreak, environmental sampling conducted and findings, and challenges in the investigation. An overview of several recent international enteric disease outbreak investigations will be provided by Dr. Ewen Todd. Outbreak investigations covered will include a variety of pathogen types and food sources. In summer of 2024, CDC, USDA, and several states investigated the largest outbreak of *Listeria* infections linked to deli meats. Representatives from CDC and USDA will discuss the epidemiology, traceback, in-plant findings, and investigational challenges. Ingredient-specific analyses can be instrumental when investigating restaurant subclusters within multistate outbreaks, as they can potentially implicate ingredients, going beyond analyses at the menu item level. A speaker from the Minnesota Department of Health will share their Ingredient Matrix CodeBuilder app, which maps ingredient-level exposures based on recipe information and patron menu-level exposures, as well as other tips for pursuing ingredient-specific analyses. A speaker from the Public Health Agency of Canada will review the response to a *Listeriamonocytogenes* outbreak in Canada, involving 20 cases who became sick between August 2023 and mid-July 2024 in four provinces. The outbreak was linked to nationally recalled plant-based refrigerated beverages.

Primary PDG Sponsor: Committee on Control of Foodborne Illness

Primary Topic Area: Epidemiology

Secondary Topic Area: Developing Food Safety Professionals

Keywords: Epidemiology, Outbreak, Public Health

S23: FROM DATA TO DECISIONS: GENOMICS/METAGENOMICS IN FSQA PROGRAMS

Primary Author:

Jessica Chen, Centers for Disease Control and Prevention

Persons:

Jessica Chen, Centers for Disease Control and Prevention

John Donaghy, Nestlé SPN.

Celina To, Nelson Jameson

Andrzej Benkowski, Eurofins Microbiology Laboratories

Ramin Khaksar, Clear Labs

Heather Carleton, Centers for Disease Control and Prevention

Session Type: Short Symposium - 1.5 hours

Session Summary: This session focuses on the transformative role of sequencing data in food safety and quality assurance (FSQA) decision-making within the food industry. It aims to provide practical insights into leveraging advanced sequencing technologies to enhance operational efficiency and ensure product safety or quality. The central theme emphasizes integrating sequencing data into everyday FSQA practices. Through case studies and real-world applications, participants will learn how sequencing data can be integrated into current QA management systems to monitor efficacy of sanitation operational procedures, source tracking, and verification of label claims. This data-driven approach empowers organizations to make informed decisions that mitigate risks, improve product quality, and safeguard consumer health. The industry panel will help translate the hard science coming from research to allow non-scientists to understand the value and impact of genomic/metagenomic outputs, so new FSQA managers know how to use this type of data to make actionable strategies into their operational frameworks. The session will also cover affordable and practicable options in sequencing, so laboratories of all sizes can have access to the technology, along with best practices to improve standardization and reproducibility. By fostering a dialogue among industry leaders representing the private sector as opposed to historical discussions coming from academia and government, food companies that are new to sequencing can learn from their peers in how this tool can also benefit them outside of recall and outbreak scenarios.

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

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

Keywords: Data, Environmental monitoring, Genomics, Pathogens, Risk Analysis, Risk Assessment, Risk Management, Shelf-life, Spoilage, Traceability

S24: RETAIL, REGULATORY, AND FOOD RESCUE AND RECOVERY CONSIDERATIONS TO ADDRESS GLOBAL FOOD WASTE

Primary Author:

Mary Morris-Donaldson, Michigan State University Extension

Persons:

Mary Morris-Donaldson, Michigan State University Extension

Catherine Nettles Cutter, Penn State University

Lily Yang, The Acheson Group

Jennifer Quinlan, Drexel University

Jason Wadsworth, Wegmans Food Markets, Inc.

Kunna Faal, Michigan State University

Emily Broad Leib, Harvard Law School

Session Type: Short Symposium - 1.5 hours

Session Summary: The real and perceived food safety risks that contribute to the estimated 931 million tons of food wasted globally each year, make it difficult for the retail food industry to manage food donations and for policy makers to regulate the amount of food that is wasted. It is at the level of food rescue and food distribution that has created innovative ways to decrease food waste while supporting health equity and food security. The speakers in this symposium will focus on the retail food industry, charitable food rescue and distribution organizations, and regulatory considerations to address the intersection of food safety and food waste within the United States and South Africa. Symposium attendees will become aware of the challenges faced by the retail food industry, charitable food distribution, and policy makers caused by the real and perceived food safety risks that impact food waste. Attendees have the opportunity to identify solutions to counteract the perceived food safety risks perpetuated around the globe. This symposium will bring a new conversation to address varying interpretations of food safety guidance, and lack of policy harmonization for managing surplus and discarded foods at multiple nodes in the food system.

Primary PDG Sponsor: Food Safety Education PDG

Secondary PDG Sponsor: Food Law PDG

Primary Topic Area: Food Safety Education

Secondary Topic Area: Food Law

Keywords: Education, Labeling, Regulations

S25: MANAGING CHEMICAL HAZARDS IN WATER REUSED IN FOOD PRODUCTION AND PROCESSING

Primary Author:

Leon Gorris, Food Safety Futures

Persons:

Leon Gorris, Food Safety Futures

Andrew Pearson, Tonkin + Taylor

Dima Faour-Klingbeil, DFK for Safe Food Environment

Raquel Medeiros, Nestlé

Session Type: Short Symposium - 1.5 hours

Session Summary: Water is an increasingly precious commodity for food production and processing in many geographies, especially in areas facing water scarcity. Codex Alimentarius is developing new guidance on the safe use and reuse of water, aiming to balance the need for food safety with the need to address water shortages and focusing on managing microbiological hazards. However, effective risk management also requires addressing chemical hazards, including contaminants of emerging concern (CECs). For both microbiological and chemical hazards, global and regional guidelines and regulations are being developed, though mostly at the levels of identifying hazards, assessing associated risk and establishing risk management principles. Regulations and best practices for water reuse, including adequate risk management of hazards, remain insufficiently addressed in existing global and regional guidelines considering the urgency required to meet water scarcity challenges. Chemical hazards in water of concern in water reuse scenarios of food industry include pesticides, natural toxins, chemical contaminants, pharmaceuticals, and industrial chemicals. Particularly, chemicals of emerging concern (CECs) can persist in the water cycle despite efficient removal processes. Recent methodologies have provided ranked lists of CECs, which can inform regulatory and operational strategies. Even when certain chemicals are removed efficiently, residual concentrations in wastewater can still pose risks, with some residuals being related to treatment technologies. For example, quaternary treatment technologies for removing chemicals remain largely unregulated despite their potential to foster antimicrobial resistance. In practice, the food industry must address risks related to a range of chemical hazards when reusing water. Some food sectors have been developing insights and practices in that regard, but hazards and food safety challenges to water reuse may differ substantially across different food sectors. This symposium brings together three speakers to discuss the chemical hazards of concern, current risk assessment, risk management and regulatory frameworks, and operational practices established to mitigate risks to consumer safety.

Primary PDG Sponsor: Water Safety and Quality PDG

Secondary PDG Sponsor: International Food Protection Issues PDG

Non PDG Sponsor (Organization, Association, etc.): Food and Agricultural Organization

Primary Topic Area: Water Safety and Quality

Secondary Topic Area: Food Chemical Hazards and Food Allergy

Additional Topic Area (Optional): International Food Protection Issues

Keywords: Chemical Contaminant, Risk Assessment, Risk Management

S26: HARMONIZATION FOR COMMERCIAL STERILITY TESTING

Primary Author:

Jesse Miller, Bio-Rad Laboratories

Persons:

Jesse Miller, Bio-Rad Laboratories

Frederic Martinez, Neogen

Haiping Li, USDA AMS Dairy Programs

Anett Winkler, Cargill

Adrianne Klijn, Societe Des Produits Nestle SA

Session Type: Short Symposium - 1.5 hours

Session Summary: Ready-to-eat dairy products processed at Ultra-High Temperature (UHT) are extensively produced and consumed because of their convenience of storage at ambient temperatures for a long period. Sterilization and aseptic filling are critical steps, and food producers must verify their efficacy by demonstrating commercial sterility. Some of these foods, such as infant formula or food for medical purposes, could be ingested by immunosuppressed individuals. There are no harmonized standards for commercial sterility testing, and many rapid methods are available with variable sensitivities. In the FAO: Codex Code of Hygienic practice for milk products (CAC/RCP 57-2004), Annex-B and European Commission regulation (EC) #1662/2006, 15 days pre-incubation of the UHT product are required before conducting testing for sterility; in China, the GB standard expects 7 to days pre-incubation depending on the tested food. In BAM Chapter 21A only canned food testing is described with 14 days pre-incubation. Different ISO working groups started to work on harmonization by writing new standards. There is a need for rapid methods and a few of them have been recently validated and certified. This symposium will discuss the need to define what are the relevant foodstuffs and trustable protocols to guarantee their safety.

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: Acid/Acidified

Foods, Beverages, Dairy, Epidemiology, Immunocompromised, Laboratory and Detection Methods, One Health, Public Health, Regulations

S27: END TO END (E2E) PHYSICAL HAZARDS RISK MANAGEMENT IN PET FOODS FOR SAFETY AND HEALTH OF COMPANION ANIMALS

Primary Author:

Deepa Thiagarajan, Mars

Persons:

Deepa Thiagarajan, Mars

Beilei Ge, Food and Drug Administration

Michele Sayles, Diamond Pet Food

Amanda Jones, Nestle Purina

Josh Hasty, Tyson

Leslie Hancock, Hills Pet Nutrition

George Awuah, Mars Petcare US

Session Type: Short Symposium - 1.5 hours

Session Summary: Physical hazards, particularly hard and sharp foreign objects, in pet foods have always been a concern and on the increase, triggering recalls in the market. Many factors have contributed to changes in how pet food industry engages today with consumers, suppliers, and regulatory agencies on foreign body issues as everyone works toward the common goal of providing safe, wholesome foods for pets. Given the complex challenges (raw material risk increase, regulatory and consumer focus), there is no single identified technology to fully protect pet food products, hence, the solution requires a holistic strategic approach with collaboration among all functions and entities. Additionally, awareness of emerging hard and sharp contaminant horizon risk is necessary as we deal with global climate change, sustainability and non-GMO. While the target is to minimize E2E risk with a Zero Foreign Material (FM) ambition, the focus must not only be around food safety but the overall quality of products, food adulteration and potential impacts on health of pets. In this symposium, stakeholders will learn 1. Ingredient manufacturers' take on best practices for keeping foreign materials out of animal food and feed to maintain quality and food safety 2. Holistic and practical E2E strategies of a global pet food manufacturer to identify, mitigate, and manage physical hazards to maintain safety, quality and minimize adulteration risk in pet foods. 3. Veterinarian perspective on understanding the nature of existing and emerging physical hazards, taking SAHCODA approaches for effective strategies to prevent adverse impacts on pet health 4. Regulatory insights on FSMA-GMP based preventative controls and the right tools for industry to work together within the regulatory framework.

Primary PDG Sponsor: Animal and Pet Food Safety PDG

Secondary PDG Sponsor: Physical Hazards and Foreign Material PDG

Primary Topic Area: Animal and Pet Food Safety

Secondary Topic Area: Physical Hazards and Foreign Material

Additional Topic Area (Optional): HACCP Utilization and Food Safety Systems

Keywords: Animal and Pet Food Safety,HACCP,Risk Management

S28: WARMING THE FROZEN FOOD SUPPLY CHAIN: FOOD SAFETY AND SPOILAGE IMPLICATIONS

Primary Author:

Sanjay Gummalla, American Frozen Food Institute

Persons:

Sanjay Gummalla, American Frozen Food Institute

Ian Jenson, FIRST Management

Marcel Zwietering, Wageningen University

Nigel Thorgrimsson, Ag Food Assist Ltd.

Session Type: Short Symposium - 1.5 hours

Session Summary: Frozen foods are an integral category of the global retail, food service, and industrial food supply chain. As one of the most ancient forms of food preservation, freezing has helped retain the nutritional potential of seafood, meats, fruits and vegetables for centuries and in recent decades given rise to a variety of innovative frozen foods such as entrees, pizza, appetizers, etc. The global standard operating set point of the frozen supply chain is -18°C (0°F), a consequence of early studies to set shelf-life parameters across a range of frozen foods. Some countries even mandate a maximum temperature of -18°C for frozen foods through regulatory schemes. At the same time, sustainability of the broader food industry and climate change have brought attention to impacts on the food system, and the possibility of a higher operational temperature set point in the frozen supply chain. A higher frozen storage temperature implies reduced energy-use and greenhouse gas emissions; however, the proposal also elicits several questions about the impact on frozen food quality and the long-held food safety assurance expected during frozen storage and transport across the chain. In this symposium, food safety experts from academia and industry will deliberate the scientific literature as they consider the parameters that determine growth of microbial pathogens and spoilage organisms at temperatures less than 0°C (the freezing point of pure water); microbial production of toxins and other hazardous compounds such as biogenic amines; other enzymatic and chemically induced risks; and share data needs for building confidence at a new temperature threshold in support of a more sustainable frozen food supply chain.

Primary PDG Sponsor: International Food Protection Issues PDG

Secondary PDG Sponsor: Modelling and Risk Analysis PDG

Non PDG Sponsor (Organization, Association, etc.): Frozen Food Foundation

Primary Topic Area: International Food Protection Issues

Secondary Topic Area: Microbial Modelling and Risk Analysis

Additional Topic Area (Optional): Retail and Foodservice

Keywords: Climate change, Frozen Foods, Pathogens, Sustainability

S29: SHAPING TOMORROW'S TABLE: THE FUTURE OF FOOD SAFETY AND REGULATION IN A CONSTANTLY CHANGING WORLD

Primary Author:

Andrew Wilson, Cultivate SA

Persons:

Andrew Wilson, Cultivate SA

Conrad Choiniere, US FDA

Cameron Prince, The Acheson Group

Bobby Krishna, Dubai Municipality

Session Type: Short Symposium - 1.5 hours

Session Summary: Regulatory agencies that fail to respond to shifts in their operational environment present a significant risk to public safety and market confidence. The failure of financial regulators to effectively respond to the rise of subprime lending in the United States was a major factor in the collapse of the U.S. housing market during the 2008 Global Financial Crisis, leading to total losses of more than \$8 trillion USD between 2007 and 2009 (Allen, 2003). Similarly, the current rapid pace of change required to support food production for a growing global population has dramatically increased pressures on food safety controls that protect public health. Food safety, sustainability and food security remain at the forefront of both government and industry agendas across the agri-food supply chain, alongside increasing operational costs and calls for stronger assurance. The race to be “first-to-market” and leverage disruptive opportunities at scale means innovative technologies being developed by industry to address these needs are challenging conventional regulatory food safety assurance systems and adaptation is required at an unprecedented pace. Can regulatory systems and the people who implement them adapt to maintain public health protection and embrace innovation to ensure safeguards remain effective into the future? Attendees will learn about: - The top challenges faced by food safety regulators; - Opportunities for industry to proactively engage with regulators and co-design food safety control systems; - The benefits of fostering organizational food safety culture as a common foundational framework to enable collaboration between regulators and industry, illustrated through practical current examples; and, - Predictions on a future state mature regulatory regime and discuss immediate practical opportunities to integrate technology and food safety culture with current food safety systems to safeguard the future of food.

Primary Topic Area: Food Safety Culture

Secondary Topic Area: International Food Protection Issues

Keywords: Food Safety Culture, Regulations, Risk Management

S30: NAVIGATING THE PATH: DIETARY SUPPLEMENTS IN THE FOOD SAFETY REGULATORY LANDSCAPE

Primary Author:

Preetha Biswas, Neogen Corporation

Persons:

Preetha Biswas, Neogen Corporation

Carolyn Montei, Neogen Corporation

Mike Lowenstein, Q Laboratories

Andrzej Benkowski, Eurofins Microbiology Laboratories

Session Type: Short Symposium - 1.5 hours

Session Summary: Food safety initiatives in the U.S. often use the phrase “Farm to Fork,” but what about when it leads to your “Supplement Bottle”? Prior to the 1994 Dietary Supplement Health and Education Act (DSHEA), supplements were regulated similarly to food in the U.S. Over the past 30 years, dietary supplement usage has increased significantly, and so has the need to ensure their safety. Many dietary supplements are derived from food ingredients, which may contain microorganisms or chemical contaminants that can pose health risks. With an international supply chain and multiple manufacturers involved, supplements are just as vulnerable to quality issues as food products. It is estimated that over 75% of U.S. adults consume at least one supplement daily, making the production of safe supplements a top priority. Under DSHEA, U.S. supplement manufacturers are required to follow GMP guidelines to ensure product quality and safety. However, these guidelines do not explicitly define testing methodologies and specification limits, which has created the need for more research and innovation in this area. To build consumer trust and ensure consistency, some manufacturers are adopting internationally recognized testing standards and certifications. Global Pharmacopeias are also collaborating to harmonize quality standards across regions. This symposium brings together industry and regulatory experts to share insights on best practices and emerging technologies for ensuring high-quality dietary supplements. Best practices for ensuring consumer safety, along with practical strategies for integrating consumer-centric safety measures into supplement production. We will examine how GMPs can be enhanced to address emerging challenges in the industry, such as contamination risks and evolving consumer preferences. The session will also provide insights into the proactive measures taken by regulatory bodies like the FDA and industry leaders to mitigate safety risks, prevent recalls, and ensure the continued safety of dietary supplements.

Primary PDG Sponsor: International Food Protection Issues PDG

Secondary PDG Sponsor: Applied Laboratory Methods PDG

Primary Topic Area: International Food Protection Issues

Secondary Topic Area: Applied Laboratory Methods

Keywords: Laboratory and Detection Methods, Public Health, Regulations

S31: THE ROLE OF MOONLIGHTING PROTEINS IN THE ADAPTABILITY AND SUCCESS OF BACTERIAL PATHOGENS IN VIVO AND IN VITRO

Primary Author:

Byron Brehm-Stecher, Iowa State University

Persons:

Byron Brehm-Stecher, Iowa State University

Arun Bhunia, Purdue University

Constance Jeffery, University of Illinois Chicago

Session Type: Short Symposium - 1.5 hours

Session Summary: Together with Archaea, bacteria dominate the Earth, occupying almost every conceivable physical niche. Along with their short generation times and inherent mutability, bacteria leverage an array of adaptive strategies or mechanisms to achieve their successes. These include the ability to alter their biochemical composition to withstand physical or chemical extremes, to persist in the presence of antibiotics, to undergo dormancy or to exhibit and benefit from random (stochastic) behaviors. Moonlighting proteins (MP) are another arrow in the quiver of bacterial adaptability and resilience. MP are defined as proteins having two or more unrelated biological functions, with many of these having canonical functions as elements of highly conserved metabolic processes. Although they lack the signaling peptides or binding motifs present in many typical virulence factors, MPs can be released under conditions of stress or membrane damage, where they bind electrostatically to bacterial surfaces and contribute to pathogen success by promoting biofilm formation, host colonization, interfering with host immune responses, scavenging iron, or performing other virulence-promoting activities. The core objective of this symposium is to examine recent advances in our understanding of MP and how they may contribute to bacterial success in the environment, within the host and to also highlight how we may be able to copy this bacterial adaptability strategy and use phosphoglucosyltransferase, an MP important in *Yersinia pestis* virulence, to promote growth of Shiga toxin-producing *E.coli* (STEC) and *Salmonella* during pre-analytical culturing steps. A comprehensive overview of MP and their multifaceted activities will set the stage for these targeted discussions of how MP can promote foodborne pathogen adaptability and success in the environment, during infection or when used to stimulate or enhance growth during in vitro culture. This overview will also note the wide distribution of MP phenomena from microbes to multicellular organisms, and will touch on other notable roles for MP, including as potential food allergens (enolases) or toxins (plant, fungal and bacterial ureases).

Primary PDG Sponsor: Applied Laboratory Methods PDG

Primary Topic Area: Applied Laboratory Methods

Secondary Topic Area: Meat and Poultry Safety and Quality

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

Keywords: Laboratory and Detection Methods, Microbial stress, Pathogens

S32: BATTLING MOLD IN LOW-MOISTURE FOODS

Primary Author:

Surabhi Wason, Kerry

Persons:

Surabhi Wason, Kerry

Jyoti Aryal, Kerry Ingredients and Flavours

Karuna Kharel, Louisiana State University AgCenter

Jennifer Acuff, University of Arkansas

Emilia Rico-Munoz, BCN Research Laboratories, Inc

Jasmine Kataria, Kerry Ingredients and Flavors

Session Type: Short Symposium - 1.5 hours

Session Summary: Low-moisture food faces a prominent challenge in mold due to its low-water activity, emphasizing the need for enhanced microbiological safety. Mold contamination continues to be a concern, impacting the nutritional quality and producing harmful mycotoxins. Mold infestations in human foods and pet treats pose health risks and raise concerns about environmental sustainability. Various studies explore strategies to control mold, with natural alternatives gaining traction amid clean-label preferences; limited consumer and industry knowledge hinders their widespread adoption. This symposium explores the pervasive issue of mold contamination in low-moisture human and pet foods and proposes sustainable solutions to mitigate its detrimental effects. By addressing mold issues in low-moisture human and pet foods with sustainable approaches, we aim to enhance pet safety while reducing the environmental footprint of the pet food industry. This makes it important to understand the survival, characterization and control of mycotoxins in low-moisture foods. Symposium attendees will learn about: 1. Industry insights and challenges of low-moisture foods 2. Detection of molds in low-moisture foods 3. Understanding mycotoxins risk and mitigation in low-moisture foods 4. Sustainable strategies to combat mold in low-moisture pet food while highlighting the significance of pet safety. Intendent audience: Researchers, educators, food industry

Primary PDG Sponsor: Low Water Activity Foods PDG

Secondary PDG Sponsor: Animal and Pet Food Safety PDG

Non PDG Sponsor (Organization, Association, etc.): BCN laboratories

Primary Topic Area: Low Water Activity Foods

Secondary Topic Area: Animal and Pet Food Safety

Keywords: Animal and Pet Food Safety, Low-water Activity Foods

S33: ADVANCEMENTS IN HPAI RESEARCH: UPDATES ON TRANSMISSION, DAIRY SAFETY, AND RISK ASSESSMENT

Primary Author:

Stephen Walker, Food and Drug Administration

Persons:

Stephen Walker, Food and Drug Administration

Nathan Anderson, U.S. Food and Drug Administration

Sarah Murphy, FDA

Nicole Martin, Cornell University

Kristin Butler, US Food and Drug Administration

Kara Dean, U.S. Food and Drug Administration

Mark Lyons, USDA

Diego Diel, Cornell University

Session Type: Short Symposium - 1.5 hours

Session Summary: Since the first detection of Highly Pathogenic Avian Influenza (HPAI; H5N1) in dairy cattle in March 2024, federal agencies, state partners, and research entities have worked tirelessly to understand this emerging pathogen and ensure that the food supply remains safe. Through these initial efforts, we have learned that current pasteurization parameters are highly effective at inactivating the virus. At IAFP 2024, a roundtable was held to discuss the current science understanding of HPAI in dairy cattle and its implications for food safety and public health. One year later, the purpose of the IAFP 2025 session is to present new and updated research on HPAI in dairy cattle, with attention to current scientific understanding, including developments related to animals, food safety, and risk assessment. Specific topics will include (i) status of the cattle disease outbreak and overview of current understanding of transmission; (ii) an update on dairy food safety research concentrating on raw milk cheese and characterizing the thermal resistance of HPAI in milk and milk products; (iii) a rapid HPAI risk assessment integrating data on exposure and dose response to characterize risk to consumers as a case study promoting informed decision-making in an evolving outbreak investigation. Altogether, this symposium aims to enhance our understanding of HPAI and guide future strategies for managing its impact on food safety, animal health, and public health.

Primary PDG Sponsor: Dairy Quality and Safety PDG

Secondary PDG Sponsor: Viral and Parasitic Foodborne Disease PDG

Primary Topic Area: Dairy Quality and Safety

Secondary Topic Area: Viral and Parasitic Foodborne Disease

Additional Topic Area (Optional): Epidemiology

Keywords: Dairy, Epidemiology, Risk Assessment, Viruses

S34: SURFACES, THE MICROBIOME AND FOODBORNE PATHOGENS - HOW THE BACKGROUND MICROBIOME INFLUENCES PATHOGEN DETECTION

Primary Author:

Jesse Miller, Bio-Rad Laboratories

Persons:

Jesse Miller, Bio-Rad Laboratories

Jasna Kovac, The Pennsylvania State University

Abigail Snyder, Cornell University

Byron Chaves, University of Nebraska-Lincoln

Mary Gadola, Neogen Corporation

Narjol Gonzalez-Escalona, FDA/CFSAN/ORS/DMMB

Session Type: Short Symposium - 1.5 hours

Session Summary: Food producers face significant challenges in monitoring and characterizing the microbial communities present in their operations. Microbes have adapted over time to form communities resistant to cleaning and disinfection in order to survive, and these communities are ideal places for pathogens to seek refuge from producer interventions. These communities may change with the season, material being produced, the production location, preventive control scheme and many other factors. How does a producer adapt, detect and prevent pathogens in their facilities with all of the variables that are present in their unique environment? The symposia will share insights on microbial communities in various environments and how pathogens interact and survive in these environments.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: Data Management and Analytics PDG

Non PDG Sponsor (Organization, Association, etc.): Neogen Corporation

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Microbial Modelling and Risk Analysis

Keywords: Environmental monitoring, Food Safety Culture, Hygiene, Laboratory and Detection Methods, Microbiome, Pathogens, Production/Plant Design, Risk Assessment, Sanitation

S35: GENOMIC TESTING AND ITS ROLE IN FOOD SAFETY ASSURANCE

Primary Author:

Purnendu Vasavada, Univ of Wisconsin-River Falls

Persons:

Purnendu Vasavada, Univ of Wisconsin-River Falls

Robert Ferguson, Food Safety Magazine

Anett Winkler, Cargill

Eric Brown, FDA-Human Foods Program

John Donaghy, Nestlé SPN.

Jo Williams-Newkirk, US CDC

Preetha Biswas, Neogen Corporation

Session Type: Full Symposium - 3.0 hours

Session Summary: In the past decade, considerable advances have been made in the genomic testing technologies that can be applied to food safety programs. Most in food safety are familiar with the emergence and impact of whole genome sequencing (WGS), but next Gen sequencing (NGS), long-read 3rd Gen sequencing, comprises much more, including microbiota characterization through metagenomics sequencing, and further developments will provide additional tools available to food companies and governmental organizations. These technologies along with advances in computing power and database technologies present considerable advances in diagnostic specificity that serve to change and improve foodborne illness prevention and outbreak investigations forever. In this symposium, attendees will hear opinions and analysis from technology experts from the U.S. Food and Drug Administration (FDA), U.S. Centers for Disease Prevention and Control (CDC), and food industry and academic diagnostic test experts who will discuss these technological advances and the changes we have seen over the past 2–5 years, and how food companies can use them to transform the accuracy and effectiveness of their food safety systems, including better means of characterizing isolates and evaluating the risks related to them.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: Applied Laboratory Methods PDG

Non PDG Sponsor (Organization, Association, etc.): Food Safety Magazine / University of Wisconsin - River Falls

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Applied Laboratory Methods

Additional Topic Area (Optional): Food Hygiene and Sanitation

Keywords: Genomics, Laboratory and Detection Methods, Pathogens

S36: NAVIGATING FOOD SAFETY AND REGULATORY CONSIDERATIONS FOR NEW AND NOVEL INGREDIENT APPROVAL PATHWAYS FOR INNOVATIONS IN HUMAN AND ANIMAL FOODS

Primary Author:

Deepa Thiagarajan, Mars

Persons:

Deepa Thiagarajan, Mars

Aaron Pleitner, Impossible Foods

Li Ma, Oklahoma State University

Joyjit Saha, Kerry

Lily Yang, The Acheson Group

Jason Dietz, US FDA Human Foods Program CFSAN

Jasmine Kataria, Kerry Ingredients and Flavors

Session Type: Short Symposium - 1.5 hours

Session Summary: As the food industry innovates, the introduction of new and novel ingredients continues to expand both in human and animal food sectors. This symposium brings together leading experts from the U.S. FDA Human Foods Program's Center for Food Safety and Applied Nutrition (CFSAN), as well as U.S. FDA Center for Veterinary Medicine (CVM), and key industry representatives to explore the various existing and emerging regulatory approval pathways available for these ingredients. Attendees will gain insights into the shifting regulatory landscape governing the pre-market assessment of new and novel ingredients, with a focus on understanding the complexities of safety and toxicology evaluations required for compliance depending on intended use. Panel will also address post-market assessment considerations, including potential regulatory implications and industry responsibilities in ensuring the ongoing safety of food products. Symposium attendees will learn about: 1. The current and emerging regulatory frameworks for ingredient approval in both human and animal foods 2. The safety and toxicology assessments necessary for regulatory compliance 3. Case studies and examples of successful ingredient approval processes for both human and animal foods 4. Sustainable strategies to enhance food safety with new clean label ingredients in the human and animal food sectors

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

Secondary PDG Sponsor: Animal and Pet Food Safety PDG

Primary Topic Area: Plant-Based Alternative Products

Secondary Topic Area: Animal and Pet Food Safety

Additional Topic Area (Optional): Food Law

Keywords: Plant-based foods, Regulations, Risk Assessment

S37: FOOD SAFETY IN FARMERS' MARKETS AND INFORMAL OUTDOOR FOOD MARKETS AROUND THE WORLD

Primary Author:

Joshua Gurtler, USDA-ARS

Persons:

Joshua Gurtler, USDA-ARS

Abdullahi Idris Muhammad, Sultan Qaboos University

Marciane Magnani, Federal University of Paraíba

Kristen Gibson, University of Arkansas

Anderson Sant'Ana, University of Campinas

Session Type: Short Symposium - 1.5 hours

Session Summary: Informal food markets (or farmers' markets) have been a significant part of the food trade for millennia. Food safety practices and regulations have been improving for commercially marketed products in recent years. Nevertheless, a major gap still exists regarding food safety in farmers' markets, where commodities are often produced and/or sold by individuals who have no formal sanitation, hygiene, or food safety training. On the other hand, research, education and regulatory endeavors are currently being implemented in various countries and locales around the globe to improve the safety of these venues to prevent the spread of microbial foodborne pathogens. This symposium will address many of these efforts taking place in eight countries around the world by speakers representing three countries. Talks will address issues such as improving food safety practices at farmers' markets, traditional and advanced food safety training programs for educating farmers' market vendors in appropriate food safety behaviors and preventing cross-contamination, research and extension approaches to reach farmers' market participants at the local level, and the utility and efficacy of various regulatory approaches for promoting food safety among farmers' market merchants. Additionally, speakers will cover innovative strategies that are currently employed or are being developed to ameliorate significant issues related to the spread of foodborne pathogens at farmers' markets. Some of these issues include areas such as food product sourcing, sanitation and personal hygiene, segregation of high-risk from low-risk foods, special treatment of fresh produce and ready-to-eat foods, etc. Attendees will come away with a better understanding of the current state of food safety at farmers' markets including customs, habits and trends; current research being conducted; modern methods for training vendors; as well as recommendations on regulatory policy in various parts of the world.

Primary PDG Sponsor: Food Safety Education PDG

Secondary PDG Sponsor: Food Safety Culture PDG

Primary Topic Area: Food Safety Education

Secondary Topic Area: Communication, Outreach and Education

Additional Topic Area (Optional): Food Hygiene and Sanitation

Keywords: Education, Food Safety Culture, Public Health

S38: MYCOTOXIN MITIGATION AND CONTROL MEASURES IN TREE NUT PRODUCTION AND PROCESSING

Primary Author:

Guangwei Huang, Almond Board of California

Persons:

Guangwei Huang, Almond Board of California

Dawit Gizachew, Purdue University

Themis Michailides, University of California, Davis

Tim Birmingham, Almond Board of California

Session Type: Short Symposium - 1.5 hours

Session Summary: Mycotoxin mitigation and control strategies for tree nuts involve a combination of pre-harvest and post-harvest measures to ensure food safety and quality. Pre-harvest strategies focus on preventing fungal contamination in the field. This includes implementing proper irrigation and fertilization practices, and using biological control agents to suppress growth of toxigenic strains. Additionally, monitoring weather conditions and applying fungicides when necessary can help reduce the risk of mycotoxin contamination. Post-harvest strategies are crucial for controlling mycotoxin levels during storage and processing. Proper drying of tree nuts immediately after harvest is essential to prevent fungal growth. Maintaining optimal storage conditions, such as low humidity and temperature, is critical to inhibit mold development. Regular inspection and removal of contaminated nuts can further reduce mycotoxin levels. Innovative approaches, such as the use of atoxigenic biocompetitive microorganisms that suppress fungal growth of toxigenic molds in the field and advanced sorting technologies, are also being explored to enhance mycotoxin control. In the session, three presentations will focus on the following: Inhibitory (limiting) factors for mold growth and aflatoxin development in low-moisture foods and treenuts Minimization and control of toxigenic mold/aflatoxin development in the field during growing and harvest Control of aflatoxin through processing/sorting

Non PDG Sponsor (Organization, Association, etc.): Almond Board of California

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Low Water Activity Foods

Keywords: Chemical Contaminant, Intervention, Low-water Activity Foods

S39: TO ROTATE OR NOT? HOW CAN MICROBIOME ANALYSIS AND BIOFILM TOOLS BROADLY IMPROVE SANITATION AND ANSWER THIS AGE-OLD QUESTION?

Primary Author:

Josie Greve-Peterson, Fortrex

Persons:

Josie Greve-Peterson, Fortrex

David Buckley, Diversey, Inc.

Dana Dittoe, University of Wyoming

Eric Moorman, Butterball, LLC

Kirby Childs, Greater Omaha Packing, Co., Inc.

Scott King, PSSI

Session Type: Short Symposium - 1.5 hours

Session Summary: The next leap in sanitation starts with leveraging sequencing tools to understand a built environment's microbiome. However, hesitancy to adoption of these technologies is based, in part, on lack of application understanding and how insights provided by this data type enhance decision-making. There are a variety of technologies and methodologies available for data generation specific to understanding bacterial communities in food establishments. Once realized, application of these tools can provide actionable insights to a sanitation program. For instance, a routine yet controversial sanitation practice is rotation of sanitizers. How can traditional microbiological data and microbiome analysis provide improved insight to this process? Expert speakers from academia and industry will address available tools, and lab and field data to support their practical use. First, we'll establish what microbiome analysis technologies are within practical reach for routine use in the food industry and highlight resources to enable better decision making. Next, we'll hear a two-part case study that reveals how laboratory-based microbiological data and environmental microbiome data at a plant informs sanitation practices. These sequenced presentations simultaneously deliver insight to the debated topic of sanitizer rotation, while demonstrating practical use of sequencing tools. Attendees of this symposium will leave with a greater understanding of these tools, how to practically introduce this technology in food settings, and how sanitizer rotation ultimately impacts a built environment's microbiome.

Primary PDG Sponsor: Food Hygiene and Sanitation PDG

Primary Topic Area: Food Hygiene and Sanitation

Secondary Topic Area: Advanced Molecular Analytics

Keywords: Antimicrobials, Microbiome, Sanitation

S40: THE EVOLVING LANDSCAPE OF FOOD INGREDIENT SAFETY IN THE UNITED STATES

Primary Author:

Paul Hanlon, Abbott Nutrition

Persons:

Paul Hanlon, Abbott Nutrition

Christine Crincoli, Cargill

Anthony Pavel, Keller & Heckman LLP

Kirk Arvidson, FDA

Session Type: Short Symposium - 1.5 hours

Session Summary: Food ingredients must meet the regulatory standard of reasonable certainty of no harm under intended conditions of use before they can be added to food products in the United States. Recent developments at both the state and federal level have generated increased consumer interest in the safety assessments of food ingredients. This session will provide insight into this rapidly evolving space by exploring the FDA's Post-Market Chemical Assessment Program, industry best practices for safety evaluations of food substances, and the dynamic shifts in state-level regulations. The FDA's commitment to food safety extends beyond pre-market approvals, and in 2024 the Agency announced their plans for updating their approach to evaluating chemicals in the food supply. This Post-Market Chemical Assessment Program is designed to prioritize food ingredients currently in food for safety assessment, complimenting pre-market evaluations. Industry also plays an important role in ensuring the safety of food ingredients, and food industry toxicologists have created a tool designed to promote aligned practices for evaluating the safety of food ingredients. This transparent approach to evaluating the safety of food ingredients is meant to provide guidance to all food manufacturers, and compliments FDA efforts on food ingredient safety. The focus on food ingredient safety has also recently seen significant activity at the state level, not just the federal level. This session will provide insights into what states are doing in regard to regulating food ingredients, and also how these efforts overlap with the initiatives at the federal level, also tying in potential impacts to industry of having state-by-state requirements. This overview of these three aspects of food ingredient regulation will provide attendees with insight into what is already happening today, how these efforts are coordinated, and what the future of food ingredient regulation may hold.

Primary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Food Law

Keywords: Regulations, Risk Assessment, Toxicology

S41: INTERNATIONAL EFFORTS IN FOOD VIROLOGY: THE 2023-2024 FAO/WHO JEMRA EXPERT CONSULTATIONS FOR THE CODEX COMMITTEE ON FOOD HYGIENE

Primary Author:

Lee-Ann Jaykus, NC State University

Persons:

Lee-Ann Jaykus, NC State University

Kang Zhou, Food and Agriculture Organization of the United Nations

Akio Hasegawa, World Health Organization

Miranda de Graaf, Department of Viroscience, Erasmus MC

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

Session Type: Short Symposium - 1.5 hours

Session Summary: The Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA), at the request of the Codex Committee on Food Hygiene (CCFH), convened two meetings on foodborne viruses, one in Rome, Italy, in September 2023 and the second in Geneva, Switzerland, in February 2024. The expert committees reviewed recent scientific developments, data, and evidence for purposes of updating the CXG-079-2012 guidance. JEMRA was asked to provide an up-to-date review of foodborne viruses and relevant food commodities of greatest public health concern with respect to (i) product and virus attribution; (ii) analytical methods for detection of enteric viruses in at-risk foods; (iii) the utility of viral indicators; and (iv) the efficacy of current and proposed methods to prevent contamination and/or inactivate viruses in foods. The virus-commodity combinations ranked of highest priority were human norovirus (huNoV) and hepatitis A virus (HAV) in shellfish, fresh and frozen produce, and prepared/ready-to-eat foods; and hepatitis E virus (HEV), of zoonotic in origin, in pork and wild game. Over the last 15 year, ISO-standardized detection methods have been developed for all commodity-virus pairs except HEV, but effective viral indicators are still lacking. Many commodity-specific methods to control human fecal contamination (HAV and hNoV) as applied to primary production (preventing contamination of waters); and through processing and preparation (promoting personal hygiene and hand/surface disinfection), have been investigated, including emerging virus inactivation methods. Less has been done regarding control of HEV. While awareness of the importance foodborne viruses has increased, and additions or changes to some food supply chain management strategies implemented, preventing contamination remains the cornerstone of control. The results of the consultations will be detailed with a wrap-up presentation by CCFH on how these efforts will be applied to international food hygiene guidelines.

Primary PDG Sponsor: Viral and Parasitic Foodborne Disease PDG

Secondary PDG Sponsor: International Food Protection Issues PDG

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

Primary Topic Area: Viral and Parasitic Foodborne Disease

Secondary Topic Area: International Food Protection Issues

Keywords: Hygiene,Public Health,Viruses

S42: VALIDATION AND STATE-OF-THE-ART METHODS FOR FOODBORNE PARASITES

Primary Author:

Sonia Almeria, FDA

Persons:

Sonia Almeria, FDA

Monica Santin-Duran, U.S. Department of Agriculture

Laura Lalonde, CFIA

Erin Crowley, Q Laboratories

Jenny Maloney, ARS, USDA

Session Type: Short Symposium - 1.5 hours

Session Summary: Foodborne and waterborne parasites such as *Cryptosporidium*, *Giardia*, *Cyclospora*, or *Toxoplasma* are consistently found on fresh fruits and vegetables and in the environment and are associated with illnesses and outbreaks of disease worldwide. In the U.S., there are yearly seasonal outbreaks of *C.cayetanensis* and *Cryptosporidium*. A One Health approach encourages accounting for all possible sources and routes of contamination; this requires quick and accurate testing to detect these parasites using sensitive and specific detection methods. The validation of the methods for parasite detection is critical in providing evidence that those methods are fit-for-purpose. However, validated methods are only available for some parasites in specific food matrices. Even validated methods are constantly in evolution. For example, the current FDA Bacteriological Analytical Manual (BAM) Chapter 19b method for the detection of *C.cayetanensis* based on the 18S ribosomal RNA gene has been recently revised due to potential cross-reactivities. A modified real-time PCR method (qPCR) for a mitochondrial target gene (Mit1C) to detect *C. cayetanensis* was just validated. When evaluating a new method, there should be a minimum of performance requirements for standardization among laboratories. The Analytical International Methods and Standards (AIMS) Program of AOAC International recently completed and approved a Standard Method Performance Requirements (SMPRs®) for detection, identification, and characterization of *C. cayetanensis* based on the new Mit1C target. This symposium will bring together experts from the field to address those updated methods and standards, as well as to identify analytical challenges and new approaches discovered along the way. Regulators, public health practitioners, and industry are well aware of the existing and emerging risks of parasites and the need for validated methodologies to be better prepared to detect contaminated produce and to respond to outbreaks.

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

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

Keywords: Parasites,Public Health,Validation

S43: THE INVISIBLE THREAT OF MYCOTOXINS IN THE FERMENTATION INDUSTRY: A FOOD SAFETY CHALLENGE OF THE 21ST CENTURY

Primary Author:

Vijay Juneja, USDA-ARS-ERRC

Persons:

Vijay Juneja, USDA-ARS-ERRC

Shalini Sehgal, Bhaskaracharya College of Applied Sciences University of Delhi

Priyanka Gupta, University of Central Oklahoma

Lakhvinder Kaur, Manav Rachna International Institute of research and studies

Sadhana Ravishankar, University of Arizona

Session Type: Short Symposium - 1.5 hours

Session Summary: As the global fermentation-derived fine chemicals market is projected to grow to \$42.4 billion by 27, the safety of fermented food products is critical. While fermentation is a cornerstone of food production, responsible for an array of products from beer and cheese to traditional foods, the presence of mycotoxins—thermostable toxic metabolites produced by fungi—poses a significant, risk to food safety. Mycotoxins such as aflatoxins, fusarium toxins, and ochratoxin A can contaminate fermented products at various stages of production. Their presence in fermented foods threatens public health, and poses a challenge to regulatory compliance. Studies have highlighted the global nature of this risk, with mycotoxins being detected in up to 82% of tested fermented food samples. The health implications of mycotoxins are severe, while their resistance to standard food processing methods complicates efforts to ensure product safety. Objectives: (1) understand the mechanisms of mycotoxin production during the fermentation process, including the conditions and fungal species responsible; (2) explore strategies for reducing mycotoxin contamination below permissible limits, including improved fungal control and innovative processing techniques; and (3) highlight advancements in the detection and quantification of mycotoxins in fermented foods, focusing on novel analytical methods that enhance accuracy, speed, and cost-effectiveness. Overall, this symposium will explore the origins of mycotoxins, their implications for food safety, and the urgent need for comprehensive strategies to detect and control their presence in fermentation processes. This session will bring together researchers, food safety professionals, and industry experts to discuss the scientific, technical, and regulatory aspects of mycotoxin contamination in fermented foods, fostering collaboration to safeguard the future of the fermentation industry and protect public health.

Primary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Secondary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Meat and Poultry Safety and Quality

Keywords: Allergens, Mycology, Pathogens

S44: PROMISES AND CHALLENGES OF IMPLEMENTING NATURAL ANTIMICROBIALS FROM FARM TO FORK

Primary Author:

Hany Anany, Agriculture & Agri-Food Canada

Persons:

Hany Anany, Agriculture & Agri-Food Canada

Subash Shrestha, Cargill

Ismail Fliss, Laval University

Jerry Erdmann, IFF

Mark Hankins, Microcos Food Safety

Session Type: Short Symposium - 1.5 hours

Session Summary: From farm to fork, natural antimicrobials, including phages, antimicrobial peptides (bacteriocins), plant extracts (species, herbs and essential oils) and protective cultures have been shown as legitimate antibacterial alternatives to treat diseases in food producing animals and plants, reduce biofilm formation and contamination in food processing environments, and increase the safety and shelflife of food products. Current advances in the field have shown the importance of better understanding the mechanism of action of these natural antimicrobials and to bring more credible evidence of their effectiveness in complex environments as food matrixes. The proposed symposium will bring together researchers from the industry, academia, and government to discuss their research on different natural antimicrobials to tackle selected bacterial pathogens throughout the food supply chain. Suggested approaches to overcome the challenges of natural antimicrobials applications as biocontrol tools throughout the food supply chain will also be presented. Challenges faced and recommendations from industries that are trying to register or already registered commercial products will be discussed. The symposium will be of interest to academicians interested in natural antimicrobials research, industry personnel (R&D, QA, Food Safety among others) in understanding the application of the technology, and government entities especially for approval and regulatory purposes.

Primary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Primary Topic Area: Meat and Poultry Safety and Quality

Secondary Topic Area: Fruit and Vegetable Safety and Quality

Keywords: Antimicrobial Resistance, Antimicrobials, Bacteriophages

S45: TRACKING SAMPLING AND TESTING STRATEGIES DURING LIVE PRODUCTION AND PRE-HARVEST FOR SALMONELLA REDUCTION

Primary Author:

Preetha Biswas, Neogen Corporation

Persons:

Preetha Biswas, Neogen Corporation

Peggy Cook, Neogen

Nikki Shariat, University of Georgia

Anna Carlson, Cargill Research & Development

Jessie Vipham, Kansas State University

Session Type: Short Symposium - 1.5 hours

Session Summary: This symposium session will explore innovative approaches to tracking sample testing strategies from pre-harvest, processing to production, specifically aimed at reducing *Salmonella* contamination in poultry. Our discussion will center on the complexities of sampling and testing within pre-production facilities, highlighting the inconsistency of sample types from environmental areas and the ongoing questions about the most effective samples to collect in a pre-harvest setting. We will examine the limitations of environmental samples, which may not accurately reflect conditions in processing plants. Participants will gain insights into how to effectively track sample results and leverage data to influence practices that ensure the safety and quality of poultry products during post-production. The session will feature presentations on current research activities, practical applications, and the challenges faced in implementing these testing strategies. Attendees will have the opportunity to engage in discussions about best practices, share experiences, and collaborate on solutions that enhance food safety. Join us for this informative session to discover how effective tracking of sample testing strategies can lead to significant reductions in *Salmonella* levels, ultimately contributing to safer poultry products and improved public health outcomes.

Primary PDG Sponsor: Pre Harvest Food Safety PDG

Secondary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Primary Topic Area: Pre Harvest Food Safety

Secondary Topic Area: Meat and Poultry Safety and Quality

Keywords: Environmental monitoring, Meat and Poultry, Pathogens, Pre-harvest

S46: BEYOND SLIME: WHY DRY SURFACE BIOFILMS NEED A NEW APPROACH TO FOOD HYGIENE

Primary Author:

Juan Goncalves, Procter & Gamble Professional

Persons:

Juan Goncalves, Procter & Gamble Professional

David Buckley, Diversey, Inc.

Jean-Yves Maillard, Cardiff University

Haley Oliver, Purdue University

Melissa Vaccaro, National Environmental Health Association (NEHA)

Session Type: Short Symposium - 1.5 hours

Session Summary: Biofilms are structures in which microorganisms form communities that pose significant risks to food safety and quality. A newly discovered risk in food areas is Dry Surface Biofilms (DSBs). These communities have distinct characteristics, such as being challenging to detect, having lower moisture content, and higher tolerance to biocides. While research on food safety aspects of DSBs is limited, studies in healthcare settings reveal their role as unmitigated sources of infection. This complicates the hygiene process, as there is no regulatory framework for assessing efficacy against DSBs, leaving end-users uncertain. To address this, hygiene processes must be enhanced to mitigate the risks associated with these highly tolerant biofilms. In this symposium, a diverse group of speakers will discuss differentiated protocols for growing and evaluating the effectiveness of cleaners, biocides, and wiping substrates against DSBs relevant to food safety. They will present recent data that challenges the effectiveness of traditional Sanitation Standard Operating Procedures in mitigating DSB risks. Additionally, the speakers will explore the potential of leveraging cleaning agents, rather than relying solely on biocides, to reduce cross-contamination risks and DSB biocide tolerance while enhancing food safety and quality. By attending this symposium, participants will gain valuable insights into the risks associated with DSBs, identify shortcomings in current sanitation practices, and explore ways to improve hygiene outcomes.

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: Applied Laboratory Methods

Additional Topic Area (Optional): Retail and Foodservice

Keywords: Hygiene, Public Health, Sanitation

S47: ALLERGEN MANAGEMENT IN HOSPITALITY VENUES

Primary Author:

Steve Taylor, University of Nebraska

Persons:

Steve Taylor, University of Nebraska

Douglas Davis, Marriott

Robert Earl, FARE

Betsy Craig, MenuTrinfo, LLC

Amy Wise, Starbucks

Session Type: Short Symposium - 1.5 hours

Session Summary: Food allergies, especially IgE-mediated, immediate hypersensitivity reactions, have become a major global public health issue. Many public health authorities around the world have enacted improved labeling regulations on packaged foods, and the global food manufacturing sector has developed best allergen management practices. These measures have resulted in safer foods for allergic consumers. However, the level of risk remains high for allergic consumers when eating away from home. The majority of fatal reactions from inadvertent ingestion of foods containing hazardous levels of undeclared allergens have occurred in restaurants. Schools, day care facilities, airplanes, and other venues where eating occurs outside the home environment are also sources of severe and sometimes fatal allergic reactions. Control measures are more difficult to implement in hospitality settings. With high levels of employee turnover, staff training is also a challenge. Additionally, some hospitality venues make claims, i.e., gluten-free or peanut-safe, which are often incompletely substantiated. This session intends to bring awareness to the comparatively high level of risk for allergic consumers that occurs in hospitality venues. The session will provide insights on approaches to enhancing allergen control and staff training.

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

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Food Safety Assessment, Audit and Inspection

Additional Topic Area (Optional): Retail and Foodservice

Keywords: Allergens, Auditing, Food Service

S48: WHAT'S THE HOLD UP? MICROBIOLOGICAL RISKS ASSOCIATED WITH HOLDING OF PRODUCT PRIOR TO FURTHER PROCESSING

Primary Author:

Wilfredo Ocasio, IEH Laboratories and Consulting Group

Persons:

Wilfredo Ocasio, IEH Laboratories and Consulting Group

Martha Kimber, Eurofins

Ron Van Santen, Danone

George Kwabena Afari, Coca-Cola

Session Type: Short Symposium - 1.5 hours

Session Summary: Heating, drying, or acidification processes intended to provide microbial safety and stability are widely recognized as critical steps to ensure the microbiological safety and quality of finished products. However, the process steps applied upstream of the heat, drying, or acidification treatment may also affect the microbiological safety and quality of numerous products (e.g., low-acid, shelf-stable, aseptically packaged dairy and plant-based beverages, infant formula, low-moisture snacks, acidified beverages, etc.). This symposium aims to give insights on the microbial risks upstream of the final microbial stability treatment given to the food. These risks apply to low-acid, high acid, shelf-stable, refrigerated or low-water activity products. Speakers will provide information as to the formation and heat resistance of bacterial toxins such as *Bacillus cereus* emetic toxin (cereulide) and staphylococcal enterotoxins. The use of predictive models (e.g., Pathogen Modeling Program) and challenge tests to help define holding /handling conditions prior to final processing will also be discussed. Recommended actions when the designed storage conditions are exceeded (predictive models, microbiological analyses, toxin tests) will be offered. In addition, the impact of spoilage organisms (incipient spoilage or the risk of overwhelming the thermal process) will be discussed in detail. Speakers in this session will address the combined impact of inadequate handling of raw product (time and temperature) and poor hydration practices. Finally, adequate handling of raw product is also necessary to prevent the production of heat-resistant enzymes prior to the heat treatment. Such enzymes (e.g., proteases) may degrade liquid products during shelf life. This topic will also be addressed by speakers during this session.

Primary PDG Sponsor: Beverages and Acid/Acidified Foods PDG

Secondary PDG Sponsor: Beverages and Acid/Acidified Foods PDG

Primary Topic Area: Beverages and Acid/Acidified Foods

Secondary Topic Area: Food Hygiene and Sanitation

Additional Topic Area (Optional): Low Water Activity Foods

Keywords: Acid/Acidified Foods, Beverages, Controlled Environment, Dairy, Frozen Foods, Low-water Activity Foods, Pathogens, Plant-based foods, Recalls, Spoilage, Toxins (bacterial)

S49: FRAGILE YET DEVIOUS; WHAT MAKES CAMPYLOBACTER SO PERSISTENT?

Primary Author:

Heidy Den Besten, Wageningen University

Persons:

Heidy Den Besten, Wageningen University

Maarten Nauta, Statens Serum Institut

Manpreet Singh, The University of Georgia

Juan DeVillena, Wayne-Sanderson Farms

Session Type: Short Symposium - 1.5 hours

Session Summary: *Campylobacter* remains the most frequently reported agent of zoonosis worldwide. Control of *Campylobacter* along the food chain is troublesome, and there is a clear relationship between the prevalence of *Campylobacter* in broiler flocks and public health risk. Chickens serve as reservoirs, and carcasses can get contaminated during slaughter. Although fragile in lab settings, *Campylobacter* can persist on carcasses and chicken meats. *Campylobacter's* metabolism is unique as it is thought to be asaccharolytic, lacking most key enzymes to metabolize sugars, hence amino acids are its primary nutrient source. Though, recently, evidence was provided that *Campylobacter* is able to utilize some sugars. This symposium will give an update on recent insights in the ecophysiology of this notorious pathogen and the role of sugars in growth, metabolism, and virulence of *Campylobacter*. Next, the symposium will share new insights on persistence and cross-contamination of *Campylobacter* on the slaughter line and data-driven evaluations of interventions to reduce contamination levels along the food chain. Based on the available risk assessment models, the impact on public health risk of cross-contamination between broiler flocks during slaughter has been considered small. However, recent Danish data suggest that the impact of cross-contamination between broiler flocks is much larger than previously thought. Updated risk assessment models including persistence phenomena are therefore being developed and will be shared. Also, differences between geographical approaches to reduce contamination levels shed light on possible intervention approaches to reduce contamination during post-chilling stages. Differences within North America will be discussed to evaluate successful advances in reducing *Campylobacter* on chicken meat.

Primary PDG Sponsor: Modelling and Risk Analysis PDG

Secondary 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: Intervention, Meat and Poultry, Microbial stress, Pathogens, Risk Assessment

S50: ADVANCING FOOD SAFETY EDUCATION THROUGH EMPLOYEE ENGAGEMENT INITIATIVES

Primary Author:

Aaron Lavallee, USDA-FSIS-OPACE

Persons:

Aaron Lavallee, USDA-FSIS-OPACE

Lily Yang, The Acheson Group

Janeen Richey, Beech-Nut Nutrition

Vijay Vankar, Leprino Foods

Session Type: Short Symposium - 1.5 hours

Session Summary: According to the Food and Agriculture Organization of the United Nations (FAO), 1.23 billion people are employed in the global food system. To reduce or prevent adverse health impacts associated with foodborne illness outbreaks, engaging these individuals beyond production-related tasks is crucial to advance food safety knowledge and education. The majority of food safety education focuses externally on consumers, presenting a knowledge gap and missing opportunities to advance food safety on a global scale. Data from one government agency showed the seriousness of that knowledge gap with over 20% of employees stating that they did not understand how their individual work contributed to the mission and agency goals of reducing foodborne illness. Influencing food safety behavior at the employee level can have influence beyond the plant or organization and can yield exponential results. In this symposium, panelists will highlight how their organizations are educating internal colleagues and associates, the people making or inspecting the food, to influence behaviors and foster food safety practices both in the industry and at home. Presenters will discuss case studies and education initiatives from private industry and the regulatory space and how their organizations check and retain the employee competency after training to ensure the development of a food safety culture. In addition, they will share insights into how to create an emotional connection to food safety at an organizational level to drive positive change at the mission level. This symposium will discuss background, methods, findings, applications, and implications of the work from the perspective of each organization.

Primary PDG Sponsor: Food Safety Education PDG

Secondary PDG Sponsor: Food Safety Culture PDG

Primary Topic Area: Food Safety Education

Secondary Topic Area: Food Safety Culture

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

Keywords: Education, Food Safety Culture, Professional Development

S51: BEST PRACTICES FOR FOOD SAFETY COMMUNICATION: RECOMMENDATIONS AND REALITIES

Primary Author:

William Hallman, Rutgers University

Persons:

William Hallman, Rutgers University

Cara Cuite, Rutgers University

Amy Philpott, Philpott PR Solutions, LLC

Sharmi Das, FDA CFSAN

Jeffrey LeJeune, FAO

Session Type: Short Symposium - 1.5 hours

Session Summary: A review of the literature identified over 100 publications recommending “best practices” for risk communication relevant to food safety. Much of the advice given is based on experience, has face validity, and is likely worth following. However, additional research is needed to empirically validate many of the recommendations. It is also clear that not all the recommended best practices are compatible or feasible in every situation and context. For example, general recommendations to engage relevant stakeholders and to be open, transparent, and inclusive in decision-making are all valuable practices. This is especially the case when the issue prompting the communication is an ongoing one and the goal is to reduce risk over time. However, there are tradeoffs that must be made in following these principles in emergency/crisis situations such as foodborne illness outbreaks, where following the best practice of rapid communications with wide audiences is likely to take priority over extensive stakeholder engagement and open/transparent decision making. Legal considerations and other constraints on the ability to follow best practices will be addressed from academic, industry, and government perspectives.

Primary PDG Sponsor: Food Safety Education PDG

Secondary PDG Sponsor: Committee on Control of Foodborne Illness

Primary Topic Area: Communication Outreach and Education

Secondary Topic Area: Food Safety Education

Additional Topic Area (Optional): Developing Food Safety Professionals

Keywords: Education, Public Health, Risk Management

S52: MOLECULAR METHODS FOR THE DETECTION OF SPOILAGE MICROORGANISMS

Primary Author:

Mu Ye, Kraft Heinz Company

Persons:

Mu Ye, Kraft Heinz Company

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

Joelle Salazar, FDA

Sarita Raengpradub, Mérieux NutriSciences

Catherine Stewart, ConAgra

Katrina Counihan, U.S. Department of Agriculture

Session Type: Short Symposium - 1.5 hours

Session Summary: The health and economic impacts of food spoilage to both consumers and manufacturers are enormous. The consequences of microbial spoilage of food ranges from having a high level of rework and /or product disposal to having to instigate a product recall. Microbial food spoilage can be a safety concern because it may lead to the growth of pathogens and the release of toxins that can make people sick. Early detection of spoilage would be advantageous in reducing food loss. Numerous methods for detection of spoilage have been devised with the goals of determining concentrations of spoilage microbes or volatile compounds produced by these microbes. However, many of these methods are considered inadequate because they are time-consuming, labor-intensive, and/or do not reliably give consistent results. Traditional methods of estimating bacterial populations or identifying problem microorganisms do not provide results quickly enough to allow for interventions. The application of molecular methods to the detection, identification, and characterization of pathogens have greatly advanced knowledge and had a tremendous impact on public health; these same technologies can also be used to address the challenges of food spoilage. For example, PCR can be used to detect specific spoilage bacteria, and NGS tools like metagenomics can be used to profile a food's microbial community and identify spoilage yeast that may be difficult to culture. This symposium will bring together experts working in the field to provide an overview of the current approaches and to address the challenges and potentials of applying advanced molecular tools for spoilage microorganism detection through research results and case studies.

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, Spoilage

S53: SMOKING FOR FOOD SAFETY - CLEAN LABELING AND INTEGRATED STRATEGIES

Primary Author:

Lihan Huang, USDA Agricultural Research Service

Persons:

Lihan Huang, USDA Agricultural Research Service

Joshua Gurtler, USDA-ARS

Surabhi Wason, Kerry

Evelyn Watts, LSU AgCenter & Louisiana Sea Grant

Jyoti Aryal, Kerry Ingredients and Flavours

Session Type: Short Symposium - 1.5 hours

Session Summary: As food safety remains a pressing global concern, recent outbreaks of *Listeriamonocytogenes* and *Salmonella* in seafood, meat, and poultry products highlight the urgent need for innovative and comprehensive safety interventions. With seafood-associated outbreaks contributing 10-20% of foodborne illnesses in the U.S., and over 40% of *Salmonella* cases linked to meat and poultry consumption, addressing these risks requires a multifaceted approach. This symposium aims to present the latest advancements in clean-label preservation techniques that combine traditional smoking methods with modern technologies and mathematical modelling to effectively mitigate safety risks across the seafood, meat, and poultry industries. Smoking has long been used to enhance the flavor and preserve the quality and safety of poultry, fish, and red meat. This symposium will explore the powerful antimicrobial effects of natural carbonyls, organic acids, and phenolic compounds found in smoke, which synergistically act to enhance food safety and extend shelf life. Attendees will gain insights into the application and optimization of liquid and dry smoke technologies, the integration of antimicrobial agents, and the regulatory considerations surrounding their use. By leveraging these innovations, the session will provide actionable strategies to address safety challenges, improve oxidative stability, and maintain product quality, all while meeting the consumer demand for clean-label and minimally processed foods. The focus will also be on bridging the gap between traditional techniques and modern processing technologies for scalable, regulatory-compliant, and sustainable solutions in food preservation.

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: Antimicrobials, Meat and Poultry, Seafood

S54: SCIENTIFIC PROGRESS TOWARD INTELLIGENT DESIGN OF ANTI-NOROVIRAL DISINFECTION PRODUCTS AND PROCESSES

Primary Author:

Lee-Ann Jaykus, NC State University

Persons:

Lee-Ann Jaykus, NC State University

Juan Goncalves, Procter & Gamble Professional

David Buckley, Diversey, Inc.

Matthew Moore, University of Massachusetts Amherst

James Arbogast, JW Arbogast Advanced Science Consulting LLC

Session Type: Short Symposium - 1.5 hours

Session Summary: The general resistance of human noroviruses (huNoV) to disinfection is well-documented, and remains an impediment to controlling its transmission by foodborne routes. Over the last decade, scientific findings have led to a better understanding of why and how this virus remains so recalcitrant to disinfection. The purpose of this session is to explore the scientific basis for noroviral resistance and how this information can be used toward intelligent design of anti-noroviral products and processes. The first presentation will focus on exploring the biochemical basis behind huNoV resistance, with a focus on capsid composition, structure, and charge. The second will delve into ingredient selection for surface disinfectant and hand sanitizer formulation in “boosting” anti-noroviral efficacy of more traditional actives (e.g., alcohols, peroxide). In the final presentation, some of the emerging anti-noroviral actives (e.g., copper, citrate) and treatments (e.g., ultraviolet radiation) will be reviewed for efficacy, with a focus on how they might be incorporated into next-generation products and processes. The content will be connected to current regulations and be informative in support of future regulatory needs. Attendees will come away with a more comprehensive understanding of why and how huNoV are so difficult to inactivate and how emerging applied science has potential to improve surface and hand disinfection in the future.

Primary PDG Sponsor: Viral and Parasitic Foodborne Disease PDG

Secondary PDG Sponsor: Food Hygiene and Sanitation PDG

Primary Topic Area: Food Hygiene and Sanitation

Secondary Topic Area: Viral and Parasitic Foodborne Disease

Additional Topic Area (Optional): Retail and Foodservice

Keywords: Hygiene, Sanitation, Viruses

S55: SPORES...LET'S "B. CEREUS"

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

Polly Courtney, General Mills, Inc

Jasna Kovac, The Pennsylvania State University

Kristin Schill, Food Research Institute/University of Wisconsin-Madison

Stephanie Smith, Washington State University

Session Type: Short Symposium - 1.5 hours

Session Summary: *Bacillus cereus* is a common sporeforming bacteria that can cause foodborne illness if cells grow to sufficiently high levels (e.g., 10⁵ organisms per g) in a food. Symptoms are exhibited between 1 and 15 hours and typically last only 24–48 hours, depending on whether emetic or diarrheal toxins are present in the food. The agent is responsible for approximately 64,000 cases in the United States annually, but most cases go unreported because symptoms resolve without medical treatment. While starchy foods such as rice, beans, and pasta are frequently associated with outbreaks, the ubiquitous distribution of the spores makes any food susceptible. Of particular concern is the growth of psychrotrophic strains in refrigerated meals, particularly in plant-based foods including milk analogs, and mesophilic strains during extended runs or extended cooling. Production of heat-stable emetic toxin is problematic because further cooking will not inactivate the toxin. This symposium will provide an overview of the prevalence of the spores, outbreaks which demonstrate lack of control, risks associated with psychrotrophic strains, and issues associated with enterotoxin detection. Lastly, strategies for inhibition of growth during processing and storage will focus on effective formulation changes along with balancing pathogen growth vs. spoilage.

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: Plant-Based Alternative Products

Secondary Topic Area: Dairy Quality and Safety

S57: STANDING OUT IN A CROWD: WHY SOME SALMONELLA STRAINS BREAK THROUGH TO CAUSE ILLNESS

Primary Author:

Eric Brown, FDA-Human Foods Program

Persons:

Eric Brown, FDA-Human Foods Program

Mark Moorman, Food and Drug Administration

Kristin Butler, US Food and Drug Administration

Timothy Jackson, FDA CFSAN

Nikki Shariat, University of Georgia

Francisco Zagmutt, EpiX Analytics

Session Type: Short Symposium - 2.0 hours

Session Summary: Typically during a foodborne illness outbreak of salmonellosis the number of *Salmonella* strains determined to be responsible for illness is limited to one or at most a few strains. Upon investigation of the production environment of a low-moisture food implicated in a *Salmonella* outbreak, one likewise will determine one or at most a few strains of *Salmonella* have become resident stains. However, outbreak investigations of soil, agricultural water and drag samples of the produce growing environment typically reveal many *Salmonella* serovars representing multiple strains indicating a diverse, abundant and incompletely understood ecosystem. Speakers in this symposium will examine the ecosystem of *Salmonella* in the produce farm environment with the goal of helping us understand why *Salmonella* illnesses attributed to produce are not as frequent as they are recovered from these environments. This symposium will examine the role of strain virulence factors to explain disease attribution and will also examine if strain clinical relevance can be explained by adaptive mechanisms of *Salmonella* on the farm and even in the plant. This symposium will also seek to answer if genomic evaluation of *Salmonella* strains can help with farm risk assessments.

Primary PDG Sponsor: Fruit and Vegetable Safety and Quality PDG

Secondary PDG Sponsor: Pre Harvest Food Safety PDG

Primary Topic Area: Pre Harvest Food Safety

Secondary Topic Area: Fruit and Vegetable Safety and Quality

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

Keywords: Genomics, Microbial stress, Outbreak, Pathogens, Pre-harvest, Produce, Public Health, Risk Assessment, Risk Management, Water

S58: DEFINE “LOT”? UNDERSTANDING NEW REGULATORY STANDARDS FOR SALMONELLA CONTAMINATION IN POULTRY PARTS AND STRATEGIES FOR ENSURING FINAL PRODUCT SAFETY

Primary Author:

Rigo Soler, Texas Tech University

Persons:

Rigo Soler, Texas Tech University

Saurabh Kumar, KERRY

Manpreet Singh, The University of Georgia

Marcos Sanchez, Texas Tech University

Joyjit Saha, Kerry

David Vargas, Wayne-Sanderson Farms

Harshavardhan Thippareddi, University of Georgia

Session Type: Short Symposium - 2.0 hours

Session Summary: In 2024, the USDA-FSIS released a comprehensive restructuring package for regulatory pathogen control in poultry products. This package includes new performance standards for *Salmonella* that incorporate sampling schemes such as quantification, serotyping, and potentially virulence factors. Evaluating microbial population variability including indicators pathogen loads/prevalence (*Salmonella*) across different lot-to-lot definition alternatives will help discern lot independence and identify a statistically robust and commercially feasible lot definition for chicken processing operations. This definition could be utilized under the proposed regulatory performance standards modifications by FSIS. There are significant economic and logistical challenges associated with the new regulations, as poultry lots can be large due to the volume of production in commercial facilities. Therefore, a practical definition of lots must be proposed and approached with sufficient data to support any decisions regarding FSIS samplings. An impractical lot definition could imply large product holdings and significant potential recalls. The importance of collecting data, including microbial levels in each plant, particularly in the secondary processing room, along with biomapping of different poultry parts can be presented to inspection authorities and auditors. Such parameters could identify best practices and strategic intervention schemes targeted at reducing pathogen contamination in the secondary processing room of various-sized commercial operations. Sections in Symposium: FSIS regulations and the current microbial values for each evaluated plant to aid in “lot” definition, along with potential solutions for addressing microorganisms. Current efforts of the industry and the importance of data-collection for making safety decisions. Different alternatives to “lot” definition as per commercial settings based. Antimicrobial solutions to reduce microbial load on final product.

Primary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Secondary PDG Sponsor: Data Management and Analytics PDG

Primary Topic Area: Meat and Poultry Safety and Quality

Secondary Topic Area: Data Management and Analytics

Additional Topic Area (Optional): Food Hygiene and Sanitation

Keywords: Data, Meat and Poultry, Pathogens, Regulations, Sampling

S59: FOOD ALLERGIES IN THE AMERICAN HOUSEHOLD - A ROUNDTABLE DISCUSSION WITH EXPERT PERSPECTIVES FROM THE FOOD ALLERGY ADVOCACY, GOVERNMENT, AND MEDICAL COMMUNITIES

Primary Author:

Joshua Scheinberg, Godshall's Quality Meats, Inc.

Persons:

Joshua Scheinberg, Godshall's Quality Meats, Inc.

Robert Earl, FARE

Anthony Flood, IFIC

Steven Gendel, Gendel Food Safety LLC

Session Type: Short Symposium - 2.0 hours

Session Summary: Food allergies in children continue to impart a significant cost and impact on families, schools, and society as a whole. Current assessments of prevalence of pediatric food allergies in the U.S. have relied on a combination of sparse public health data and surveys of parents, caregivers, and households of individuals and school aged children living with food allergies. Diagnoses of food allergies in children continue to evolve and can vary depending on parental choices, available testing resources, and the specialization of diagnosing practitioners. There continues to be a critical need for research to better assess attitudes, beliefs and behaviors to better understand the true prevalence and overall burden, and to improve diagnoses of food allergies in children. In this symposium, representatives from federal government, non-profit organizations, and academia will provide their perspectives on pediatric food allergies, our current understanding of the prevalence and burden, and present findings from new research and initiatives. Specifically, USDA-FNS will present results of a current study utilizing insurance claim data to assess prevalence and costs of medically diagnosed pediatric food allergies. Medical researchers specializing in this area will present current approaches to measuring prevalence and diagnosing food allergies; FARE representatives will present current approaches to supporting research and education in pediatric food allergies; and IFIC will present consumer insights from an IFIC/FARE consumer research collaboration. Note: I defer to the collective expertise and familiarity with this PDG's programming to provide changes or feedback to enhance this proposed symposium. I look forward to working with the PDG and speakers on this symposium.

Primary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Food Safety Education

Keywords: Allergens, Education, Public Health

S60: NOVEL FOODS, SAFETY, SHELF LIFE, AND RAPID METHODS - APPROACHES TO TEST METHOD DESIGN, VALIDATION AND APPLICATION IN ALTERNATIVE PROTEIN-BASED PRODUCTS

Primary Author:

Andrew Morin, Mérieux NutriSciences

Persons:

Andrew Morin, Mérieux NutriSciences

Chio Saeteurn, BlueNalu

Catharine Carlin, Merieux NutriSciences

Alex Brandt, Food Safety Net Services / Certified Group

Kristin Schill, Food Research Institute/University of Wisconsin-Madison

Session Type: Short Symposium - 2.0 hours

Session Summary: Alternative protein based food categories continue to rise in terms of source type, sales, and overall ubiquity and diversity on shelves. Multiple companies have now received FDA pre-market consultations and USDA approval to produce cell cultured meats. Cell culture derived food products in the U.S. lie in a novel regulatory landscape, overseen by both the USDA and FDA. Required testing such as virus detection and genome stability have few to no analogs in food safety testing. At the same time, a smaller production scale means validating testing with smaller sample amounts in mind. In plant based products, formulations for novel product development have a high potential for astringency and assay interference given the vast array of ingredient types. More novel food sources (Microalgae, Duckweeds, and Dairy analogue based) have resulted in more novel food products. These new substrates and products require rigorous method validation and development to ensure that testing is fit-for-purpose. New products may also contain novel spoilage organisms, or offer unique opportunities for foodborne pathogens, creating new challenges in shelf life and food safety. As a result, it is essential that innovations in product development are met with similar innovations in food testing. In this symposium, testing considerations and rapid methods will be discussed from the perspective of ingredient suppliers, CPG producers, test method developers and third party testing labs. Insight into the overall landscape, approaches, challenges for validation and market demand will be presented. The intended outcome of this symposium is to serve as insight, education and overall awareness for audience members as their companies pursue these novel portfolios.

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

Secondary PDG Sponsor: Advanced Molecular Analytics PDG

Primary Topic Area: Plant-Based Alternative Products

Secondary Topic Area: Advanced Molecular Analytics

Additional Topic Area (Optional): Applied Laboratory Methods

Keywords: Laboratory and Detection Methods, Plant-based foods, Validation

S61: CAN EXCEPTIONAL LETHALITY DURING THERMAL PROCESSING ACT AS A PREVENTIVE CONTROL?

Primary Author:

Rico Suhalim, PepsiCo

Persons:

Rico Suhalim, PepsiCo

Ariel Buehler, General Mills

Lakshmikantha Channaiah, University of Missouri

Nathan Anderson, U.S. Food and Drug Administration

Lisa Lucore, Shearer's Foods

Bradley Marks, Michigan State University

Session Type: Short Symposium - 2.0 hours

Session Summary: Thermal processing of food has a long history of satisfying the hunger of people worldwide. Historically, food processors have been using thermal processes such as baking, frying, popping, and extrusion to convert raw materials into quality and safe foods. With the advent of FSMA, food companies are required to validate these processes. However, validation studies can be costly, time consuming, and in some cases can be challenging to execute. In general, some thermal processes to manufacture foods employ exceptionally lethal temperatures to produce consumable finished products. Raw materials are converted into quality products by undergoing transformations in texture, color, and flavor to meet key finished product quality attributes for an acceptable product. The use of very high heat during some processes (e.g., frying, extrusion, baking, etc.), destroys pathogenic and spoilage microorganisms, resulting in extended shelf lives of finished food products. An exceptionally lethal process eliminates any known or reasonably foreseeable biological hazards for these food products, thus reducing/eliminating the need to provide evidence that the process delivers a specific level of lethality. While exceptionally lethal processes and safe harbors exist, each food manufacturer must establish their process threshold to determine that their process can provide adequate lethality. Although exceptional lethality can be achieved by some thermal processes, food companies should not overlook the importance of other food safety programs, such as sanitation, good manufacturing, and product handling practices to prevent potential post-process cross contamination. In addition, chemical and physical hazards are outside the scope of exceptional lethality and must be managed accordingly. The speakers will provide real-life examples and address i) exceptional lethal processes, ii) risk analysis for exceptional lethal processes, iii) how to demonstrate processes meet exceptional lethality or "do not," and iv) how to interpret validation study results.

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): Food Safety Culture

Keywords: Low-water Activity Foods, Modeling, Risk Assessment, Risk Management

S62: INTEGRATING MULTIDISCIPLINARY PRODUCE SAFETY RESEARCH TO INFORM REGULATION

Primary Author:

Manan Sharma, USDA/ARS

Persons:

Manan Sharma, USDA/ARS

Kalmia Kniel, University of Delaware

Michelle Danyluk, University of Florida

Laurel Dunn, University of Georgia

Channah Rock, University of Arizona

Faith Critzer, University of Georgia

Donald Schaffner, Rutgers University

Natalie Dyenson, International Fresh Produce Association

Session Type: Short Symposium - 1.5 hours

Session Summary: Managing risks associated with foodborne pathogens on produce requires an understanding of drivers for produce contamination. Well planned and executed research should result in relevant findings that can apply to produce safety regulations and guidelines in different regions and at different scales. Projects have historically addressed isolated survival, mitigation, and transfer of pathogens in waters, soils containing biological soil amendments of animal origin (BSAAO), and on packing equipment. Few projects have integrated these findings. The development of food safety metrics applicable to different growing regions, commodities, and farm sizes can provide “metrics-centric” approaches to cost-effective mitigations. Coordinated research can collect data and create models that accurately quantify the levels of risk faced by consumers. This approach requires cooperation among geographically diverse researchers using common methods, microorganisms, and collecting environmental data and metadata. This symposium describes a framework addressing produce safety challenges in an integrated manner: The CONTACT project (Scientific Challenges and Cost-Effective Management of Risks Associated with Implementation of Produce Safety Regulations) funded by the USDA SCRI in 2020, as a multi-institution, multi-region CAP project. Work on water, BSAAOs, transfer, and integrated modeling approaches will be described in a relevant manner to current produce safety regulations and guidelines, and integrated across objectives within CONTACT. This work spans multiple regions and laboratories using standardized testing, and “real world” production conditions. Data are modeled and analyzed accounting for the step-wise survival or mitigation of pathogens. We seek to inspire industry, government and academic professionals to use integrated approaches to projects by showcasing best practices for collaboration in applied research focused on finding solutions to stimulate meaningful change.

Primary PDG Sponsor: Fruit and Vegetable Safety and Quality PDG

Secondary PDG Sponsor: Water Safety and Quality PDG

Primary Topic Area: Fruit and Vegetable Safety and Quality

Secondary Topic Area: Water Safety and Quality

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

Keywords: Modeling,Pre-harvest,Produce,Water

S63: LFFM: FIVE YEARS OF SUCCESS IN STRENGTHENING FOOD SAFETY

Primary Author:

Ruiqing Pamboukian, FDA/ORA

Persons:

Ruiqing Pamboukian, FDA/ORA

Beilei Ge, Food and Drug Administration

Michele Sayles, Diamond Pet Food

Christopher Waggener, FDA

Lauren Yeoung, FDA

Maria Ishida, New York State Department of Agriculture and Markets

Sinisa Urban, Maryland Department of Health Laboratories Administration

Sara Sklenka, FDA

Ruth Timme, US Food and Drug Administration

Jessica Price, Virginia Division of Consolidated Laboratory Services

Katie Arnold, FDA

Session Type: Short Symposium - 2.0 hours

Session Summary: The Laboratory Flexible Funding Model (LFFM) is a cooperative agreement designed to enhance the capacity and capabilities of state human and animal food testing laboratories in support of the National Integrated Food Safety System (IFSS). Through targeted sample testing in microbiology, chemistry, radiochemistry, and special projects, LFFM strengthens the collaboration between FDA and states by increasing testing capacity in partner laboratories and contributing to strengthening a risk-based, prevention-focused food safety system. During the first four years, over 71,000 samples have been analyzed for pathogens, toxic elements, and more. The current year-five work will add ~13,000 samples. LFFM data has driven compliance actions, including 90 product recalls, consumer advisories, adding firms to import alerts, and warning letters. Besides surveillance, LFFM leverages the nation's food testing laboratory network for emergency response needs and to strengthen testing in support of foodborne outbreak and illness investigations. Together, valuable information have been gained about hazards in our nation's food supply which can be used to develop risk prevention and mitigation strategies. This symposium will overview and highlight several success stories and showcase the power of LFFM collaboration between FDA and states. Attendees will gain a deep understanding of how the FDA and state partners work together to better ensure food safety and protect public health via LFFM.

Primary PDG Sponsor: Animal and Pet Food Safety PDG

Secondary PDG Sponsor: Advanced Molecular Analytics PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Animal and Pet Food Safety

Keywords: Chemical Contaminant, Genomics, Pathogens

S64: THE FRONTLINES OF FOOD SAFETY EDUCATION: CHALLENGES AND OPPORTUNITIES

Primary Author:

Aaron Lavalley, USDA-FSIS-OPACE

Persons:

Aaron Lavalley, USDA-FSIS-OPACE

Shauna Henley, University of Maryland, Extension

Britanny Saunier, Partnership for Food Safety Education

H. Lester Schonberger, Virginia Tech

Ellen Thomas Shumaker, NC State University

Lisa Peterson, University of Illinois Urbana-Champaign

Emily Marrison, The Ohio State University

Jennifer Quinlan, Drexel University

Session Type: Short Symposium - 2.0 hours

Session Summary: Community-based food safety educators and professionals in extension are essential to helping people understand steps that reduce their risk of foodborne illness. Consumer education has evolved with the last decade accelerating that evolution faster than any point in history. With illness rates still high, efforts to engage the public with science-based information is still relevant and critical to good health. Consumer education must evolve in the face of challenges threatening its efficacy, reach, and future and the extension network is key to delivering food safety education. The science behind safe food handling has remained consistent over the years, but the way information is delivered has not. Food safety educators are facing an evolving audience and landscape. This translates to targeted approaches that are specific to regions. Beliefs, communication styles, and other factors affect how individuals receive, understand, and respond to information. Rapidly changing communication channels from print to digital and traditional to social media, and the presence of misinformation, mean that educators are constantly adapting. Not only are channels for information delivery and the audiences changing but so is the network that serves these communities. Many are at or close to retirement age and less and less resources are provided from local, state, and federal government to serve their communities. This symposium will focus on collaborations across academia, government agencies, and industry by: (a) reviewing the state of consumer education on the front lines (b) highlighting the resource needs, compounding priorities, and funding; (b) exploring case-studies from the field where educators have evolved their approaches to reach consumers; (c) dissecting the rapidly evolving social media ecosystem and how educators can compete with mis and disinformation; (d) recruiting the next generation of educators and influencers; (e) exploring the challenges of measuring effectiveness of food safety education on consumer behavior change.

Primary PDG Sponsor: Food Safety Education PDG

Primary Topic Area: Food Safety Education

Secondary Topic Area: Communication, Outreach and Education

Keywords: Education,Inclusion,Public Health

Roundtable Abstracts

RT1: CHALLENGES AND OPPORTUNITIES AROUND THE NEW FSMA PRE-HARVEST AGRICULTURAL WATER RULE: SAFETY, SUSTAINABILITY, AND THE NEED FOR INTEGRATED WATER RESOURCE MANAGEMENT

Primary Author:

Elizabeth Bihn, Cornell University

Persons:

Elizabeth Bihn, Cornell University

Elisabetta Lambertini, GAIN

Robert Sakata, Colorado Department of Agriculture

Charles Gerba, University of Arizona

Joan Rose, Michigan State University

Dr. Siddhart Kishore, UC Merced

Session Type: Roundtable - 1.5 hours

Session Summary: Water is a limited and valuable natural resource. Water is also critical to the production of fruits and vegetables. Since pre-harvest agricultural water can be a vector of microbial contaminants for fresh produce, water quality standards have been developed to protect food safety. In May of 2024, the U.S. FDA released new agricultural water use requirements for the primary production of fresh produce, applicable to U.S. producers as well as foreign producers exporting into U.S. markets. Though these requirements are no longer only based on water testing metrics, they allow growers to be exempt from the responsibility of completing an agricultural water assessment if they use municipal treated water or groundwater that meets the standard of no generic *E. coli* detected in 100 ml, or if they treat their water. These options may incentivize growers to use more ground water or attempt to treat surface water to meet quality expectations. Using municipal potable water is most often not an option due to high costs, distance, or availability. Groundwater overdraft, land subsidence, and aquifer contamination have been worsening for decades across the nation. Surface water is subject to regional variability and competing demand for multiple uses (e.g., environmental flows, urban, agricultural, energy, food processing, industrial). Harmonization and trade-offs are needed between water quality, availability, and costs. This roundtable will bring together experts from food safety, food security, integrated water resources management, and public communication to discuss approaches to quantitatively manage agricultural water use and its quality, risks from unsustainable water use, and the potential impact of new water quality demand and standards on food safety, food security, and water availability for multiple uses. The roundtable will start with each speaker introducing their topic for 5–10 minutes, followed by a facilitated discussion and Q&A with the audience.

Primary PDG Sponsor: Water Safety and Quality PDG

Secondary PDG Sponsor: Fruit and Vegetable Safety and Quality PDG

Primary Topic Area: Fruit and Vegetable Safety and Quality

Secondary Topic Area: Water Safety and Quality

Additional Topic Area (Optional): Pre Harvest Food Safety

Keywords: Climate change,Produce,Water

RT2: LISTERIA QUANTIFICATION IN DAIRY AND RTE: CHALLENGES, INNOVATIONS, PERSPECTIVES

Primary Author:

Craig Jewell, Hygiena LLC

Persons:

Craig Jewell, Hygiena LLC

Olivia Arends, The Kraft Heinz Company

Julie Weller, Hygiena

Staci Richardson, Schreiber Foods

Brian Kraus, Wells Enterprises, Inc.

Alex Brandt, Food Safety Net Services / Certified Group

Jeffrey Kornacki, Kornacki Microbiology Solutions, Inc.

Sanjay Gummalla, American Frozen Food Institute

Session Type: Roundtable - 1.5 hours

Session Summary: The U.S. maintains a “Zero tolerance” policy for *Listeria monocytogenes* in ready-to-eat (RTE) foods and dairy products that do not support growth, while other nations (such as the EU, Canada, Australia, and New Zealand) adopt more flexible regulatory limits. For Example, the EU uses a limit of 100 CFU/g of *L. monocytogenes* in RTE foods, provided the limit is not exceeded throughout the product’s shelflife. As new *L. monocytogenes* quantification methods emerge, emphasis can be placed on more robust risk assessment and thereby support risk management in production facilities. This roundtable unites experts to discuss the evolution and rising importance of *L. monocytogenes* quantification in dairy and RTE foods, aiming to align safety protocols with evolving industry practices. Key topics to be explored include innovative quantification technologies, where advanced tools such as qPCR, biosensors, and CRISPR-based detection are assessed for their speed, accuracy, and potential for industry adoption. Additionally, discussions will focus on regulatory frameworks and implementation, comparing current and future guidelines for *L. monocytogenes* quantification, particularly evaluating the differences between the U.S. zero-tolerance policy and the EU's tolerance-based regulations. Finally, the roundtable will examine how improved quantification can refine risk assessment models, influence policy decisions, and assess the economic impact on dairy and RTE food production and international trade. This roundtable discussion seeks to balance the scientific, regulatory, and economic aspects of *L. monocytogenes* management and drive ongoing and future research, define best practices, and explore how improved *L. monocytogenes* quantification methods may allow for regulatory reform, potentially shifting from a zero-tolerance policy to CFU/g limit. Together we can continue the advancement of *L. monocytogenes* food safety in dairy and RTE products, on a global scale.

Primary PDG Sponsor: Dairy Quality and Safety PDG

Secondary PDG Sponsor: Applied Laboratory Methods PDG

Primary Topic Area: Dairy Quality and Safety

Secondary Topic Area: Applied Laboratory Methods

Additional Topic Area (Optional): Food Law

Keywords: Dairy, Laboratory and Detection Methods, Pathogens

RT3: DEFINING A FOOD SAFETY DATA STANDARD

Primary Author:

Joseph Heinzelman, Neogen

Persons:

Joseph Heinzelman, Neogen

Angela Anandappa, Alliance for Advanced Sanitation

Mark Carter, MC Squared

Robert Salter, Charm Sciences, Inc.

William Melnyczenko, Merieux NutriSciences - Silliker Labs

Melissa Calicchia, Food Microbiological Laboratories, Inc.

Hannes Pouseele, BioMerieux, Inc.

Session Type: Roundtable - 1.5 hours

Session Summary: As food safety continues its journey to digitalization, one missing element to accelerating the aggregation of data is a food safety data standard. The roundtable will hear from experts and drive towards defining new data standard for those participating in the industry. A new data standard would allow new technologies to easily be interpreted, integrated, and utilized in a more meaningful capacity. This would also provide a unique framework between stakeholders of those companies using, manufacturing, and regulating food safety results from various testing devices, 3rd party testing labs, and food safety professionals. A unified data standard for routine execution of food safety testing will ultimately provide a data taxonomy that will be able to be leveraged with new Artificial Intelligence and Machine Learning techniques. Defining a data standard at the forefront of the digitalization of food safety will provide those that supply the industry with a defined standard to improve the ease of use and applicability of diagnostic results in a more meaningful capacity. This session will provide a necessary set of recommendations for a charter, working members, and objectives and goals for this important milestone to be achieved.

Primary PDG Sponsor: Data Management and Analytics PDG

Primary Topic Area: Data Management and Analytics

Secondary Topic Area: Food Hygiene and Sanitation

Keywords: Data, Environmental monitoring

RT4: CROSSING THE FINISH LINE: INDUSTRY'S RACE TO FSMA 204 COMPLIANCE

Primary Author:

Lisa Lupo, The Acheson Group

Persons:

Lisa Lupo, The Acheson Group

Benjamin Miller, The Acheson Group

Raquel Maymir, General Mills

Eric Marshall, Leavitt Partners

Patrick Guzzle, National Restaurant Association

Quincy Lissaur, SSAFE

Session Type: Roundtable - 1.5 hours

Session Summary: Although compliance with the Traceability Rule (FSMA 204) has been extended by 30, the complexity of full supply-chain traceability critical that the industry continue on pace to the finish line. The readiness of food companies currently varies from fully implemented to nonawareness of the rule. What does this mean for the effectiveness of traceability from farm to retail? And what is being done to bring the industry together? Although the rule does require that covered facilities implement enhanced traceability recordkeeping, what it doesn't do is unify the industry in a single, comprehensive approach to enable efficient interaction between food businesses and industry sectors. So the industry is taking this into its own hands. In 2024, a team of industry, association, supplier, and government representatives joined together to discuss the lack of consistency and how to unite all facets of the industry. The result was the non-profit Partnership for Food Traceability (PFT) a public-private partnership with FDA participation, association involvement, and cross-sector membership of both large and small businesses. The roundtable will feature experts from various industry segments, using the PFT as a primary example. These experts will explore why collaboration and interoperability are essential for achieving full traceability. They'll discuss how a shared forum for exchanging, gathering, and using traceability data can help create a unified vision across the supply chain. Additionally, they'll explain how a well-organized plan can support the industry's transition to enhanced traceability practices. Participants will gain valuable insights into current efforts by industry, associations, and government agencies. They'll also learn about recommended actions for businesses, both foreign and domestic, at this stage and what to expect as the compliance deadline approaches.

Primary PDG Sponsor: Food Law PDG

Primary Topic Area: Food Law

Secondary Topic Area: Data Management and Analytics

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

Keywords: Outbreak, Regulations, Traceability

RT5: LISTERIA CONTROL IN READY-TO-EAT FOODS: ADDRESSING THE CONTINUED CHALLENGE

Primary Author:

John David, Neogen

Persons:

John David, Neogen

Denise Eblen, USDA/FSIS

Peggy Cook, Neogen

Sharon Beals, BeaconPoint Labs

Martin Wiedmann, Cornell University

Gabriela Lopez Velasco, NEOGEN

Session Type: Roundtable - 1.5 hours

Session Summary: Recent outbreaks of *Listeria monocytogenes* in ready-to-eat (RTE) foods have underscored the critical need for enhanced control measures. These incidents, involving various deli meats and other RTE products, highlight the persistent threat posed by this pathogen and the importance of continued focus and innovation in food safety strategies and practices. This roundtable will bring together experts from government, academia, and industry to engage in an interactive discussion on solutions for improving *Listeria* control. Guided discussion will address key topics such as: • Recent regulatory updates and their implications for *Listeria* control strategies • The persistence of *Listeria monocytogenes* in food processing environments and effective mitigation approaches • Industry challenges and lessons learned in controlling *Listeria* in RTE meat processing • Advances in rapid molecular diagnostics and their role in transforming *Listeria* detection and prevention This session aims to provide practical insights and innovative strategies for reducing the risk of *Listeria* contamination in RTE foods by facilitating an open exchange of knowledge and expertise. Audience engagement is encouraged, with opportunities for attendees to ask questions, share experiences, and explore solutions.

Primary PDG Sponsor: Applied Laboratory Methods PDG

Secondary PDG Sponsor: Data Management and Analytics PDG

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, Pathogens, Ready to Eat Foods

RT6: PRACTICAL ASSESSMENT OF RISK: WHAT MODELING TOOLS AND TECHNIQUES TO USE?

Primary Author:

Yuhuan Chen, FDA CFSAN

Persons:

Yuhuan Chen, FDA CFSAN

Vijay Juneja, USDA-ARS-ERRC

Donald Schaffner, Rutgers University

Panagiotis Skandamis, Agricultural University of Athens

Bala Kottapalli, T.Marzetti

Marcel Zwietering, Wageningen University

Girvin Liggans, FDA

Session Type: Roundtable - 1.5 hours

Session Summary: Practical and rapid assessment of risk or its elements are often necessary to inform food safety management decisions. But what modeling tools are readily available to facilitate timely and practical assessment of risk? How can modeling tools, data, and other relevant information be made accessible within an organization or to the broader food safety community? This roundtable brings together a panel from government, industry and academia to share their perspectives and examples of relevant tools and resources. The roundtable will start with a brief overview of established modeling tools and databases, such as FDA-iRISK[®] FDA-OC App sampling tool (expanding upon the ICMSF sampling spreadsheet), ComBase, USDA-PMP/PMIP and other tools, as well as considerations on emerging Large Language Model and Machine Learning tools. The panel will share case studies grounded in real world scenarios, including challenges and opportunities for conducting practical risk assessments to inform decisions in data scarce and evolving situations. The panel members will present brief remarks to spark discussions, and the audience will be invited to pose questions and share comments. This engaging session will offer insights on modeling tools and data accessible to aid practical and efficient assessment of risk for timely decisions to enhance food safety.

Primary PDG Sponsor: Modelling and Risk Analysis PDG

Primary Topic Area: Microbial Modelling and Risk Analysis

Secondary Topic Area: HACCP Utilization and Food Safety Systems

Keywords: Pathogens,Risk Assessment,Risk Management

RT7: DECODING FOOD ALLERGEN METHODS: WHY, WHEN, AND HOW TO IMPLEMENT ANALYSIS

Primary Author:

Melanie Downs, University of Nebraska-Lincoln

Persons:

Melanie Downs, University of Nebraska-Lincoln

Jodi Nickerson, Eurofins Microbiology Laboratories

Laura Allred, Gluten Intolerance Group

Markus Lacorn, R-Biopharm

Gabriela Lopez Velasco, NEOGEN

Simon Flanagan, Mondelez International

Jasmine Lacis-Lee, BVAQ and The Allergen Bureau

Session Type: Roundtable - 1.5 hours

Session Summary: Analytical methods to detect and quantify food allergens can be critical to validate and verify the efficacy of allergen control programs.

However, the complexity of obtaining accurate results for food allergen measurements across a wide range of applications can often be underestimated, leading to issues with understanding how to implement food allergen analysis and interpret results. These challenges are likely to be heightened with developments at the international level towards use of reference doses based on quantitative risk assessment to make decisions about precautionary allergen labeling. A substantial number of commercial methods are available to detect and/or quantify food allergens, but users are left to make many critical decisions with minimal guidance or data. How should users select methods, verify performance, and compare results among methods? How should food safety professionals develop and implement sampling and testing programs? This roundtable session will bring together a group of experts to translate guidelines for food allergen method validation, performance criteria, and usage into actionable best practices. Panelists will share perspectives from international standards organizations, regulatory agencies, method development companies, third-party laboratories, and food manufacturers on the important factors to consider in determining which food allergen methods are fit for purpose.

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, Risk Assessment, Sampling, Validation

RT8: CAREER GPS: GUIDING YOUR PROFESSIONAL JOURNEY

Primary Author:

Sarah Smith-Simpson, Nestle Nutrition

Persons:

Sarah Smith-Simpson, Nestle Nutrition

Nadia Narine, Lumar Food Safety Services Ltd.

Staci Richardson, Schreiber Foods

Donald Schaffner, Rutgers University

Karuna Kharel, Louisiana State University AgCenter

Gustavo Reyes, Western Growers Science

Trushenkumar Shah, University of Connecticut

Session Type: Roundtable - 1.5 hours

Session Summary: Food safety professionals often face the challenge of navigating their career growth and professional development in a fast-paced industry. This session aims to address the question of "What's next?" and provide guidance on taking the next steps in the food safety field. We will explore strategies for identifying the next career step, whether it be transitioning roles within Quality Assurance or pursuing a role as a microbiologist in the lab. Participants will learn how to reflect on their career goals, assess their strengths, and advocate for roles that align with their long-term aspirations. Seeking guidance and mentorship is crucial in career development, and this session will provide insights into the individuals, resources, and networks that offer valuable advice and support. We will emphasize the importance of seeking advice from experienced professionals and industry leaders, as well as building a supportive professional network. Participants will also engage in discussions about potential missteps and challenges in career development. We will explore the balance between being selective and open to new opportunities, evaluating career moves strategically, and advocating for roles that align with personal and professional development goals. In addition to professional growth, personal development is equally important. The session will highlight the significance of ongoing learning, skill enhancement, and personal well-being. Overall, this session aims to provide food safety professionals with valuable insights, practical strategies, and the keys to developing a supportive network to navigate their career paths and achieve their professional development goals. By joining this session, participants will gain the tools to take control of their career development in the dynamic food safety industry.

Primary PDG Sponsor: Developing Food Safety Professionals PDG

Secondary PDG Sponsor: Student PDG

Primary Topic Area: Developing Food Safety Professionals **Secondary**

Topic Area: Food Safety Education

Additional Topic Area (Optional): Food Safety Culture

Keywords: Education, Food Safety Culture, Professional Development

RT9: 20 YEARS OF CONSUMER INSIGHTS: WHAT IFIC'S FOOD & HEALTH SURVEY TELLS US ABOUT CONSUMERS AND FOOD SAFETY

Primary Author:

Anthony Flood, IFIC

Persons:

Anthony Flood, IFIC

Christine M. Bruhn, Emerita, University of California, Davis

Aaron Lavalley, USDA-FSIS-OPACE

Pamela Wilger, Post Consumer Brands

Kris Sollid, IFIC

Session Type: Roundtable - 1.5 hours

Session Summary: Consumer research regarding food safety is a vital asset for all food safety professionals. It can be used to assess and understand the diverse food safety needs of the consumer as well as provide a road map for developing and promoting safety enhanced products. Consumer research enables better understanding of consumer perceptions about food safety and food-handling behaviors that can impact food safety regulatory and policy decisions. The International Food Information Council (IFIC) has a rich history of conducting consumer research on food and nutrition related issues. For the past 20 years, IFIC's signature consumer research project, the Annual Food & Health Survey, has been referenced and utilized in numerous thought leading discussions among academicians, policy leaders, consumer advocacy groups and the food and beverage industry. As part of this roundtable, IFIC will reveal new food safety data (results) stemming from the 2025 Food & Health Survey. Attendees at this roundtable will be the first to glean insights on the new 2025 data. Panelists representing diverse sectors of the food safety community: government, industry and academia along with IFIC will share perspectives on the Food & Health Survey data and will lead the attendees in an interactive discussion on past research successes, current research gaps and future educational needs.

Primary PDG Sponsor: Food Safety Education PDG

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

Primary Topic Area: Communication Outreach and Education

Secondary Topic Area: Food Safety Education

Keywords: Education, Professional Development, Public Health

RT10: COMPARING DIFFERENT APPROACHES TO IDENTIFY SALMONELLA SEROTYPES OF CONCERN IN MEAT AND POULTRY

Primary Author:

Katherine Marshall, CDC

Persons:

Katherine Marshall, CDC

Nikki Shariat, University of Georgia

Francisco Zagmutt, EpiX Analytics

Matthew Stasiewicz, University of Illinois

Kathryn McCullough, North American Meat Institute

Hilary Whitham, Centers for Disease Control and Prevention

Drew Posny, USDA

Session Type: Roundtable - 1.5 hours

Session Summary: A variety of approaches have been developed to identify and examine the top *Salmonella* serotypes of concern for specific commodity groups, particularly poultry. In this roundtable session, authors of several approaches will briefly describe their approach including the data sources used and findings, and then participate in a discussion of the strengths and limitations of each approach, compare similarities and differences between the approaches, and approaches moving forward. Special attention will be paid to objectively examine and discuss strengths and weakness of each method, with less focus on the serotypes themselves and differing results, to best inform methodological updates moving forward. The goal of this session is to provide attendees with a deeper understanding of the different approaches to identify top *Salmonella* serotypes of concern that have been developed, describe critical differences and use cases for each, and describe potential future work.

Primary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Secondary PDG Sponsor: Data Management and Analytics PDG

Primary Topic Area: Meat and Poultry Safety and Quality

Secondary Topic Area: Data Management and Analytics

Keywords: Data, Meat and Poultry, Risk Assessment

RT11: ADDRESSING THE EMERGING THREAT OF PSYCHOACTIVE COMPOUNDS IN FOOD

Primary Author:

Carrie Rigdon, Association of Food & Drug Officials

Persons:

Carrie Rigdon, Association of Food & Drug Officials

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

Hillary Booth, Washington State Department of Health

Sinisa Urban, Maryland Department of Health Laboratories Administration

Margaret Thelen, Council of State and Territorial Epidemiologists

Samantha Lee, Minnesota Regional Poison Center/Hennepin Healthcare

Jodi Taylor, Ohio Department of Agriculture

Melanie Firestone, University of Minnesota School of Public Health

Session Type: Roundtable - 1.5 hours

Session Summary: Recent outbreaks potentially associated with psychoactive compounds in food, such as the multi-state Diamond Shroomz™ incident, present significant challenges to public health and food safety systems. This outbreak, which sickened at least 169 and hospitalized at least 66 individuals experiencing severe neurological and psychiatric symptoms, highlighted gaps in our existing foodborne illness detection and response frameworks. Illnesses potentially related to consuming Diamond Shroomz™ chocolate bars, cones, and gummies were detected using surveillance mechanisms that are uncommon for multi-state foodborne disease outbreaks. This led to delayed identification, inconsistent reporting, and challenges in coordinating multi-agency efforts. These outbreaks are particularly challenging because the products often operate within legal gray areas, complicating regulatory oversight and public health interventions, evading traditional surveillance mechanisms and impeding coordinated multi-agency response. This session will explore the five primary challenges: - Delayed detection and reporting: Neurological symptoms, unlike traditional foodborne illnesses, cause delayed recognition and attribution to food products. - Regulatory and legal ambiguities: Legal ambiguities hinder timely regulatory action. - Complex contaminant identification: Novel compounds evade routine testing methodologies. - Coordination challenges: Unconventional detection and regulatory ambiguities slow down effective interventions. - Product traceability: The use of unregulated supply chains complicates recall efforts. Panelists will discuss the importance of developing new surveillance tools, improving laboratory capacity for detecting psychoactive substances, and fostering stronger inter-agency collaborations to mitigate these emerging threats. They will provide concrete recommendations to enhance preparedness and protect public health in future outbreaks involving psychoactive compounds in food.

Primary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Non PDG Sponsor (Organization, Association, etc.): Association of Food and Drug Officials

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Epidemiology

Additional Topic Area (Optional): Applied Laboratory Methods

Keywords: Chemical Contaminant, Epidemiology, Laboratory and Detection Methods

RT12: EFFORTS TO REDUCE FOOD SAFETY RISKS IN THE PRODUCTION OF WHEAT FLOUR

Primary Author:

Scott Osborne, The Mennel Milling Company

Persons:

Scott Osborne, The Mennel Milling Company

Teresa Bergholz, Michigan State University

Nathan Anderson, U.S. Food and Drug Administration

Molly Miller, North American Millers' Association

Luis Sabillon, New Mexico State University

Kelly Stevens, General Mills

Session Type: Roundtable - 1.5 hours

Session Summary: This roundtable will provide insights into the progress made across industry, academia and regulators in the safe production of wheat flour. Because wheat is a raw agricultural commodity, there are associated risks of pathogenic microorganisms making their way into refined wheat flour. Learn what some of the leading food scientists are doing to produce a refined flour product that maintains all its functionality while mitigating food safety risks.

Primary PDG Sponsor: Low Water Activity Foods PDG

Non PDG Sponsor (Organization, Association, etc.): North American Milling Association (NAMA)

Primary Topic Area: Low Water Activity Foods

Secondary Topic Area: Food Chemical Hazards and Food Allergy

Keywords: Low-water Activity Foods, Pathogens, Public Health

RT13: EMPOWERING EDUCATION: CREATING INCLUSIVE AND ENGAGING TRAINING PROGRAMS

Primary Author:

Christina Allingham, University of Massachusetts Amherst

Persons:

Christina Allingham, University of Massachusetts Amherst

Amanda Kinchla, University Massachusetts

Shauna Henley, University of Maryland, Extension

Angela Walla, Texas Tech University

Joseph Meyer, Kerry

Teresa McCoy, The Ohio State University

Ruth Torres Castillo, New Mexico State University

Tia Glave, Catalyst, LLC

Session Type: Roundtable - 1.5 hours

Session Summary: Food safety educators, including professors, curriculum developers, subject matter experts, industry managers, and student mentors are often not formally trained in effectively communicating food safety knowledge to others engagingly and inclusively. The top challenges to successful content delivery include a lack of course adaptation to DEI principles, creative activities to engage diverse audiences, and the inability to impart lasting behavioral changes to food safety employees. Through this roundtable, we will discuss best practices and share strategies for teaching target audiences (college students, adult audiences, and employees in industry), and discuss ways to adapt teaching materials to fit the needs of diverse audiences. This session features a diverse panel discussing relevant case studies on effective food safety communication, and offering insight into curriculum development and effective execution from different perspectives. Speakers include: An internationally recognized industry professional who will share expertise in designing inclusive curricula and adapting to meet diverse operator needs; A Director of Learning and Organizational Development, who will share experiences with training educators on effective program development and evaluation practices that result in evidence-based, learning outcomes; A University Outreach Specialist from an HBCU discussing integrating DEI efforts in programming/mentorship opportunities; A Post-Doctoral Researcher discussing strategies for effectively mentoring students and innovative media approaches for curriculum development. The topics presented in this roundtable will benefit all food safety professionals aiming to effectively develop or deliver food safety curricula, training, mentorship, or general expertise. Panelists will provide strategies to enhance mentorship within processing facilities and the classroom, ultimately improving training and achieving more effective and measurable learning outcomes.

Primary PDG Sponsor: Developing Food Safety Professionals PDG

Secondary PDG Sponsor: Food Safety Education PDG

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

Primary Topic Area: Communication Outreach and Education

Secondary Topic Area: Developing Food Safety Professionals

Additional Topic Area (Optional): Food Safety Education

Keywords: Education,Inclusion,Professional Development

RT14: SUSTAINING FOOD SAFETY IMPROVEMENT INITIATIVES IN LOW- AND MIDDLE-INCOME COUNTRIES (LMICS): INSIGHTS FROM RESEARCH AND PRACTICAL ENGAGEMENTS

Primary Author:

Kebede Amenu, International Livestock Research Institute

Persons:

Kebede Amenu, International Livestock Research Institute

Arie Havelaar, University of Florida

Haley Oliver, Purdue University

Vivian Hoffmann, International Food Policy Research Institute (IFPRI)

Ariel Garsow, The Global Alliance for Improved Nutrition

Hung Nguyen-Viet, International Livestock Research Institute

Amare Ayalew, The Partnership for Aflatoxin Control in Africa (PACA), African Union

Barbara Kowalczyk, George Washington University

Session Type: Roundtable - 1.5 hours

Session Summary: The publication of foodborne disease (FBD) burden estimates by the World Health Organization Foodborne Disease Burden Epidemiology Reference Group (WHO/FERG, 2015), was an important milestone in generating evidence in terms of the health burden associated with the consumption of unsafe foods. Specifically, the publication revealed the high disparity in the burden of FBD between high-income countries (HICs) and low- and middle-income countries (LMICs) with clearly higher burden in LMICs. The publication has drawn attention to FBD, highlighting various food safety research and development initiatives in LMICs. As a result, various food safety improvement initiatives have been designed and implemented, although their scope has not matched the scale of the underlying issues in LMICs. Food safety has direct or indirect relations with several sustainable development goals (SDGs). This roundtable discussion will bring various international experts with long years of experience working in LMICs (Africa and Asia) with direct engagement with stakeholders to discuss the sustainability of food safety initiatives in terms of research for development projects. The discussion will focus on Stakeholders' engagements, project management, research ethics, capacity building, and evidence generation. The RT will bring together various food safety professionals and researchers involved in food safety projects in LMICs in various capacities. The following questions will be specifically addressed: • What would be the barriers or challenges for sustainability in food safety improvement? • How have previous projects impacted the food safety landscape of target countries? How can the target countries sustain project initiatives? • What are the missing gaps and best model of sustainable improvements? • Food safety in the international context and the implications • The implications of food safety related to food defense and international human mobility (HICs versus LMICs)

Primary PDG Sponsor: International Food Protection Issues PDG

Secondary PDG Sponsor: Food Defense PDG

Primary Topic Area: International Food Protection Issues

Secondary Topic Area: Food Defense

Keywords: Epidemiology, Food Defense, Food Security, Hygiene, One Health, Risk Analysis, Sustainability

RT15: STRATEGIES FOR MANAGING FOREIGN MATERIAL INCIDENTS IN FOOD PRODUCTION

Primary Author:

Suzanne Finstad, Tyson Foods, Inc.

Persons:

Suzanne Finstad, Tyson Foods, Inc.

Amanda Fischer, Schreiber Foods

Michele Sayles, Diamond Pet Food

Kurt Westmoreland, FlexXray, LLC

Jeff Varcoe, PhD, J.M. Smucker Co.

David Rasmussen,

Session Type: Roundtable - 1.5 hours

Session Summary: Foreign material contamination continues to be an underdiscussed food safety issue that has only become more challenging as processing and production operations become more complex and larger in scale. The development of better in-line technology has helped many producers to understand and evaluate the challenge more deeply, but setting up a food safety culture that reduces these risks and accounts for all potential sources of foreign material in product is critical to reducing the number of incidents that occur and ultimately the number of recalls each year while also helping mitigate food waste challenges. This panel discussion will feature leaders from a range of industries and will discuss how practices like preventative maintenance, supplier monitoring and asset risk assessments can all contribute to improvements in foreign material and waste reduction. Our panelists will discuss how to respond in the immediate aftermath of an incident, run a robust root cause investigation, and make a proper disposition of affected product. We will also discuss how initiatives such as food waste reduction can be integrated into the decision-making process, and solutions for moving away from immediate disposal of product when an incident occurs. Key takeaways will include:

- Discussion of the most common sources of risk for foreign material
- How suppliers play a role in helping reduce contamination challenges
- What are areas to pay attention to within a plant setting for foreign material reduction
- What options exist for remediating after an incident occurs
- How to think about root cause investigations in order to improve future outcomes
- Nonproprietary case studies, including possible contributing causes to illustrate how incidents may occur.

Primary PDG Sponsor: Physical Hazards and Foreign Material PDG

Secondary PDG Sponsor: Animal and Pet Food Safety PDG

Primary Topic Area: Physical Hazards and Foreign Material

Secondary Topic Area: Animal and Pet Food Safety

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

Keywords: Animal and Pet Food Safety, Meat and Poultry, Risk Management

RT16: DECODING REGULATORY AND PUBLIC HEALTH USES OF WGS: WHAT FOOD PRODUCERS SHOULD KNOW

Primary Author:

Jessica Chen, Centers for Disease Control and Prevention

Persons:

Jessica Chen, Centers for Disease Control and Prevention

Marc Allard, FDA

Bill Marler, Marler Clark

Mark Moorman, Food and Drug Administration

William K. Shaw, Jr., USDA FSIS

Heather Carleton, Centers for Disease Control and Prevention

Joelle Mosso, Western Growers Association

Brendan Ring, Creme Global

Celina To, Nelson Jameson

Emily Butler, Abott Nutrition

Session Type: Roundtable - 1.5 hours

Session Summary: Genome sequencing has revolutionized food safety microbiology having many real-world applications ranging from outbreak detection, ensuring compliance to regulations, or as part of an environmental monitoring program in a processing plant. This session will open with a brief overview of the current technological landscape and language before moving into roundtable discussion with regulators, public health, testing service providers, trade associations, and legal entities provide on how this data is used to inform decision making. Discussion will be centered around how WGS data is used over the course of an outbreak investigation or during an inspection, what types of conclusions are drawn from the data, what other types of evidence is considered, and what this means for food producers. This session aims to build transparency on how WGS data is used, identify what is needed for food producers to be able to confidently leverage the promise of WGS without fear of retribution, and identify what roles third party laboratories and academia can play. This session will be of interest to food producers, third party labs that service food producers, academics, regulators, and those in public health.

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

Secondary PDG Sponsor: Food Law PDG

Non PDG Sponsor (Organization, Association, etc.): Advanced Molecular Analytics PDG also supports this proposal.

Primary Topic Area: Food Safety Assessment Audit and Inspection

Secondary Topic Area: Food Law

Additional Topic Area (Optional): Advanced Molecular Analytics

Keywords: Genomics,Public Health,Regulations

RT17: LINGERING HAZARDS: CONQUERING THE PERSISTENT THREATS OF LISTERIA AND SALMONELLA IN DELI MEATS

Primary Author:

Carrie Rigdon, Association of Food & Drug Officials

Persons:

Carrie Rigdon, Association of Food & Drug Officials

Shecoya White, Mississippi State University

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

David Nicholas, New York State Department of Health

John Jarosh, United States Department of Agriculture

Sarah Kozak-Weaver, Wegmans

Briana Lorenzo, Food Safety & Quality

Jack Burnett, Vikan

Angela Montalbano, NYS Dept. of Ag & Mkts

Session Type: Roundtable - 1.5 hours

Session Summary: Despite advancements in sanitation, environmental monitoring, and regulatory oversight, the persistent threats of *Listeria monocytogenes* and *Salmonella* in deli meats and charcuterie continue to cause outbreaks. The 2024 listeriosis outbreak linked to ready-to-eat deli meat, which sickened 61 people in 19 states and resulted in 10 deaths, highlights the ongoing challenges in controlling *Listeria*. Similarly, *Salmonella* outbreaks, such as the 2021 Italian-style meats outbreak, demonstrate the persistent risks associated with charcuterie. These events raise critical questions about why preventable incidents continue to occur and what steps are needed to avoid them in the future. This roundtable will gather experts from public health and regulatory agencies, RTE deli meat production, retail grocery delis, and sanitation consulting to explore key barriers to preventing recurring outbreaks. The discussion will prioritize identifying root causes and actionable steps to prevent history from repeating itself. Key topics will include: • Understanding the factors that led to high-profile incidents, such as inadequate sanitation protocols, gaps in environmental monitoring, or missed warning signs; • The role of Food Safety Culture in controlling *Listeria* and *Salmonella*, emphasizing training, consistent sanitation, and accountability; • The need for improved public communication on the risks associated with deli meats and charcuterie, helping consumers make informed choices; and • Collaborative solutions between regulators, industry, and sanitation experts to improve equipment design, sanitation protocols, and environmental controls across the supply chain. By reflecting on past outbreaks and lessons learned, this session aims to provide actionable recommendations for reducing pathogen persistence and preventing the recurrence of outbreaks in deli meats and charcuterie.

Primary PDG Sponsor: Retail and Foodservice PDG

Secondary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Non PDG Sponsor (Organization, Association, etc.): Association of Food and Drug Officials

Primary Topic Area: Retail and Foodservice

Secondary Topic Area: Meat and Poultry Safety and Quality

Additional Topic Area (Optional): Food Hygiene and Sanitation

Keywords: Food Service, Meat and Poultry, Sanitation

RT18: AI IN ACTION: TRANSFORMING FOOD SAFETY WITH SMART DETECTION, AUTOMATION, AND ETHICAL SOLUTIONS

Primary Author:

Jyoti Aryal, Kerry Ingredients and Flavours

Persons:

Jyoti Aryal, Kerry Ingredients and Flavours

Nitin Nitin, Department of Food Science and Technology, University of California, Davis

Martin Wiedmann, Cornell University

Mariam Ellouze, Ferrero

Jeyamkondan Subbiah, University of Arkansas

Kaitlyn Casulli, University of Georgia

Abhinav Mishra, University of Georgia

Surabhi Wason, Kerry Ingredients and Flavors

Session Type: Roundtable - 1.5 hours

Session Summary: Artificial Intelligence (AI) is reshaping the food safety industry, providing advanced tools for detecting, preventing, and managing foodborne risks. AI's real-world applications include predictive analytics for outbreak prevention, automating food safety audits, and enhancing pathogen detection. These innovations can lead to improved food quality and safety across supply chains. However, AI adoption is slowed by limited data sharing, regulatory challenges, and ethical concerns surrounding data privacy, algorithmic transparency, and bias. This roundtable will bring together experts from academia and industry working in food safety and AI technology to discuss how AI can be integrated into food safety systems, the importance of addressing ethical concerns, and the collaborative efforts between industry and government to facilitate commercialization. Key topics will include predictive analytics for outbreak prevention, automation in food safety auditing, and the critical need for data sharing and regulation to optimize AI use. Roundtable attendees will learn about: 1. How AI can predict foodborne outbreaks by analyzing vast datasets, identifying key risk factors, and enabling timely interventions. 2. How AI-powered tools, including machine learning, are automating quality control and food safety audits, enhancing accuracy and precision. 3. AI applications in the detection, identification, and characterization of pathogens. 4. Data sharing, ethical concerns, and regulatory challenges in using AI for food safety, such as data privacy, algorithmic transparency, and accountability for AI-driven decisions.

Primary PDG Sponsor: Modelling and Risk Analysis PDG

Secondary PDG Sponsor: Data Management and Analytics PDG

Primary Topic Area: Microbial Modelling and Risk Analysis

Secondary Topic Area: Data Management and Analytics

Keywords: Data, Modeling, Regulations

RT19: TRUSTED DATA SHARING: COLLECTIVE LEARNING FOR FOOD SAFETY INSIGHTS

Primary Author:

Nathan Anderson, U.S. Food and Drug Administration

Persons:

Nathan Anderson, U.S. Food and Drug Administration

James Doyle, Creme Global

Carrie Rigdon, Association of Food & Drug Officials

De Ann Davis, Western Growers Association

Dr. Shelby Hollenbeck, FMI

Martin Hahn, Hogan Lovells

Saskia van Ruth, University College Dublin

Clare Narrod, USDA

Session Type: Roundtable - 1.5 hours

Session Summary: Data sharing is proving to be a highly effective and efficient tool across the food supply chain, bridging knowledge gaps and providing critical insights into food safety, food fraud, and food integrity. Collaboration between industry, global trade organizations, and regulators is gaining traction, accelerating our collective understanding of key challenges that would be difficult to address using isolated data. The benefits of shared data include optimizing resources, improving productivity, enhancing transparency, building trust, enabling benchmarking, providing early warnings, and informing rulemaking. Additionally, data sharing supports predictive modelling to detect food fraud and protect food integrity. This roundtable will explore business benefits gained from platforms like GreenLink®, the FDA Food Safety Data Analytics Sharing Hub (DASHboard), the Food Industry Intelligence Network (fiin), and contributions to FSIS regulatory analysis. Panelists representing academia, legal, regulatory and global trade organizations will share real-world examples and discuss overcoming barriers, shared value, how the rewards outweigh risks, and how data insights have improved food safety, mitigated supply chain risks, and strengthened fraud prevention efforts. Additionally, speakers will address possible mechanisms to reduce bias in decisions derived from diverse datasets and how data sharing helps address the digital divide.

Primary PDG Sponsor: Data Management and Analytics PDG

Secondary PDG Sponsor: Food Fraud PDG

Primary Topic Area: Data Management and Analytics

Secondary Topic Area: Food Fraud

Keywords: Data, Produce, Seafood

RT20: EDIBLES AND DRINKABLES - FOOD SAFETY EXPLORATIONS AT THE INTERSECTION OF FOOD AND CANNABIS

Primary Author:

Lily Yang, The Acheson Group

Persons:

Lily Yang, The Acheson Group

Steven Gendel, Gendel Food Safety LLC

Shannon McCoy, Organigram Global

Deepa Thiagarajan, Mars

Aaron Pleitner, Impossible Foods

Alex Tudor, Bio-Rad

Session Type: Roundtable - 1.5 hours

Session Summary: As the global cannabis market – both medical and recreational – gains momentum regionally and globally, with projected global growth to \$444B by 2030, there are growing concerns about the safety of cannabis-derived (THC, its psychoactive compounds, and derivatives) and cannabis-infused foods and beverages (for humans, animals, and pets). Manufacturers should implement GMPs, supply chain controls, label controls, and preventive controls to protect all consumers from biological hazards, food poisoning, allergic reactions, and exposure to toxic chemicals. Yet, regulations in the U.S. and internationally are highly inconsistent regarding requiring food safety controls for edibles, and, in many cases, the existing requirements are not adequate. This globally diverse roundtable will include overviews of current food safety practices and regulatory allowances for cannabis-infused edibles/beverages, explore efforts by non-governmental organizations such as ASTM to supplement the regulatory gaps, and encourage/include active audience participation to promote the exchange of experiences, ideas, and learnings.

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

Secondary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Non PDG Sponsor (Organization, Association, etc.): The Acheson Group

Primary Topic Area: Plant-Based Alternative Products

Secondary Topic Area: Food Chemical Hazards and Food Allergy

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

Keywords: Allergens, Animal and Pet Food

Safety, Beverages, Cannabis, Intervention, Low-water Activity Foods, Pathogens, Pet

Food, Plant-based foods, Ready to Eat Foods, Regulations, Risk

Management, Toxicology

RT21: COMBATTING FOOD FRAUD: LEVERAGING INNOVATION, TRACEABILITY, AND AI FOR A SAFER GLOBAL FOOD SUPPLY

Primary Author:

James Doyle, Creme Global

Persons:

James Doyle, Creme Global

Angela Anandappa, Alliance for Advanced Sanitation

Sharmeen Khan, OpsSmart Global

Fernando Avelleyra, Wal-Mart Stores, Inc.

Maryam Blythe, Mars Inc.

Katie Zammit, Cargill

Cronan McNamara, Creme

Vijay Krishna, Glanbia Performance Nutrition

Session Type: Roundtable - 1.5 hours

Session Summary: This session will explore cutting-edge strategies and emerging technologies to address food fraud in today's complex supply chains. As incidents like the fipronil egg contamination and horsemeat scandal demonstrate, food fraud is not only a financial risk but also a critical food safety concern. With food fraud on the rise globally, panelists will discuss how advancements such as DNA metabarcoding, Nuclear Magnetic Resonance (NMR), and isotopic testing are reshaping fraud detection by offering more precise and proactive solutions. The session will consider how pilot programs using isotopic testing to verify the product origin could be a critical model for how businesses can utilize scientific methods to enhance traceability and prevent fraud at the source. Additionally, the use of NMR will be highlighted for its ability to detect adulteration in products where DNA analysis may not be effective, ensuring food safety and authenticity. An example of collaboration between companies will be shared, showcasing how businesses can work together to address food fraud challenges, exchange insights, and explore practical best practices that could contribute to a stronger, more resilient global food supply chain. Beyond technological advancements, the session will emphasize the critical importance of supply chain auditing and the need for regulatory compliance to prevent fraud at multiple stages. A strong collaboration between food businesses, regulatory agencies, and third-party labs is essential for staying ahead of fraud risks. Consumer education will also be explored as an important tool to empower individuals to recognize fraudulent products, thus driving demand for greater transparency and accountability. Attendees will gain actionable insights into how businesses can integrate these technologies, strengthen their fraud prevention strategies, and ultimately usher in the era of Food Fraud 2.0, where predictive intelligence and proactive measures define the future of food safety.

Primary PDG Sponsor: Food Fraud PDG

Secondary PDG Sponsor: Data Management and Analytics PDG

Primary Topic Area: Food Fraud

Secondary Topic Area: Data Management and Analytics

Keywords: Modeling,Risk Analysis,Risk Management

RT22: WHAT DO YOU NEED FROM YOUR VALIDATED MICROBIOLOGICAL METHODS?

Primary Author:

David Legan, Eurofins Scientific Inc.

Persons:

David Legan, Eurofins Scientific Inc.

Arpan Bhaghat, Saputo

Laura Bleichner, Gold Standard Diagnostics

Catharine Carlin, Merieux NutriSciences

Daniele Sohier, Hygiena

William K. Shaw, Jr., USDA FSIS

Session Type: Roundtable - 1.5 hours

Session Summary: Method validation is the key to accurate, reliable, actionable results. Microbiological method validation procedures are detailed in the ISO 16140 series, AOAC International's "Appendix J" and internal guidelines by U.S. FDA, Health Canada, and other bodies. For developers of rapid pathogen screening methods, achieving certification against these standards is a costly but essential commercial requirement. Additionally, laboratories operating under ISO 17025 must use validated methods to maintain their accreditation. Existing validation standards and certification schemes compare novel "candidate" methods against cultural "reference" methods. This built confidence when technologies such as PCR were new and unproven but experience over the past 30 years has shown that many molecular methods are more precise and sensitive than the cultural reference methods. Are method users and end-users of data still being served by current validation standards? AOAC International recognizes that Appendix J does not adequately address rapid confirmation or sequence-based identification methods, and this creates an opportunity for the broad interested community of method users to comment on their needs and preferences. Panelists in this roundtable will bring a broad and deep experience as decision makers, method users and method developers ready to engage with questions such as: • Is it time to abandon the concept of cultural methods as "the gold standard"? • Is it necessary to detect only a single viable cell in a 375 g (or larger) test portion? • Does comparing one rapid method against multiple local reference methods add value? • Is a single, global reference method possible? In not, why not? • Does the market focus on detection speed damage method efficacy? • And ultimately: what do method users expect from validations? This panel may be the first opportunity for all method users and not just the small group of standards setters to voice an opinion.

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

Keywords: Laboratory and Detection Methods, Pathogens, Validation

RT23: A GOOD FIT - LEVERAGING EMP INTO RETAIL AND FOOD SERVICE OPERATIONS

Primary Author:

Jill Hollingsworth, Ecolab

Persons:

Jill Hollingsworth, Ecolab

David Buckley, Diversey, Inc.

Donald Schaffner, Rutgers University

Sarah Morrison, IKEA North America Services

Thomas Ford, Compass Group

Dr. Viktoria Wagner, Ecolab Europe

Natalie Seymour, Ecolab

Michelle Leger, Lunds & Byerlys

Session Type: Roundtable - 1.5 hours

Session Summary: Environmental contamination is an issue that impacts all food facilities, but there are differences in how the environment and sanitation are monitored across the food continuum. An environmental monitoring program (EMP), used by many food manufacturers, can be an essential tool for monitoring and verifying the efficacy of a sanitation program. Further, environmental monitoring can provide critical data regarding facility and equipment performance, corrective action effectiveness, areas of concern, and continuous improvement. However, many food establishments such as food retailers and food service operations believe they lack the tools, knowledge, programs, or resources to implement an EMP. This panel will explore opportunities for using EMP in retail food establishments around the world. They will address some of the most common concerns and provide answers to frequently asked questions about EMP such as: How do you determine if EMP is right for your business? What organisms should you be looking for in the environment – aerobic plate count, indicator organisms, specific bacteria? What actions should you take after receiving the results? How can audit data be used to focus your efforts on what and where to sample? How can the data be organized and analyzed to make it meaningful and effective? This diverse panel will be comprised of experts who will share their perspectives, pain-points, and success stories for how they are using an EMP. Attendees will leave with a better understanding of the options available and how any grocery or food service facility can implement an EMP and leverage microbiological data to improve sanitation and reduce food safety risks.

Primary PDG Sponsor: Retail and Foodservice PDG

Secondary PDG Sponsor: Food Hygiene and Sanitation PDG

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

Primary Topic Area: Food Hygiene and Sanitation

Secondary Topic Area: Retail and Foodservice

Additional Topic Area (Optional): Data Management and Analytics

Keywords: Data,Environmental monitoring,Retail

RT24: MICROBIOME AND METAGENOMIC DATA ARE CHEAP AND DETAILED: WHAT NOW?

Primary Author:

David Legan, Eurofins Scientific Inc.

Persons:

David Legan, Eurofins Scientific Inc.

M. Laura Rolon, California Polytechnic State University

John Donaghy, Nestlé SPN.

Isabel Walls, USDA-FSIS

Michele Sayles, Diamond Pet Food

Joelle Mosso, Western Growers Association

Jerome Combrisson, MARS

Karen Jarvis, U.S. Food and Drug Administration

Session Type: Roundtable - 1.5 hours

Session Summary: It is now easy and (relatively) cheap to generate microbiome or metagenomic data for an agricultural environment, food processing operation, or food product. Researchers have shown both stable and transient components of the microbiome of individual food processing facilities. The ability of sanitation to temporarily modify processing facilities' microbiomes has been documented and transfer of microbes from the facility to the product has been demonstrated. Modifying the food product microbiome to retard or modify spoilage by adding "good bacteria" has grown from traditional fermentation processes to a deliberate strategy. However, beyond limited applications in spoilage diagnosis, the food industry seems to struggle with applying metagenomic approaches in their operations or does not find value in generating microbiome data. Does this reflect a lack of imagination, a concern about inadvertent pathogen detection, or something else? What is the industry missing? Should microbiome analysis be integrated into routine environmental monitoring programs? Or how, otherwise, can this technology be applied proactively to improve food safety? Panelists will bring their diverse viewpoints and discuss the path forward for this technology.

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): Data Management and Analytics

Keywords: Environmental monitoring, Microbiome, Risk Management

Technical Abstracts

T1-01: DECODING THE SURVEILLANCE PYRAMID: LABORATORY TESTING FOR ACUTE GASTROENTERITIS

Primary Author:

Alexandra Edmundson, University of Minnesota

Persons:

Alexandra Edmundson, University of Minnesota

Craig Hedberg, UMN School of Public Health

Melanie Firestone, University of Minnesota School of Public Health

Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Clinical laboratory tests for enteric disease form the basis of public health surveillance and outbreak detection, but only a fraction of patients with acute gastroenteritis submit specimens for testing.

Purpose: The objective of this study is to characterize the sociodemographic factors associated with laboratory test orders placed for patients with acute gastroenteritis.

Methods: Medical records from encounters of patients with acute gastroenteritis within one Minnesota-based healthcare system from 2017–2023 were reviewed. Encounters were included if they met any of the following criteria: diagnoses of enteric diseases or diarrhea of unknown cause, patient complaints of diarrhea, or orders of laboratory tests for enteric pathogens. Patients were excluded if they had diagnosed chronic conditions that cause diarrhea. Encounters per patient were grouped into illness events, resulting in 254,629 illness events in this sample. A log binomial regression model was used to calculate relative risks of orders placed across racial/ethnic groups, adjusting for age, sex, insurance coverage, and first encounter type.

Results: Of the 254,629 cases of acute gastroenteritis, 24.5% (n=62,282) had orders placed for enteric pathogen laboratory tests. Across all encounter types, Non-Hispanic (NH) White patients had orders placed 26.1% (47,679/182,551) of the time, which was higher than any other group. After adjusting for age, sex, insurance coverage, area-level poverty, and first encounter type, NH Asian, NH Black or African American, and Hispanic patients were significantly less likely (20%, 14%, and 5% respectively) to have laboratory test orders placed than NH White patients.

Significance: Laboratory test ordering practices directly affect public health surveillance and outbreak detection. When certain demographic groups are less likely to be tested for reportable enteric pathogens, it makes identifying and controlling outbreaks of foodborne disease more difficult within these communities.

Award Application: Developing Scientist Award

T1-02: GLOBAL FOODBORNE OUTBREAKS ASSOCIATED WITH NUTS AND NUT PRODUCTS FROM 2000 TO 2024

Primary Author:

Joanna Rothwell, University of California Davis

Persons:

Joanna Rothwell, University of California Davis

Linda J. Harris, University of California-Davis

Alda Pires, Dept. Population Health & Reproduction, UC Davis School of Veterinary Medicine

Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Consumption of contaminated nuts and nut products has led to substantial foodborne disease outbreaks globally, causing illnesses, hospitalizations, and fatalities, while also damaging product reputation and agricultural trade.

Purpose: Outbreaks of foodborne illness associated with contaminated nuts and nut products from 2000 to 2024 were reviewed to identify pathogens, affected products, illness outcomes, geographical distribution, and contributing factors.

Methods: Outbreaks (evidence linking two or more cases to the consumption of a nut or nut product as the principal ingredient) were identified through a literature review of databases, including Google Scholar, PubMed, and Web of Science, using search strings related to foodborne illnesses and nuts from January 2000 to December 2024. Outbreak data were also obtained from the Publicly Available International Foodborne Outbreak Database and the National Outbreak Reporting System. Outbreaks were analyzed using descriptive statistics by nut type, product category, etiological agent, and temporal trends, with geographic distributions mapped.

Results: A total of 42 outbreaks across 16 countries were identified, affecting over 2,900 individuals with ~500 hospitalizations (17%). *Salmonella* was the most frequently implicated pathogen (83%, n=35), followed by *Escherichia coli* O157:H7 (9%, n=4). Outbreaks were more commonly associated with peanuts (21%, n=9) compared to other nut types including cashews (17%, n=7), mixed nuts (14%, n=6), and almonds (14%, n=6). Peanuts were also linked to the highest number of cases (62%, n=1,816) followed by almonds (10%, n=286). While outbreaks were most commonly associated with whole nuts, those with nut butters and flavored/salted nuts occurred periodically, and those involving nut-based dairy analogs, particularly cashew-based products, were more prevalent in recent years.

Significance: This study highlights the importance of international collaboration,

investment in outbreak investigation resources, strengthening food safety standards, and ensuring adherence to good processing and environmental control practices to help reduce the incidence and severity of future outbreaks.

T1-03: HARNESSING THE POWER OF 10 MEV ELECTRON BEAM TECHNOLOGY TO REDUCE ALLERGEN LEVELS IN PEANUTS

Primary Author:

Tonali Lara-Ramos, Texas A&M University

Persons:

Tonali Lara-Ramos, Texas A&M University

Suresh Pillai, Texas A&M University

Chandni Praveen, Texas A&M University

Presentation type/Format: Technical

Category: Food Allergens

Abstract:

Introduction: Peanut (*Arachis hypogaea*) allergy is one of the most prevalent and serious food allergies; despite this, there is no established processing method that can reduce the sensitization of peanut protein.

Purpose: This study aimed to evaluate the effects of 10 MeV Electron Beam on the reduction or elimination of peanut allergens.

Methods

: 10 Grams of each peanut sample were prepared under six different irradiation conditions: dry with shell, dry without shell, immersed in 15 mL of water, and immersed in 15 mL of 20 mM buffer solutions at pH 4.5, 6.5, and 8.5. Each condition was tested on three different peanut forms: powder, crushed, and whole. The samples were irradiated at five doses, including a control (0, 5, 10, 15, and 20 kGy), in triplicate for each combination. A peanut lateral-flow device was used to detect peanut proteins. 0.2 Grams of peanut samples were suspended in an extraction buffer and then transferred to a vial containing a biotinylated antibody. During incubation, if peanut proteins are present, the antibody-gold complex binds to immobilized antibodies at the test line, resulting in a visible red line.

Results: Among all the tests conducted: dry samples in the three forms: whole, crushed, and powdered treated at doses greater than 10 kGy consistently fell below the detection limit (LOD) of the test: 2.5 ppm, resulting in negative test outcomes. Falling below this LOD suggests a significant reduction in the presence of peanut allergens, making these peanuts potentially safer for allergic individuals.

Significance: Peanut consumption in sensitized individuals can trigger severe allergic reactions, including anaphylactic shock. As it rapidly emerges as a major global medical concern, decreasing peanut allergenicity is a critical food safety priority.

Award Application: Developing Scientist Award

T1-04: POTENTIAL OF GUAR GUM IN ACTIVE PACKAGING OF FRUITS AND CONFECTIONERY

Primary Author:

Rowaida Khalil, Faculty of Science, Alexandria University

Persons:

Rowaida Khalil, Faculty of Science, Alexandria University

Presentation type/Format: Technical

Category: Packaging

Abstract:

Introduction: Guar gum (GG); an edible water soluble polysaccharide, has emerged as a prominent thickener for many food applications due to excellent physicochemical properties and cost-effectiveness, however, the high water solubility (WS) of GG-based films limits their application in food packaging. Extracts of propolis (PE); a natural resinous material collected from plant buds and exudates by honey bees, has low water solubility and strong flavor/aroma restraining its direct use as a natural food preservative.

Purpose: This study aimed to develop and evaluate novel edible coating and film prototypes made from guar gum (GG) and encapsulated propolis extract (PE), to improve the barrier properties, shelf-life, and microbial safety of both high- and low-moisture food products, such as fresh-cut kiwi and chocolate chips.

Methods: PE encapsulated using gum arabic (GA; 6%w/v) was incorporated into GG coating (1.5% w/v) and films (2% w/v) which were reinforced with microcrystalline cellulose and montmorillonite and plasticized with glycerol. Physicochemical/antimicrobial/antioxidant/thermal/mechanical/structural characterizations of coatings/films were followed by their application to preserve fresh-cut kiwi and milk chocolate chips that were assessed for quality parameters upon storage at 5°C for 8 days and room temperature for 16 weeks, respectively.

Results: GA-PE nanocapsules significantly improved viscosity, antimicrobial/antioxidant capacity of the GG hydrocolloid, substantially inhibited the growth of *Rhodotorula muscilaginosa* (an indigenous spoilage yeast) by 2.0 log units, slowed down moisture loss (8.23%), and pH/TSS change in kiwi fruit after 8 days of chilled storage. The novel active GG-based film displayed an enhanced water vapor barrier ($5.05 \times 10^{-11} \text{ g.m}^{-1}.\text{s}^{-1}.\text{Pa}^{-1}$), WS (45.40%), moisture (8.80%), tensile (18.89 MPa), elasticity (54.20%), hydrophobicity (WCA= 67.04°), and UV-blocking (99.50%). The novel film applied as a single-layer overwrap prevented weight loss (0.70%), firmness loss (< 3.0 N), lipid oxidation (1.33 meq O₂/Kg oil), and a_w (0.410) change of chocolate chips for more than 90 days at room temperature.

Significance: This is the first study on the fabrication of two packaging prototypes; active edible coatings and films, prepared entirely from GG and encapsulated PE,

designed to keep the freshness/visual appeal of high and low moisture food commodities, demonstrate high-barrier properties, and safety, in addition to its low cost and minimal environmental impact upon disposal.

T1-05: INACTIVATION OF MULTIDRUG-RESISTANT SALMONELLA ENTERICA IN FOODS USING TiO₂-TCPP INCORPORATED CELLULOSE NANOFIBRIL-BASED PHOTOCATALYTIC PACKAGING

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Presentation type/Format: Technical

Category: Packaging

Abstract:

Introduction: *Salmonella enterica* is estimated to cause 1.2 million foodborne illnesses in the United States annually. Exposure to antibiotics in therapeutical and environmental practices drives a surge of multidrug-resistant (MDR) strains, increasing the prevalence of MDR *S. enterica* infections. This calls for alternative strategies to prevent contamination and inactivate MDR *S. enterica* in foods.

Purpose: Evaluate a novel antibacterial packaging material incorporating photocatalytic conjugates into cellulose nanofibrils that generate reactive oxygen species (ROS) under visible light to inactivate MDR *Salmonella* on food surfaces.

Methods: Packaging material was developed by incorporating TiO₂-tetra(4-carboxyphenyl) porphyrin (TcPP) conjugates into cellulose nanofibrils. Generated ROS under different light intensities were qualified by electron paramagnetic resonance (EPR). log CFU reduction under different light intensities at 4°C (n=3) was determined by submerging film in liquid MDR *S. enterica* serovar Typhimurium BAA-190. The antimicrobial properties of films were assessed on *S. enterica*-inoculated mango slices and cooked chicken breast under 6000 lux light intensity for 48 hours.

Results: TcPP-TiO₂ conjugates expanded the responsive light wavelength of TiO₂ from the UV to the visible spectrum, resulting in ROS production. Under 6000 lux light, the film caused a 4.2 ± 0.6 log reduction in *S. enterica* after 72 hours

compared to the control ($p < 0.05$). The antibacterial efficacy decreased as the light intensity decreased with only a 1.8 ± 0.03 log reduction in *S. enterica* under 1000 lux light ($p < 0.05$). on mango slices, a 3 ± 0.07 log reduction ($p < 0.05$) in *S. enterica* occurred under 6000 lux within 48 hours. However, the film showed no significant antibacterial effect on inoculated chicken breast.

Significance: The newly developed photocatalytic packaging film effectively inactivates *S. enterica* under low-intensity visible light by generating ROS. This innovative solution offers a promising approach to combating MDR bacteria in the food industry.

Award Application: Developing Scientist Award

T1-06: CARBON DOTS FROM RHIZOME PEELS FOR MULTIFUNCTIONAL FOOD SAFETY TECHNOLOGIES: ACTIVE PACKAGING INDICATORS, ANTI-COUNTERFEITING SOLUTIONS, AND FUNCTIONAL COMPOUNDS

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Presentation type/Format: Technical

Category: Packaging

Abstract:

Introduction: Carbon dots (CDs) are eco-friendly nanomaterials with exceptional optical properties, versatile surface chemistry, and minimal cytotoxicity, making them promising for advanced food packaging applications.

Purpose: This study aimed to develop smart packaging, anti-counterfeiting, and functional materials using CDs derived from agro-waste.

Methods: CDs were sustainably synthesized from rhizome peels of ginger, galangal, and turmeric using a one-step hydrothermal process. The CDs, along with black plum anthocyanins (BPA), were incorporated into a chitosan (CS)/fish gelatin (FG) matrix to create a smart packaging system for Asian seabass. Antioxidant activities were evaluated using DPPH and ABTS assays, while antimicrobial efficacy was tested against *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, and *Escherichia coli*. The mechanical, UV-barrier, and pH-responsive properties of the films were also analyzed, alongside their ability to preserve seafood quality during refrigerated storage.

Results: The CDs exhibited strong antioxidant activity and potent antimicrobial effects against all tested pathogens. Incorporation of CDs (1, 3, and 5 wt%) significantly improved the mechanical properties of the CS/FG films, including tensile strength and flexibility ($P < 0.05$). Films with BPA and 5% CDs demonstrated superior UV-barrier, antioxidant, and antimicrobial properties. These films also exhibited distinct pH-responsive color changes (pH 2–12), confirming their suitability for intelligent packaging. Additionally, the films featured anti-counterfeiting properties with a fluorescent code under UV light. When used to package Asian seabass, the films effectively reduced bacterial growth and preserved the fish's color and quality during storage at 4 °C for 16 days.

Significance: This study highlights the potential of CS/FG-based films containing CDs and BPA as sustainable active and intelligent packaging solutions, offering enhanced food safety by mitigating oxidation and microbial spoilage. By leveraging agro-waste-derived CDs and natural compounds, this approach promotes eco-friendly and cost-effective food packaging technologies, contributing to public health and environmental sustainability.

Award Application: Developing Scientist Award

T2-01: EVALUATING SANITIZER EFFICACY AGAINST SALMONELLA IN LOW-MOISTURE FOODS AND VALIDATION OF SURROGATES FOR EFFECTIVE SANITATION

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Presentation type/Format: Technical

Category: Low-Water Activity Foods

Abstract:

Introduction: Persistent bacterial populations (PBPs) in low-moisture food (LMF) environments, particularly *Salmonella* spp., can pose significant food safety risks since traditional cleaning and sanitizing methods may not be suitable or effective. Understanding sanitizer's efficacy and limitations in these contexts is critical for developing tailored sanitation and improving food safety.

Purpose: Three sanitation protocols were evaluated to inactivate LMF PBPs and to identify suitable surrogate.

Methods: LMF PBPs of *Salmonella enterica* or *Enterococcus faecium* were formed on stainless steel coupons with a food matrix (non-fat dry milk powder or peanut butter powder) in a ratio of 0.5 mL to 0.4 g and subjected to storage (one or four days) in controlled relative humidity (30 or 70%). Coupons were cleaned by scraping and/or sanitized with treatment of 62% isopropyl alcohol (IPA; 1 min), aqueous quaternary ammonium compound (QUAT; 10 min), or thermal intervention (115–135°C). Following treatment (no-treatment, clean, sanitize, clean+sanitize), coupons were sampled by submerging in chilled peptone or neutralizing broth for 15 minutes before diluting and plating. Pre- and post-cleaned and sanitized populations were enumerated and compared with ANOVA and Tukey's HSD to determine significant differences.

Results: The IPA treatment achieved a 1.5-log reduction of *Salmonella* without prior cleaning, which significantly increased to an 8.3-log reduction when the surface was cleaned first ($P < 0.05$). The thermal treatment of 135°C for 30 minutes achieved an 8-log reduction when cleaning was performed, which was significantly higher than when not pre-cleaned (5.2 log CFU) ($P < 0.05$). QUAT

demonstrated lower efficacy than both IPA and thermal treatments (1.3-log reduction without cleaning and a 4.5-log reduction when cleaned and sanitized)

Significance: Traditional sanitation strategies were less effective against LMF PBPs, highlighting the need for tailored approaches. The findings show success with IPA or thermal treatments to sanitize surfaces, and the importance of thorough cleaning prior to sanitation protocols.

Award Application: Developing Scientist Award

T2-02: PILOT-SCALE VALIDATION OF STEAM PRE-TREATMENT AND TWO-STAGE DRYING FOR ENHANCED SALMONELLA CONTROL IN APPLE DRYING

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Presentation type/Format: Technical

Category: Low-Water Activity Foods

Abstract:

Introduction: Research has shown that increasing humidity during apple drying improved pathogen inactivation; however, there are insufficient resources for processors to optimize their apple drying preventative controls to comply with the Food Safety Modernization Act.

Purpose: This study was designed to evaluate the efficacy of steam pre-treatment and two-stage drying on *Salmonella* inactivation during apple drying in a pilot-scale impingement oven.

Methods: Apples (cv. Gala) were rinsed, sliced (4-mm thick, 18.66 ± 1.33 g/slice), and inoculated with a 6-strain *Salmonella* cocktail (9.5 ± 0.2 log CFU/g). Inoculated slices were steam-treated under three conditions (dew points of 55, 65, and 75°C) for 1, 2, 3, and 4 min (3 replications). Two-stage drying was conducted in triplicate using 65°C dew point, 2 min pretreatment, followed by hot-air convective drying in a pilot-scale impingement oven (1–2 h) at 80, 110, or 140°C and 20, 50, or 80% fan speed (dew point ~24°C). Sample temperature, browning index (BI), water activity (aw), and moisture content (MC) were measured, and survivors were enumerated on a differential/non-selective medium (37°C, 48 h). ANOVA, Tukey's multiple comparison test, and t-tests ($\alpha = 0.05$) were used to evaluate treatment effects and compare survival data.

Results: Increasing humidity and steam pre-treatment duration resulted in higher *Salmonella* inactivation ($P < 0.05$) with no change in aw or MC ($P > 0.05$). No *Salmonella* reductions were observed after 55°C dew point pre-treatment ($P > 0.05$); however, 2 min of steam treatment at 65 and 75°C dew points resulted in 4.4 ± 0.6 and 8.4 ± 0.7 log reductions, respectively. Following a 65°C dew point, 2 min pre-treatment, drying apples at 80°C and 20% fan speed (2 h) yielded an overall >5 log *Salmonella* reduction ($P < 0.05$), in which the aw (0.186 ± 0.008), MC ($7.47 \pm 0.78\%$ wb), and BI (39.1 ± 4.0) were similar to commercial products ($P >$

0.05).

Significance: Steam pre-treatment can enhance pathogen control in pilot-scale two-stage apple drying while maintaining product quality.

Award Application: Developing Scientist Award

T2-03: THERMAL INACTIVATION PARAMETERS FOR SALMONELLA IN LIPID-BASED NUTRIENT SUPPLEMENTS

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Presentation type/Format: Technical

Category: Low-Water Activity Foods

Abstract:

Introduction: *Salmonella* contamination in lipid-based nutrient supplements (LNS) poses a significant health risk to vulnerable populations such as malnourished individuals, young children, or people in humanitarian settings. Thermal inactivation is a critical intervention for mitigating this hazard. This study evaluates the thermal resistance of *Salmonella* in three distinct LNS matrices under various thermal processing conditions.

Purpose: Determining D- and Z-values for *Salmonella* in three LNS matrices to inform thermal processing parameters and guidelines for enhanced food safety.

Methods: Eight *Salmonella* strains were cultured, centrifuged, normalized by cell density (OD600), then resuspended as an oil-base cocktail (canola oil). The inoculum cocktail was used to inoculate three LNS formulations: peanut butter-based, chickpea-based, and a modified peanut butter-based product with increased sugar and oil. Samples allowed to adapt at room temperature for at least a week. Adapted samples were treated in water and oil baths at temperatures ranging from 87 °C (30 s – 20 min) to 115 °C (5 s – 30 s). Reduction of *Salmonella* was quantified by spread plating on Tryptic Soy Agar (TSA) with Xylose-Lysine-Deoxycholate (XLD) overlay. D- and Z-values were determined using linear regression (first-order kinetics).

Results: Thermal inactivation results varied depending on product matrix and treatment medium. The linearity of the D- and Z-values was calculated and assessed for each individual treatment and their combination ($R^2 > 0.95$). D-values ranged from 388.86 s at 87 °C (water bath) to 9.65 s at 115 °C (oil bath). Calculated Z-values were 17.83 °C (peanut butter-based), 23.44 °C (chickpea-based), and 19.50 °C (modified peanut butter-based).

Significance: These findings provide essential thermal processing parameters for

mitigating *Salmonella* risks in LNS. The results support the development of tailored thermal inactivation guidelines for diverse LNS formulations, depending on specific inactivation requirements.

Award Application: Developing Scientist Award

T2-04: THE EFFECT OF TEMPERATURE, BED DEPTH, AND AIR VELOCITY FOR THE PREDICTION OF SALMONELLA INACTIVATION DURING HOT-AIR APPLE DRYING

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Presentation type/Format: Technical

Category: Low-Water Activity Foods

Abstract:

Introduction: *Salmonella* has been linked to dried fruits, including dried apples. Most fruit drying processes were designed and implemented to achieve quality outcomes, not Food Safety and Modernization Act requirements that came later. Therefore, the effect of drying process parameters on pathogen inactivation is generally lacking.

Purpose: The purpose of this study is to predict *Salmonella* inactivation at specific apple water activity (a_w) levels and to explore the effect of temperature (T), bed depth (B), and air velocity (A) on the predicted inactivation.

Methods: *Salmonella* cocktails were harvested from lawn culture and inoculated into fresh-cut apple cubes. A single layer of inoculated apple cubes was placed atop uninoculated cubes in a drying chamber to achieve bed depths of 5.1, 8.9, or 12.7 cm. Apple cubes were dried at 88, 104, or 120°C with air velocities of 2.10(25%), 2.95(37.5%), or 3.82(50%) m/s. A Box-Behnken Design was utilized for selecting drying conditions ($n=15$). At multiple time points ($n \geq 5$), samples were collected from the top layer for a_w measurement and *Salmonella* enumeration. Three independent trials were completed.

Results: *Salmonella* inactivation during drying was normalized (initial population = 9.39 ± 0.30 log CFU/4 cubes). A strong linear correlation ($R^2 > 0.86$) was found between *Salmonella* inactivation and apple a_w in all conditions. The linear regression model predicted *Salmonella* inactivation in 0.6 a_w apples to range from 2.25 ± 0.11 to 4.97 ± 0.21 log CFU/4 cubes. RSM analysis showed significance ($p < 0.05$) in the linear effect of T, B, and A, the quadratic effect of A, and the interaction between B and A on the predicted *Salmonella* inactivation. The adjusted R^2 of 0.99 indicated a good RSM fit.

Significance: Apple drying might not always achieve a 5-log reduction. Apple a_w

may be an indicator of process-specific lethality. The RSM results could aid in optimizing the drying process for improved lethality.

Award Application: Developing Scientist Award

T2-05: ASSESSMENT OF AQUACULTURE PRACTICES ON OYSTER AND WATER MICROBIOMES USING SHOTGUN METAGENOMICS IN THE DELMARVA PENINSULA

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Presentation type/Format: Technical

Category: Seafood

Abstract:

Introduction: Oyster aquaculture provides a sustainable and reliable food supply. Limited information is available regarding the effects of aquaculture practices on oyster and water microbiomes in the Delmarva Peninsula.

Purpose: This study aimed to evaluate the effects of aquaculture practices (bottom and floating cages) on oyster and water microbiomes.

Methods: Eastern oysters (*Crassostrea virginica*) [Floating (n=75), Bottom (n=75)] and water [Floating (n=76), Bottom (n=76)] samples were collected from the Chesapeake Bay [Tangier Sound (TA), Honga River (HR)], and Delaware inland Bays (DE) between April and October of 2022 and 2023. DNA was extracted from oyster homogenate (n=6) and 500 mL of water per practice, followed by shotgun metagenomic sequencing using an Illumina NextSeq 2000. Taxonomic profiling was performed using the EzBioCloud platform. Statistical significance of alpha and beta diversity was tested using Wilcoxon Rank Sum test and PERMANOVA on Bray-Curtis dissimilarity, respectively.

Results: Shotgun metagenomic analysis revealed that Bacteria (81.8%) predominated in the community, followed by viruses (18.2%), archaea (0.01%) and fungi (0.0008%). Alpha and beta diversity indices revealed a distinct and significantly higher microbial diversity in water microbiomes compared to oyster microbiomes ($p < 0.05$). Floating oysters had comparatively higher relative abundances of *Vibrio* (2.56%) and *Shewanella* (0.8%) compared to bottom oysters, which had 0.04% and 0%, respectively. The predominant phyla in oysters were Cyanobacteria (60.67%), followed by Phixviricota (26.19%), Uroviricota (7.05%), and Proteobacteria (3.57%), while water microbiomes were dominant by Proteobacteria (38.68%), Cyanobacteria (33.48%), and Actinobacteria (12.39%). DE microbiomes exhibited diverse communities, with higher *Vibrio* in oysters,

while HR showed higher *Vibrio* in water. Foodborne pathogens such as *Salmonella*, *Listeria*, *Clostridium*, and *Escherichia* were not detected, however, various bacteriophages, including *Vibrio* phages, were observed.

Significance: This study revealed site- and aquaculture practice-specific microbial variations, with higher *Vibrio* abundance in floating oysters. These findings provide insights into microbiome dynamics to enhance seafood safety and public health.

T2-06: EVALUATION OF THE THERMAL TOLERANCE MEDIATED BY CROSS-PROTECTION FROM THE CHASRI IN *SALMONELLA*

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Presentation type/Format: Technical

Category: Low-Water Activity Foods

Abstract:

Introduction: *Salmonella enterica* Senftenberg strain CFSAN045763 has been implicated in multiple pistachio-associated outbreaks. Genomic analysis reveals it contains the Copper Homeostasis and Silver Resistance Island (CHASRI), which confers resistance to copper and silver. The role of CHASRI in *Salmonella*'s stress response requires further investigation.

Purpose: This study determines if the CHASRI in this strain confers thermal tolerance in inoculated pistachios.

Methods: A CHASRI knockout strain was constructed using a scarless red recombinase method and confirmed by PacBio sequencing. Growth kinetics of the wildtype and knockout strains were assessed. Pistachios were inoculated (~8 log CFU/g) with the strains in four replicates and stored at 40% relative humidity for 3 days prior to thermal treatment. Two technical replicates of 20 g were heated in an oven set to 118°C for up to 180 minutes, and survivors were determined periodically by direct plating on mTSAYE. Inactivation data were fitted into a Weibull model and α and β parameters were statistically analyzed using ANOVA.

Results: Long-read sequencing confirmed the deletion of the CHASRI without introduction of any indels. The growth kinetics of the two strains were not significantly different ($P > 0.05$). There was no difference in the reduction after desiccation (~1 log CFU/g) between the wildtype and knockout strains, indicating similar levels of desiccation tolerance. The wildtype strain survived the thermal treatment for the entirety of the testing time (180 minutes), with an average reduction of 3.9 log CFU/g. The log reductions at 60 and 180 minutes were significantly different between the two strains ($p < 0.05$), with the wildtype being more thermally tolerant. The time to 5-log reduction based on the alpha/beta parameters was significantly different between the two strains ($p = 0.04$), with an

average of 230 minutes for wildtype and 159 minutes for knockout.

Significance: This is the first study that illustrates cross-protection from the CHASRI towards thermal inactivation.

Award Application: Developing Scientist Award

T3-01: THE CHALLENGE OF CROWDSOURCING A FOODBORNE ILLNESS OUTBREAK

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: User-generated data and artificial intelligence may be useful tools to rapidly identify outbreak signals, but proven regulatory confirmation models should be used to ensure that a product is not misattributed as the source of the outbreak.

Purpose: This mixed-method research assessed if and why someone would self-identify with a publicized foodborne outbreak.

Methods: The qualitative research used a comparative case study of three cereal incidents: the 2018 Honey Smacks *Salmonella* recall, the 2022 Lucky Charms incident, and the 2023/2024 Quaker Oats *Salmonella* recall. The case analysis included interviews and a review of regulatory documents and media publications. The quantitative research was a discrete choice experiment using a publicized foodborne illness vignette survey. Each scenario included four attributes: the number of people to assess social influence, symptoms to evaluate symptom attribution or nocebo effect, FDA to assess authoritative figure impact, and a call to action to determine the effect of creating urgency.

Results : The qualitative research found that the Lucky Charms incident was discovered by user-generated data amplified by media reports before confirming the outbreak. investigators found no link between consumption of Lucky Charms and illnesses. The quantitative research, with 1036 participants, found that all four attributes are positively associated with a person's willingness to self-identify with a publicized foodborne illness. Reporting a large number of illnesses (OR = 2.42, CI [0.77, 1.00], $p < 0.001$) and including symptoms (OR = 2.21, CI [0.70, 0.88], $p < 0.001$) in the publicized headlines more than doubles the odds that someone will self-identify as ill from an outbreak of foodborne illness.

Significance: Results from this research can directly inform how public health campaigns and media strategies are designed, provide insight to improve regulatory policies to protect consumers and food organizations, and highlight regulatory investment needed to improve current foodborne illness outbreak management.

T3-02: RISK-BASED APPROACH TO PRIORITIZE CONTROL OF SALMONELLA SEROVARS IN FOODS

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Interest in *Salmonella* regulations focusing on serovar virulence in animal products calls for robust risk-based methods to identify target serovars with the highest public health impact.

Purpose: To identify higher virulence (HV) *Salmonella* serovars using a risk-based probabilistic method that incorporates exposure from foods and attributed illnesses while accounting for statistical uncertainty in data sources.

Methods: We identified candidate HV serovars associated with beef and chicken products if they were: 1) >0.5% of weighted *Salmonella*-positive regulatory samples and 2) attributed to outbreak illnesses between 2015-2021. For each commodity, we used hierarchical Bayesian models to compute a risk multiplier (RM) based on each serovar's attributable disease incidence relative to frequency in product. Serovars were ranked based on the RM, and the confidence in ranks was estimated using a probabilistic ranking. A RM >1 indicates a serovar is, on average, riskier than other serovars, while a rank of 1 represents full certainty in a serovar having the highest RM. Final HV serovars from beef and chicken were selected if the RM >1 and confidence in ranking >50%.

Results: Of serovars both in product and attributed to illnesses (n=22), 15 were present in >0.5% of *Salmonella*-positive samples (chicken = 5,882, beef = 741). We identified five HV serovars from chicken and beef: *S. Typhimurium*, *Enteritidis*, *Monophasic*, *Newport*, and *Braenderup*. *S. Newport* had 6.2 (95% CrI: 4.16–9.00; confidence = 0.96) times more risk than the average serovars in beef, followed by *S. Enteritidis* (RM = 4.5, 1.54–13.70; 0.79), *Typhimurium* (3.8, 2.36–5.76; 0.76), and *Monophasic* (2.1, 0.98–4.32; 0.50). In chicken, *S. Monophasic* was the riskiest serovar (13.4, 9.64–19.14; 1.00), followed by *S. Braenderup* (2.9, 1.85–4.70; 0.82) and *Enteritidis* (2.0, 1.82–2.18; 0.63).

Significance: Our risk-based approach identifies HV serovars by considering both

presence in food and attributable illnesses. This enables interventions to efficiently target serovar virulence and effectively reduce salmonellosis.

T3-03: MACHINE LEARNING IDENTIFICATION OF GENETIC VIRULENCE PROFILES OF HIGHER VIRULENCE SEROVARS OF NON-TYPHOIDAL SALMONELLA ENTERICA SUBSPECIES ENTERICA

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: The varied epidemiological virulence of *Salmonella* serovars can be partially explained by genetic differences, but the underlying virulence factor gene (VF) profiles driving these differences are understudied.

Purpose: To identify VFs and VF categories that differentiate higher virulence (HV) serovars previously identified based on epidemiological criteria (*S. Typhimurium*, *S. Enteritidis*, *S. Monophasic*, *S. Newport*, and *S. Braenderup*) from other lower virulence (LV) serovars.

Methods: We used protein clustering to screen 64,284 *Salmonella* isolates from US sources (human (n=30,442), chicken (24,552) and beef (9,290)) for the presence of 33,007 VFs from eight categories: 'nutrition', 'effector delivery system', 'exotoxin', 'motility', 'regulation', 'stress response', 'antimicrobial resistance' and 'other'. We trained and validated a supervised random forest (SRF) to identify serovars in HV and LV groups based on VF carriage, and identified VFs and VF categories differentiating virulence groups based on mean decrease in Gini impurity.

Results: of the initial 33,007 VFs, 350 were informative enough to include in the SRF, i.e., present in between 100 and 61,070 (95%) isolates. The 98% (2.06% out-of-bag error) accuracy of the SRF indicated that the VFs were highly predictive of HV serovars. 'Exotoxins' and 'effector delivery system' VFs were the most strongly associated categories. 'Exotoxins' comprised six of the fifteen top-ranked individual VFs. The importance of 'exotoxins' varied, indicating some 'exotoxins' may disproportionately impact virulence. in contrast, all 'effector delivery system' VFs had similar importances, suggesting similar roles in virulence. intriguingly, 'antimicrobial resistance' was found to be the least associated VF category, suggesting a limited impact on food-attributed disease incidence.

Significance: HV serovars encode an identifiable genetic profile that distinguishes them from LV serovars. 'Exotoxin' and 'Effector delivery system' VFs stand out as categories for future *Salmonella* surveillance approaches that consider genetic virulence, rather than serovar alone.

T3-04: INVESTIGATION OF A MULTI-YEAR LISTERIA MONOCYTOGENES OUTBREAK LINKED TO COTIJA AND QUESO FRESCO-STYLE CHEESES

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: in 2018, 2021, and 2024, FDA, CDC, and state/local partners investigated a multi-year *Listeria monocytogenes* outbreak linked to cotija and queso fresco cheeses, resulting in 26 ill people, 23 hospitalizations, two deaths, four pregnancy-associated illnesses, and one fetal loss.

Purpose: To review the multi-year investigation efforts to identify the potential source of the illnesses and contributing factors to contamination at the production facility.

Methods: A case was defined as a laboratory-confirmed infection of *L. monocytogenes* with an isolate related by wgMLST within 0-21 alleles collected between June 15, 2014 and December 10, 2023. Ill people or their proxies were interviewed with the standard Listeria initiative case report form. Environmental and product sampling for *L. monocytogenes* was conducted following guidance from FDA BAM. All isolates were sequenced and uploaded to PulseNet for analysis. FDA conducted traceback analysis and a full-scope Preventive Controls inspection at a manufacturer of interest (Manufacturer A).

Results: of the 22 people interviewed, 16 (73%) reported eating queso fresco, cotija, or similar cheeses. Among people who remembered specific brands, four reported eating brands made by Manufacturer A. FDA and state partners conducted traceback investigations for queso fresco-style cheese associated with

illnesses. Routine sampling conducted by Hawaii in 2024 detected a match to the outbreak strain in cotija produced by Manufacturer A. An FDA inspection initiated at Manufacturer A in 2024 noted sanitation issues; the outbreak strain was also detected in the production environment. Manufacturer A recalled all dairy products, and a consent decree was entered in federal court against the firm.

Significance: This outbreak highlights how continued investigational work can detect a source of contamination and illnesses. investigators were able to identify a potential food source based on interviews, but sampling of foods at retail and traceback were the key to identifying the ultimate source of illnesses.

T3-05: VIBRIO VULNIFICUS EPIDEMIOLOGY & PREDICTORS OF MORTALITY, 2000-2022

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: *Vibrio vulnificus* (V.v.) can cause life-threatening infection through foodborne and waterborne routes, but risk factors for mortality are not fully described.

Purpose: To inform prevention efforts, we aimed to characterize V.v. epidemiology and identify correlates of mortality.

Methods: Data were analyzed for all culture-confirmed V.v. cases reported to CDC from 2000-2022. Frequencies of patient characteristics and medical outcomes were calculated overall and by transmission route (foodborne vs. non-foodborne). To identify correlates of mortality, bivariate comparisons were conducted, interactions were explored, and multivariate logistic regression models were fit.

Results: from 2000-2022, 2,989 V.v. cases were reported to CDC, including 656 foodborne (22%) and 1,619 non-foodborne (54%) cases. Five-year case total increased 70% from 2000-2004 (n=487) to 2018-2022 (n=827). Overall, 2,484 (83%) patients were male, 2,166 (73%) were non-Hispanic White, and 2,064 (76%) reported an underlying condition; 2,493 (83%) were hospitalized and 692 died (23%). Both number of deaths (260 vs. 200) and fatality rate (40% vs. 12%) were higher among foodborne vs. non-foodborne cases. In multivariate models, risk factors for mortality included history of liver disease/alcoholism (odds ratio [OR]=6.5, $P<0.001$), age 45-59 (OR=11.3, $p=0.001$), foodborne transmission (OR=1.3, $P=0.006$), and Black (OR=1.8, $P=0.03$) or Asian (OR=2.5, $P=0.009$) race/ethnicity. Antibiotic use was protective (OR=0.33, $p=0.001$). Diabetic liver disease was associated with lower mortality than other forms of liver disease (interaction OR=0.5, $P=0.04$).

Significance: V.v. infections confer a substantial and increasing public health burden. Although non-foodborne transmission caused 2.5 times more cases,

foodborne transmission was associated with 30% more deaths and over 3 times higher fatality rate in the observed period. Identification of older age, history of liver disease/alcoholism, and minority race/ethnicity as risk factors for mortality can inform public health and medical interventions.

T3-06: TRANSMISSION OF *C. JEJUNI* BETWEEN HUMANS AND LIVESTOCK IN RURAL ETHIOPIA

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: *Campylobacter jejuni* and *C. coli* are the most common causes of bacterial enteritis worldwide. Symptomatic and asymptomatic infections are associated with stunting in children in low- and middle-income countries. However, little is known about their sources and transmission pathways in these settings.

Purpose: To quantify the sources of *C. jejuni* infection in infants under 1 year of age in Eastern Ethiopia.

Methods: We isolated *C. jejuni* from feces of infants, their mothers and siblings, and livestock using Chromagar *Campylobacter*. A total of 380 isolates were sequenced using Illumina short-read sequencing. Four attribution models incorporating different genomic profiling methods, assumptions, and estimation approaches were used to determine likely sources.

Results: Among 287 *C. jejuni* isolates, 48 seven-gene sequence types (STs) were identified, including 11 previously unreported STs. Within an ST, core genome STs of multiple isolates differed by fewer than five alleles. Isolates from the same infant were closely related, while isolates from consecutive samples of the same

infant often had different STs, suggesting rapid clearance and new infection. All models predicted chickens as the primary reservoir, with or without humans (mothers or siblings) as intermediaries. Model predictions varied in the relative contributions of cattle vs. small ruminants.

Conclusions: Transmission pathways of *C. jejuni* in this setting are highly complex and interdependent. Chickens are the dominant reservoir, but other livestock and environmental sources also contribute. Candidatus *C. infans*, a currently nonculturable species, was prevalent and likely transmitted between humans. Reducing infant colonization and stunting in low-resource settings requires integrated interventions targeting proximate sources—such as caretakers' hands, food, and indoor soil—through nutrition, food safety, and WASH (water, sanitation, and hygiene) strategies.

T4-01: EFFECT OF BIOPROTECTIVE CULTURES ON LISTERIA MONOCYTOGENES IN HIGH MOISTURE CHEESE

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: *Listeria monocytogenes* (Lm) contamination of high moisture cheese continues to cause outbreaks of foodborne illness.

Purpose: To evaluate the use of commercial protective cultures (PC) to control Lm during the production and storage of queso fresco cheese as an intervention to improve food safety.

Methods: Pasteurized milk was inoculated with a six-strain cocktail of Lm at ~2 log CFU/mL. PCs were then individually added in freeze-dried format to milk at ~7 log CFU/mL. Initial screening of four PCs was conducted in milk following a cheesemaking time-temperature incubation profile of 35°C for 4 h, followed by 20°C for 20 h, and then 12°C for 7 days. Two selected PCs were then used in the production of queso fresco following the same inoculation methodology and a typical queso fresco cheesemaking process. Samples were collected for pH and enumeration throughout the process and weekly during storage at 7°C for 4 weeks. Experiments were repeated at least three times and data were analyzed by repeated measures ANOVA.

Results: Treatment effects were observed with LALCULT LC1 and Lyofast LPAL in milk when compared to control and other PCs ($P < 0.0001$), such that mean Lm counts were ~1 and 2 log CFU/mL lower than control at 7 days (7.73 log CFU/mL), respectively. Similarly, addition of LC1 or LPAL resulted in Lm counts lower than control during the production and storage of queso fresco ($P < 0.001$), with mean counts ~3 and 1 log CFU/g lower than control (7.61 log CFU/g) at the end of storage, respectively. The change in pH with LPAL differed from both LC1 and control ($P < 0.0001$), reaching mean pH values of 5.99, 6.26, and 6.24, respectively.

Significance: These findings identify LC1 as a viable intervention to reduce the growth of Lm in queso fresco without altering pH, warranting additional research on the use of PCs to control Lm in other high moisture cheeses.

Award Application: Developing Scientist Award

T4-02: MICROBIOLOGICAL HYGIENE OF MINAS ARTISANAL CHEESE (QUEIJO MINAS ARTESANAL), A RAW MILK CHEESE PRODUCED IN BRAZIL

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: Minas Artisanal Cheese (MAC) is a raw milk cheese produced in Brazil, widely consumed and theme of constant arguing and discussion regarding their regulatory guidelines, safety profiles and hygiene parameters for production.

Purpose: Here, we assessed the microbiological profiles of MAC samples, in order to demonstrate the hygienic profiles of production and to provide reliable parameters for official guidelines.

Methods: 113 MAC samples were collected from 8 producing regions in Minas Gerais, Brazil (Serro, Canastra, Cerrado, Araxá, Triângulo, Vertentes, Entre-Serras, non-registered regions). Samples were subjected to analysis to enumerate lactic acid bacteria (LAB, MRS, pH 5.7, 30°C 72h), mesophiles (Petrifilm™ AC, 32°C 48h), Enterobacteriaceae (Petrifilm™ EB, 35°C 24h) and coliforms and *Escherichia coli* (Petrifilm™ EC, 35°C 24-48h). The obtained counts were converted to log CFU/g and subjected to statistics to check significant differences among producing regions (Kruskall-Wallis and Dunn, $p < 0.05$).

Results: MAC samples presented mean counts of 7.7 ± 0.6 for LAB, 8.3 ± 0.9 for mesophiles, 3.8 ± 1.5 for Enterobacteriaceae and coliforms and 3.0 ± 0.9 for *E. coli* (Results in log CFU/g). Samples produced in Cerrado and non-specified regions presented higher counts of Enterobacteriaceae and coliforms when compared to other producing regions ($p < 0.05$), while similar distributions were observed for

LAB, mesophiles and *E. coli* ($p > 0.05$). Based on hygienic reference values, 49/113 and 48/113 MAC samples presented Enterobacteriaceae and coliforms counts, respectively, higher than 5.000 CFU/g, while only 11/113 MAC samples presented *E. coli* counts higher than 500 CFU/g. All MAC samples presented mesophiles counts higher than 100.000 CFU/g, an expected result due to the ripening process (LAB counts higher than 100.00 CFU/g in 112/113 MAC samples).

Significance: The data highlight the poor hygienic conditions in the MAC samples, supporting the development of adequate procedures and guidelines for MAC production. Acknowledgments to CAPES (finance code 001), CNPq and FAPEMIG.

T4-03: EVALUATION OF A MULTIPRONGED TRAINING APPROACH TO ASSIST SMALL AND MEDIUM DAIRY PROCESSORS IN LISTERIA ENVIRONMENTAL MONITORING USING SURVEY AND MOLECULAR METHODS

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: A multipronged training approach comprised of workshops on environmental monitoring proGrams (EMPs) and essential technical skills to sustain EMPs may provide small and medium dairy processors (SMDPs) with the support needed to develop and implement tailored and effective *Listeria* EMPs.

Purpose: To evaluate the use of a novel multipronged training approach to advance the technical skills and attitudes of SMDPs in their development and implementation of *Listeria* EMPs.

Methods: The performance and attitudes related to *Listeria* EMP practices were assessed for 10 SMDPs from 3 US states using survey and molecular methods. The presence of *Listeria* in each facility was evaluated prior to training using environmental sampling following the FDA-BAM method of detection of *Listeria* in environmental samples. During training, all 10 SMDPs completed a series of 4 workshops as part of a multipronged approach that covered topics related to FSMA preventive controls, basic dairy science and sanitation, technical writing, and EMPs. Surveys were administered via email to all processors to assess their (i) pre-training *Listeria* EMP-related attitude and practices for 7 topic areas and (ii) post-training confidence in performing tasks and intent for improvements in each area.

Results: Pre-training survey responses indicated that all SMDPs found EMPs, sanitation, equipment design, written policy, record keeping, and employee training and food safety attitude important but varied in their practices. The initial environmental sampling found that 90% (N=10) of the SMDPs had *Listeria* present in their facility. There was no difference in the proportion of *Listeria* positive sites between facilities with (N=4) and without (N=6) an EMP prior to training ($p = 0.59$, Wilcoxon).

Significance: Multipronged training may provide the support needed to empower

SMDPs to develop, implement, and improve EMPs in their own facilities to limit the risk of human illness due to *L. monocytogenes* presence in the processing environment.

Award Application: Developing Scientist Award

T4-04: BIOFILM DYNAMICS OF LISTERIA MONOCYTOGENES AND RALSTONIA INSIDIOSA UNDER CHEESE PROCESSING AND AGING CONDITIONS

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: *Ralstonia insidiosa* (Ri) enhances *Listeria monocytogenes* (Lm) biofilms, enabling Lm to better survive and persist in nutrient-deficient food processing environments, increasing the risk of contaminating finished processed foods.

Purpose: This study investigated *Listeria monocytogenes*-*Ralstonia insidiosa* biofilm dynamics and sanitizer tolerance under cheesemaking (22°C) and cheese aging (11°C) temperature conditions.

Methods: Three Lm strains isolated from cheese processing environments and two Ri strains were paired and simultaneously grown on stainless steel coupons at 11°C and 22°C, in 0.05X Trypticase Soy Broth. The biofilms were imaged on Days 1, 3, and 10 using Scanning Electron Microscopy and enumerated on Chromogenic *Listeria* Plating medium and on 0.05X Trypticase Soy Agar supplemented with 1.5mg/L Crystal Violet for Lm and Ri, respectively, after treatment with sodium hypochlorite or quaternary ammonium compounds. The study was replicated on two separate occasions. Repeated measures ANOVA assessed the effects of temperature, time, and strain combinations on growth and sanitizer effectiveness.

Results: Temperature impacted Ri growth ($p=0.0003$); Ri grew significantly better at 11°C. Lm grew equally well at 22 and 11°C. For Ri, higher growth temperatures improved sanitizer efficacy ($p=0.034757$), while longer growth times reduced it ($p=0.000536$). These factors did not significantly affect sanitizer performance on Lm. Biofilm architecture at 11°C was characterized by a scant distribution of singular layers of cell aggregates connected by web-like extra-cellular polymeric substances (EPS). At 22°C, the cell aggregates were copious, had more layers and denser EPS. At both temperatures, biofilms became more three-dimensional over time with 10-day biofilms comprising of multiple-layered cell aggregates wrapped in dense gel-like EPS with comparatively wider surface areas.

Significance: While room temperature conditions favor more complex Lm-Ri

mature biofilm structures, cooler cheese aging temperatures may promote higher cell densities within the biofilms, conferring greater sanitizer tolerance. This emphasizes the need for strict sanitation of cheese aging surfaces/environments.

Award Application: Developing Scientist Award

T4-05: RELATIONSHIP BETWEEN ENTEROBACTERIACEAE AND CRONOBACTER AS DETERMINED BY THE APPLICATION OF A STANDARDIZED QUALITATIVE METHOD

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: Enterobacteriaceae (EB) is widely used in powdered infant formula (PIF) industry to assess process hygiene conditions and for compliance. In general, no overall correlation exists between EB and *Cronobacter* (EB-C) in PIF and environmental samples (ES). Data obtained using different methods from different samples could have contributed to the observed lack of correlation.

Purpose: Assess relationship between EB-C by applying a standardized qualitative method.

Methods: An alternate real time PCR based method, foodproof Enterobacteriaceae plus *Cronobacter* Detection Kit, is previously validated as per ISO-16140-2:2016 for EB-C detection in PIF, ES and others. This qualitative method allows EB-C testing from the same sample as it employs a single DNA extract obtained after enrichment for their detection. The method was used to analyze 209 non product contact factory environmental samples for EB-C detection and the

Results were used to determine the relationship between them.

Results: The following results were obtained (i) 175 samples were EB-C negative, (ii) 33 samples were EB positive while testing negative for *Cronobacter*, (iii) 1 sample was EB-C positive and (iv) 0 sample was *Cronobacter* positive while being EB negative. While specificity, i.e. the proportion of samples that tested negative for EB among samples that tested negative for *Cronobacter*, was calculated to be at a statistically acceptable 84.1%, sensitivity i.e. the proportion of samples that tested positive for EB among samples that tested positive for *Cronobacter*, was 100%. The statistical validity of sensitivity has to be confirmed through additional samples as this estimate is based on a single *Cronobacter* positive sample.

Significance: Results obtained by the application of a standardized method on the

same sample indicates that EB absence can confidently indicate *Cronobacter* absence. Therefore, taking corrective action after a positive finding of EB will enhance the food safety and is more effective than looking only for *Cronobacter*, due to the much higher prevalence of Enterobacteriaceae in these environments.

T4-06: PREVALENCE, CHARACTERIZATION, AND ANTIMICROBIAL RESISTANCE (AMR) PROFILING OF SALMONELLA FROM BACKYARD CHICKENS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The growing interest in organic, fresh, and locally sourced poultry leads to the expansion of backyard poultry farming. However, recent backyard poultry-linked *Salmonella* outbreaks have raised significant public health concerns.

Purpose: To assess the prevalence, serotypes, and antimicrobial resistance of *Salmonella* from backyard chickens.

Methods: Samples were collected at three stages from three small backyard chicken farms (A, B, and C) managed by 4-H participants. Pre-harvest samples (28, 53, and 56d) included cloacal swabs (n=53), environmental (n=14), feed (n=2), and litter (n=5), and post-harvest samples at 57d involving eight whole carcasses—two each from post-scalding, pre-dip, post-dip, and post-chiller stages—were collected. All samples underwent pre-enrichment in buffered peptone water, enrichment in Rappaport Vassiliadis broth, and streaking on XLT4 agar. Carcass analysis used the whole carcass enrichment method. Presumptive *Salmonella* colonies were confirmed by PCR targeting the *invA* gene and characterized for serotype and antimicrobial susceptibility using the disc diffusion method. Data were analysed using descriptive statistics and Fisher's Exact test.

Results: A total of 13 *Salmonella* isolates were identified, including *S. infantis* (54%, n=7), *S. Newport* (30%, n=4), *S. Kentucky* (8%, n=1), and *S. Bareilly* (8%, n=1). Prevalence was 6.75% (5/74) in pre-harvest samples and 100% (8/8) in post-harvest samples. All isolates were multidrug-resistant (MDR) with resistance to >3 antibiotic classes, including penicillins, macrolides, and tetracyclines, but remained susceptible to cefepime, meropenem, fosfomycin, and colistin. Post-harvest isolates showed significantly higher resistance ($P < 0.05$). No *Salmonella* was detected in pre-harvest samples from farms A and C. In contrast, five pre-harvest samples from farm B tested positive.

Significance: These findings highlight the considerable prevalence of multidrug-resistant (MDR) *Salmonella* in backyard poultry, underscoring the need for rigorous biosecurity measures as farm B, where pre-harvest samples were *Salmonella* positive, was in proximity to a wooded area, with domesticated hamsters, rabbits, and frequent wild rabbit visits.

Award Application: Developing Scientist Award

T5-01: PRODUCT FORMULATION AND RUBBING TIME IMPACT THE INACTIVATION OF VIRUSES ON HANDS BY FOAM-BASED HAND SANITIZERS

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: Effective hand hygiene, including hand washing and hand sanitizer use, is crucial in reducing infectious disease transmission via the hands. While the efficacy of hand washing is well-documented, hand sanitizers remain less extensively studied but are considered a reliable alternative to soap and water when the hands are not visibly dirty or greasy.

Purpose: Examine the efficacy of five commercially-available foam hand sanitizers—four alcohol-based and one non-alcohol-based—against enveloped and non-enveloped viruses using bacteriophage phi6 ($\Phi 6$) and bacteriophage MS2 as surrogates, respectively.

Methods: A cocktail of MS2 and $\Phi 6$ (8 log PFU/mL) was inoculated on the palmar surface of the hands of 30 volunteers and exposed to 3 mL or 6 mL of hand sanitizer product followed by rubbing the palmar surface of the hands together for 10 s or until dry. Remaining virus was recovered by a modified dish method and assayed using the double agar overlay assay. Each volunteer received 2 treatment combinations for a total of 60 samples per virus.

Results: Significant log reductions were observed among the viruses ($P \leq 0.05$), with $\Phi 6$ consistently showing higher susceptibility across all factors compared to MS2 with log reductions of 2.83 ± 1.98 and 0.50 ± 0.53 log reduction, respectively. While dosing volume did not significantly impact log reduction ($P = 0.1$), rubbing time significantly affected virus inactivation ($P \leq 0.05$). Higher log reduction was observed when hands were rubbed until dry (2.69 ± 2.06), as compared to the typical 10 s rubbing time (0.65 ± 0.75).

Significance: This study revealed that the efficacy of commercially-available foam hand sanitizers depends on rubbing time in which the hands should be rubbed together until dry for maximum efficacy. Additionally, overall product formulation, rather than exclusively active ingredient concentration, impacts product efficacy.

Award Application: Developing Scientist Award

T5-02: DETERMINING THE IMPACT OF SURFACE ROUGHNESS ON DISINFECTION EFFICACY OF COMMERCIAL PRODUCTS AGAINST PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS WET SURFACE BIOFILMS

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: Biofilms can contain pathogenic and spoilage microorganisms and resist stressors like disinfectants, which may be impacted more by damaged surfaces compared to new surfaces.

Purpose: This study aimed to develop a reproducible method for scratching surfaces and determine the efficacy of disinfectants on damaged surfaces.

Methods: A Sutherland Rub Tester was modified to standardize surface scratching for creating surface roughness to simulate a worn surface, and the surface damage was analyzed via microscopy. EPA methods MB 19-06 and MB 20-04 were then modified for disinfection efficacy testing against biofilms formed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* on scratched and non-scratched high-density polyethylene (HDPE) and stainless steel (SS) coupons. Three commercial disinfectants were tested: hypochlorite (CL), hydrogen peroxide (HP), and quaternary ammonium (QA). Three trials were completed for CL and HP, and five trials for QA.

Results: Microscopy results indicated average surface roughness of SS and HDPE increased by 0.82 μm and 12.69 μm , respectively, after scratching with 80 grit sandpaper. Average biofilm reductions ranged from 3.23 to 6.22 logs per coupon for *P. aeruginosa* and 2.92 to 5.62 logs per coupon for *S. aureus* on all surfaces using the three disinfectants. Although biofilm formation was more pronounced on 80-grit-scratched surfaces during early phases of growth compared to smooth surfaces, no significant efficacy difference against mature biofilms was detected between scratched and non-scratched surfaces. Compared to the QA-based disinfectant, both HP- and CL-based disinfectants performed significantly better ($p < 0.05$) on HDPE coupons. However, only one CL-based disinfectant achieved the desired 6 log/coupon reduction against *P. aeruginosa* biofilms on non-scratched

HDPE.

Significance: This research is particularly relevant to healthcare and food processing, where worn fomites heighten public health risks by harboring bacterial biofilms..

Award Application: Developing Scientist Award

T5-03: A DATA-FOCUSED ANALYSIS OF LEAFY GREEN HARVEST EQUIPMENT CLEANING & SANITATION PRACTICES

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: Field harvesting and packing equipment have been implicated as potential sources of microbiological contamination; equipment complexity and variations in use have led to varied cleaning and sanitizing practices.

Purpose: Assess leafy green harvester cleaning and sanitation programs to identify opportunities for improvement.

Methods: An assessment of cleaning and sanitation practices used for leafy green harvest machines was completed across three states: AZ, CA, and FL. Self-Propelled Belt and 3-Point harvesters were sampled for ATP, Aerobic Plate Count (APC), Total Coliform (TC), and Generic *E. coli* (gEC) at three time points, Post-Harvest(PH), Post-Detergent(PD), and Post-Sanitization(PS). Harvest machine sampling locations were standardized across events, with each machine having up to five locations. Metadata was recorded for each sampling, including practices, location, equipment type, condition, cleaning accessibility, surface type, environmental data, and parameters that could have impacted sample outcomes. Swab samples were analyzed using AOAC standardized methods; T-tests and ANOVAs were performed (n=30).

Results: Leafy green harvest machines (23 machines) were assessed over 30 sampling events across three states (11-AZ, 9-CA, and 10-FL). Considerable variability was observed in detergents, sanitizers, crew sizes, and water usage. PH, 94% of swabs across all areas were above the ATP threshold (20,000 RLU; n=660); this decreased to 47% of swabs after sanitation (PS)(n=382). *E. coli* detection (≥ 10 CFU/swab) was infrequent and decreased after cleaning and sanitizing: PH, 6.2%; PD, 5.3%; PS, 1.1%. TC reductions after cleaning (n=493) and

sanitizing (n=208) ranged from -3.54 to 6.13 log CFU/swab; occasionally, TC populations increased after cleaning (36.1%) and sanitizing (18.3%) compared to swabs immediately following harvest. Significant differences ($p<0.05$) between detergent and sanitizer combinations were observed in ATP, APC, TC, and gEC reductions.

Significance: Practices and challenges were similar across all states, and opportunities (sometimes very simple) exist for improvement. Detergent type, application, and equipment used during cleaning had a greater impact on microbial reduction than sanitizers alone.

T5-04: BIOFILM FORMATION OF TYPHOIDAL AND NONTYPHOIDAL SALMONELLA SEROVARS AND THEIR SENSITIVITY TO AMYGDALIN AND LACTIC ACID TREATMENTS

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: As high as 80% of bacterial infections could be linked to sessile microbial cells and biofilms. Various serovars of typhoidal and nontyphoidal *Salmonella* could form biofilm on biotic and abiotic surfaces.

Purpose: The current study compared biofilm formation of typhoidal and nontyphoidal *Salmonella* serovars for up to two weeks and investigated the sensitivity of planktonic and sessile cells of the pathogen to amygdalin and lactic acid.

Methods: Four-strain mixtures of typhoidal and nontyphoidal *Salmonella* serovars were spot-inoculated on surface of stainless-steel coupons (type 304, 2b finish) and were half-submerged in sterilized milk (3% fat) at 25 °C for up to two weeks for biofilm formation. After removal of loosely attached cells, the coupons were then treated with distilled water (control), 20% amygdalin, and 20% lactic acid (immersion for 90 seconds) to evaluate the sensitivity of the planktonic and sessile cells to the antimicrobials. Sessile cells were recovered from coupons using glass beads and sonication. Results of the trials were statistically analyzed using Tukey and Dunnett's-adjusted ANOVA at type I error level of 5%.

Results: During the two-week aerobic storage at 25°C, both typhoidal and nontyphoidal serovars of the pathogen illustrated comparable ($p \geq 0.05$) biofilm formation and sensitivity to amygdalin and lactic acid. While treatment with

amygdalin resulted in modest reductions of <1 log CFU/cm² of the pathogen, treatments with 20% lactic acid resulted in microbiologically significant reductions ($p < 0.05$) of more than 99.9% of the inoculated pathogen.

Significance: Under the conditions of our experiments we observed that typhoidal and nontyphoidal *Salmonella* serovars have comparable biofilm formation and sensitivity to selected antimicrobials. Thus, an antimicrobial treatment validated against nontyphoidal *Salmonella* could almost certainly be equally effective against typhoidal serovars as well. This could be of importance in preparation of Sanitation Standard Operating Procedures, as prerequisite proGram of HACCP and FSMA-based food safety management systems.

Award Application: Developing Scientist Award

T5-05: NOVEL DRY SURFACE INOCULATION METHODOLOGY USING ELECTROSTATIC POWDER COATING TECHNIQUE FOR LOW-MOISTURE FOOD SANITATION EXPERIMENT

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: Recurring outbreaks of foodborne illnesses linked to cross-contamination in low-moisture food products underscore the criticality of better understanding pathogen transfer on food contact surfaces in dry food processing environments.

Purpose: This study aimed to develop and standardize a non-aqueous inoculation method for dry food contact surfaces using electrostatic powder coating techniques, simulating realistic dry contamination environments.

Methods: A blend of hard winter red and soft white wheat berries (80/20 ratio, 1000 g) was inoculated with a six-strain *Salmonella* cocktail and equilibrated to 0.45 aw. The inoculated wheat berries were ground, milled, and sieved to produce standard wheat flour. This flour was then applied on #304 stainless steel coupons (64.3 mm diameter) either directly by using a powder coating gun or a custom-built powder coating system comprising a rotating fan and high voltage (30 kV) electrode inside a metal canister that charged the powder electrostatically or triboelectrically. Powder-coated coupons were brushed 20 times using a soft brush until visually clean. Triplicate samples were serially diluted and plated on differential media, and *Salmonella* colonies were enumerated.

Results: The custom-built powder coating system was not significantly different (p -value < 0.05) in the *Salmonella* population (3.51 ± 0.32 log CFU/cm²) as compared to the powder coating gun (3.62 ± 0.07 log CFU/cm²). The high voltage charging method (3.45 ± 0.35 log CFU/cm²) also resulted in no significant difference as compared to the triboelectric charging method (3.40 ± 0.10 log CFU/cm²). Double applications of powder coating did not significantly increase population (3.57 ± 0.36 log CFU/cm²), compared to single applications.

Significance: The new custom-built electrostatic powder coating technique is considered more efficient and safer compared with the conventional electrostatic gun method, which will ensure reliable and realistic simulation of low-moisture

food contamination environments.

Award Application: Developing Scientist Award

T5-06: AN ALTERNATIVE TO CHEMICAL SANITATION FOR THE EFFECTIVE CONTROL OF LISTERIA MONOCYTOGENES BIOFILMS IN THE FOOD INDUSTRY

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: *Listeria monocytogenes* biofilms pose a persistent challenge in the food industry, contributing to contamination and compromising global food safety.

Purpose: To develop and evaluate an enzymatic-bacteriocin solution designed to (1) hydrolyze biofilms and (2) control *Listeria monocytogenes*.

Methods: A modified microplate biofilm model, based on Iglesias et al. (2019), was employed to test the efficacy of the enzymatic-bacteriocin solution. The model used simple biofilms (*Listeria innocua*) and mixed biofilms (*Listeria innocua* + *Pseudomonas* sp.) grown in 24-well microplates. Biofilm hydrolysis was assessed via crystal violet staining, while bacteriocin efficacy was measured by quantifying *Listeria innocua* concentration after biofilm disruption by sonication. The tested conditions included: Water (control), Enzymatic cocktail solution alone, bacteriocin solution alone and sequential application of enzymatic solution followed by bacteriocin. Each condition was tested in quadruplicate and repeated across three plates (n=192). The total microbial flora was enumerated on Plate Count Agar (PCA), while *Listeria innocua* was quantified on CHROMagar. Statistical analysis was performed using nonparametric tests (Kruskal-Wallis and Mann-Whitney).

Results: Biofilm elimination, measured by crystal violet staining, showed significant differences across the tested conditions ($p < 0.05$). These results were consistent in both simple biofilms (n=48) and mixed biofilms (n=48). The greatest reduction in biofilm biomass was observed when the enzymatic solution was used either alone or in combination with bacteriocin, with reductions exceeding 50%. The enzyme-bacteriocin combination further enhanced the bactericidal effect of bacteriocin, reducing *Listeria* sp. counts by over 5 logs CFU/mL, with a significant difference with bacteriocin alone. This effect was consistent across both simple biofilms (n=24, $p < 0.05$) and mixed biofilms (n=24, $p < 0.05$).

Significance The combination of enzymatic and bacteriocin treatments offers a promising alternative to chemical sanitation for controlling and eliminating *Listeria monocytogenes* biofilms in the food industry.

T6-01: PERCEPTIONS OF FOOD INDUSTRY LEADERS ABOUT INDUSTRY-TO-INDUSTRY CONFIDENTIAL FOOD SAFETY DATA SHARING: AN INTERVIEW STUDY

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Presentation type/Format: Technical

Category: Data Management and Analytics

Abstract:

Introduction: Data sharing can improve food safety, particularly regarding rare food safety issues, through (i) trend analyses that will allow for more rapid identification of food safety issues and (ii) development of models and food safety decision support tools.

Purpose: This study explores perceptions of industry-to-industry food safety data sharing in the food industry sectors.

Methods: Participants with at least two years of experience in a leadership role within US food companies were invited to participate in a semi-structured interview study. The recorded interviews were transcribed using NVivo Transcription and manually validated. Thematic analysis was performed with NVivo 14 using an iterative coding process.

Results: Interviews, ranging 19-52 minutes, were completed with 27 of 87 invited food industry leaders . Participants represented food industry sectors, including dairy, food manufacturing, meat and poultry, produce, and laboratory services. Thematic analysis revealed four main themes: 1) industry leaders recognize the potential benefits of engaging in food safety data sharing, which include the opportunity to benchmark practices and allow for better predictive modeling capabilities due to access to more data points, 2) technical barriers, such as limited digitalization, standardization of data collection and management practices, limit the feasibility of data sharing, 3) trust, or lack thereof, is a key factor influencing data sharing decisions, often underlying specific concerns such as confidentiality, liability risks, and data misuse, and 4) the need for sustained collaboration between academia, government, and industry to identify trade-offs

in data sharing and enable improved food safety through information pooling.

Significance: Our study emphasizes the benefits, barriers, and solutions for industry-to-industry sharing of confidential food safety data. By identifying industry motivators and barriers, our findings provide evidence to inform data sharing initiatives and facilitate practices, allowing for collective analysis of localized incident data and reducing the risk of future food safety incidents.

Award Application: Developing Scientist Award

T6-02: RAPID SALMONELLA SEROVAR CLASSIFICATION USING AI-ENABLED HYPERSPECTRAL MICROSCOPY WITH DIFFERENT DATA PREPROCESSING APPROACHES

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Traditional methods for *Salmonella* serovar detection and identification often require prolonged enrichment on selective media, extending sample preparation and detection times that can compromise timely interventions in food safety.

Purpose: This study aims to demonstrate a one-step method for detecting and identifying *Salmonella* serovars using hyperspectral microscope imaging (HMI) and artificial intelligence (AI), comparing manual feature selection with statistical feature extraction for machine learning.

Methods: Five *Salmonella* serovars (Enteritidis, infantis, Kentucky, Johannesburg, and 4,[5],12:i:-) were freshly cultured overnight in tryptic soy broth with yeast extract at 32°C prior to imaging. Minimal sample preparation involved placing 2 µL of culture on glass slides, drying, then rehydrating with 2 µL of sterilized deionized water. Raw high-dimensional data were preprocessed to compare two sets of input features: i) three manually selected spectral wavebands based on domain knowledge, and ii) abstract representations derived from principal component analysis (PCA) explaining 99% of total variance. Classification performance of k-nearest neighbors (k-NN), support vector machine (SVM), and random forest (RF) was evaluated by accuracy, precision, and recall.

Results: For the manually selected wavebands, k-NN, SVM, and RF achieved classification accuracies of 60.13%, 54.05%, and 59.45% (n=500). In contrast, using 18 PCA-derived features increased accuracies to 73.65%, 75.00%, and 77.70%, respectively. All models showed comparable improvements in precision and recall with these PCA-derived features, leveraging statistically identified linear combinations of all original wavebands.

Significance: These findings highlight that AI-enabled HMI can accelerate *Salmonella* serovar classification by minimizing sample preparation. They also underscore the importance of data preprocessing, enabling machine learning models to extract critical insights from high-dimensional data.

Award Application: Developing Scientist Award

T6-03: COMPARATIVE DETECTION OF THE DECAY OF PROTOZOAN PARASITES IN SOIL

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Category: Viruses and Parasites

Abstract:

Introduction: Protozoan parasites remain infectious in soil for long periods of time and are challenging to detect. Reliably detecting these organisms, and measuring their viability, is critical to assessing their risk in growing environments.

Purpose: This study seeks to correlate viability of *Cryptosporidium parvum* and *Eimeria tenella* (a surrogate for *Cyclospora cayetanensis*) oocysts, as determined in cell culture, with levels of gene expression.

Methods: Agricultural soil was sent for chemical analysis and then distributed into 4oz sampling bags (120g/bag, n=130 bags). Basil and cilantro were procured from a local distributor with 6g weighed into individual petri dishes (n=65/plant type). Oocysts of *C. parvum* and *E. tenella* (90% sporulated, 10^6 oocysts in 100uL each), were then inoculated dropwise onto each plant sample and airdried for 120min. *E.coli* TVS355 (10^7 CFU) was also added to serve as a reference for expected organismal decay. one plant sample was added to each bagged soil sample and hand-massaged to a depth of 17cm. Samples were incubated at 12 or 32°C. Samples, taken at 30d intervals, were assessed for infectivity and decay through cell culture infection coupled with qPCR, and RNA extraction followed by RT-qPCR to measure gene expression. Statistical analysis was performed using JMP via ANOVA.

Results: After 4 months of incubation, RNA decay methods detected significantly ($p<0.05$) fewer viable oocysts than cell culture for both organisms. After 10 months, oocysts no longer infected mammalian cells but remained detectable by RNA quantification, with 1.79 and 1.34 log oocysts/sample for *C. parvum* and *E. tenella*, respectively. *E. coli* remained cultivable for 10 months, with 1.19 log CFU/sample detected at that time.

Significance: Comparing infectivity-based methods with novel RNA-based methods may simplify quantifying viable protozoan parasites and improve the monitoring of organisms like *Cyclospora cayetanensis*, which lack infectivity-based assays.

Award Application: Developing Scientist Award

T6-04: FISHING FOR ANSWERS: A COMPARATIVE STUDY OF ZEBRAFISH EMBRYO AND LARVAE MODELS IN HUMAN NOROVIRUS REPLICATION

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: Zebrafish embryo and larvae models are promising tools for studying human norovirus (HuNoV) replication, the leading cause of viral gastroenteritis worldwide. A comprehensive evaluation is needed to assess their efficiency and suitability.

Purpose: To compare zebrafish embryo and larvae models by screening HuNoV GI and GII strains (n=20) for their replication in both models.

Methods: Stool samples positive for HuNoV (100 mg each) were resuspended in 1 mL of sterile phosphate-buffered saline, vortexed for 1 min, and centrifuged at 1,000×g for 5 min. The supernatant was collected, and 3 nL was microinjected into either fertilized zebrafish egg (within 4-hour post-fertilization) or the yolk of 3-day post-fertilization larvae. Each day post-infection (dpi), 10 embryos/larvae were pooled as a single sample, and two samples were collected daily until 5 dpi. RNA was extracted from the harvested embryos/larvae and quantified using droplet digital PCR. Seven GI and 13 GII genotype strains were analyzed. Two trials were conducted for each strain, with group means, 95% confidence intervals, and statistical differences analyzed in R using estimated marginal means and pairwise comparisons.

Results: Zebrafish embryos did not survive microinjection with clarified HuNoV samples, requiring stool sample dilution to reduce toxicity. However, this dilution sometimes reduced the norovirus concentration below the infectious threshold of 8 log genome copies per gram, preventing infection after microinjection. In contrast, zebrafish larvae showed no mortality after microinjection with clarified sample. GII HuNoV strains replicated significantly in larvae (typically >4-log increase in viral RNA at 1–2 dpi), while GI strains replicated less efficiently in larvae (1–3-fold RNA increase at 1–2 dpi). This mirrors human infections, where GII strains exhibit greater replication than GI strains.

Significance: Given the effective replication of a wide range of HuNoV strains in the zebrafish larvae model, this model can potentially aid in validating interventions to mitigate HuNoV transmission.

Award Application: Developing Scientist Award

T6-05: EVALUATING THE IMPACT OF GENOMIC ANALYSIS TOOLS ON MACHINE LEARNING MODELS FOR VIBRIO PARAHAEMOLYTICUS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Bioinformatics analysis combined with machine learning has gained traction recently. However, results obtained from using various genomic analysis tools can potentially affect machine learning model performance, thereby impacting meaningful inferences for addressing food safety issues.

Purpose: This study aims to investigate how the choices of various genomic analysis tools could impact the machine learning classification performance for source attribution of *Vibrio parahaemolyticus* (Vp).

Methods: Genome assemblies for Vp (176 environmental, 960 seafood, and 853 clinical sequences) were downloaded from the National Center for Biotechnology Information Pathogen Detection database. Pangenome analyses were performed via nine pangenome pipelines using combinations of three annotation tools (Bakta v1.9.4, Prokka v1.14.5, PGAP v6.4) and three pangenome construction tools (Panaroo v1.5.1, PPanGGOLiN v2.2.1, Roary v3.13.0). Core genome Multi Locus Sequence Typing (cgMLST) and whole genome MLST were conducted using chewBBACA (v3.3.10). Workflow efficiency was assessed by comparing computational runtime and memory usage. Shannon entropies were calculated from outputs of each tool to estimate diversity. Random forest models predicting isolation sources were built in R (v4.3.2) using genomic features, and evaluated for sensitivity, specificity, balanced accuracy, AUROC, and Area Under the Precision-Recall Curve.

Results: Among the pangenome pipelines, PGAP-Roary required the most runtime (23 hours), while PGAP-PPanGGOLiN consumed the most memory (103 GB). Roary pipelines generated the highest number of total genes. The cgMLST matrix had the highest mean Shannon entropy. Models developed using Bakta-PPanGGOLiN and PGAP-Roary outperformed others, with average AUROC values of 0.90 and 0.91, respectively.

Significance: The choice of genomic analysis tools significantly impacts source attribution accuracy in machine learning pipelines, highlighting the need for

optimized workflows.

Award Application: Developing Scientist Award

T7-01: MITIGATING LISTERIA PREVALENCE IN COLD ROOMS USING 222-NM FAR UV-C RADIATION

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: *Listeria* species can survive in cold rooms of food processing industries. Far UVC (222-nm) exposure inactivates bacterial cells and can be used to sanitize cold rooms.

Purpose: In this study, the effect of UVC 222-nm light on *L. innocua* populations seeded on stainless steel (SS) and plastic coupons (PC) was evaluated at different distances and exposure durations.

Methods: The coupons were inoculated with a suspension of *L. innocua* (9.04 ± 0.02 log CFU/ ml) and dried overnight (12-16h) in the biosafety cabinet. The dried coupons were then placed under the UVC 222-nm light in a cold room at distances of 25.4 cm, 101.6 cm, and 203.2 cm and exposure times of 0, 1, 4 and 8h. After exposure, the coupons were sonicated in 100ml of 1x PBS for 2 min at 42 kHz and enumerated on MOX (Modified Oxford) agar. The plates were incubated at 37 °C for 48h.

Results: PS had higher reductions of *L. innocua* occurred on SS ($p \leq 0.05$). Bacterial reductions increased with exposure durations ($p \leq 0.05$). Significantly higher *L. innocua* reductions occurred at 203.2 cm and 101.6 cm than at 25.4 cm ($p \leq 0.05$). The highest reduction of *L. innocua* on PS (6.98 ± 0.70 log CFU/ ml) occurred at 25.4 cm after 8h. The highest reduction of *L. innocua* on SS (4.78 ± 1.89 log CFU/ ml) occurred at 25.4 cm after 8h. Lowest reductions were observed from SS (1.27 ± 0.22 log CFU/ ml) and PS (1.33 ± 0.37 log CFU/ ml) coupons placed at 203.2 cm and 101.6 cm respectively.

Significance: UVC 222nm can be used as a potential intervention against *L. monocytogenes* in food industry cold rooms. Height of the surface from the lamp and duration of exposure can significantly influence the potency of the treatment.

Award Application: Developing Scientist Award

T7-02: TARGETED VISIBLE-LIGHT-INDUCED NANO-SANITIZERS FOR ULTRAFAST INACTIVATION OF MICROBES AND THEIR BIOFILMS

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Current sanitation methods have significant limitations, including the risk of promoting biocide-resistant bacteria, biofilm persistence, lack of food-grade options, limited pathogen inactivation efficacy in the presence of residual organic matter and corrosive nature of chemical sanitizing agents. Moreover, addressing the growing challenge of antimicrobial resistance in microbes requires the development of innovative strategies to manage microbial risks within food and agricultural systems.

Purpose: This research focused on developing innovative targeted, light-active nano-biosanitizers to improve the inactivation rate against a wide range of target microbes and their biofilms and to overcome key limitations of conventional sensitizers.

Method: Amine-enriched Nano Bio-Metal-Organic Frameworks (Nano-BioMOFs) were synthesized in an aqueous medium based on food-grade organic ligands. Nano-BioMOFs were then functionalized with mannose as bio-affinity ligands to capture diverse pathogens. The bio-affinity ligands functionalization increased the localization of target microbes in the vicinity of the photoactive species, thereby enhancing the antimicrobial activity of reactive oxygen species generated upon light exposure. The antimicrobial activity and role of affinity ligands in improving the inactivation rate were evaluated against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Candida albicans* and bacterial biofilms.

Results: Nano-BioMOFs demonstrated enhanced antimicrobial activity under daylight irradiation. Mannose-functionalized Nano-BioMOFs exhibited a high binding affinity for diverse microbial targets, resulting in a 30-fold increase in the inactivation rate compared to non-functionalized Nano-BioMOFs. Under daylight exposure, the engineered Nano-BioMOFs achieved more than 6 log (>99.9999%) microbial inactivation within a short treatment time of 2-3 minutes. In bacterial biofilm models, over 4 log CFU/cm² of biofilm were inactivated using mannose-

functionalized Nano-BioMOFs within 30 minutes of daylight irradiation.

Significance: This work highlights developing next-generation visible-light-activated bio-sanitizers designed for ultrafast and targeted inactivation of diverse microbes and their biofilms on various surfaces. This innovative approach paves the way for advanced food-grade antimicrobial materials for diverse applications.

T7-04: APPLICATION OF PERACETIC ACID BY SPRAY OR IMMERSION ON CHICKEN BREASTS INDUCED CAMPYLOBACTER VIABLE-BUT-NOT-CULTURABLE

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: When exposed to the oxidizing and acidifying properties of peracetic acid (PAA), *Campylobacter* can enter a viable-but-not-culturable (VBNC) state, becoming undetectable through standard culture-based methods.

Purpose: This study aimed to assess whether PAA effectively inactivates viable *Campylobacter* or partially induces a VBNC state, thereby only reducing the culturable cell count.

Methods: We inoculated retail chicken breast slices (25 g) with a three-strain *Campylobacter jejuni* cocktail and treated it with 500 ppm PAA spray or immersion for ten seconds. Slices were then shaken in neutralizing buffered peptone water (nBPW) immediately ($n = 6$), one hour ($n = 3$) or 24 hours ($n = 3$) after treatment. Rinsing (treated with water), untreated positive and uninoculated controls were also included. Culturable and viable *Campylobacter* was quantified using qPCR and direct plating on mCCD agar, respectively.

Results: After PAA treatment, the culturable cell concentration was significantly lower (5.38 ± 0.36 log CFU/mL) than the viable cell concentration (6.40 ± 0.42 log CFU/mL), suggesting that PAA induced VBNC state in a subpopulation of *Campylobacter*. Spray (0.17 log CFU/mL reduction; $p = 0.66$) and immersion (0.09 log CFU/mL reduction; $p = 0.97$) treatments did not significantly reduce viable cell counts compared to the untreated control (6.28 ± 0.56 log CFU/mL). However, both spray and immersion significantly reduced culturable cell counts compared to the untreated control (5.20 ± 0.59 log CFU/mL and 4.98 ± 0.50 log CFU/mL vs. 5.82 ± 0.45 log CFU/mL, respectively). Lastly, neither treatment significantly reduced culturable or viable *Campylobacter* after 24 hours post-treatment cold storage compared to immediately after treatment, while storage in aerobic and cold temperature conditions significantly reduced both viable and culturable concentrations.

Significance: Ten-second spray or immersion treatment with 500 ppm PAA significantly reduced culturable, but not viable, *Campylobacter* on chicken breasts,

suggesting that culture-based methods overestimate PAA's antimicrobial activity.

Award Application: Developing Scientist Award

T7-05: POLICIES TO REDUCE ANTIMICROBIAL USE IN LIVESTOCK HAVE NOT RESULTED IN CONSISTENT DIFFERENCES IN AMR IN BEEF-ATTRIBUTED SALMONELLOSIS

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Policies that restrict antimicrobial use in livestock (AMU) to reduce antimicrobial resistant (AMR) bacteria including Non-Typhoidal *Salmonella* (NTS) differ between countries, but quantifying the impact on public health impact is complex due to limited availability of appropriate data.

Purpose: We applied a Quantitative Microbiological Risk Analysis (QMRA) approach to evaluate differences in AMR NTS risk from consuming beef produced in the EU and US due to differing AMU policies.

Methods: Annual beef-attributable AMR NTS illnesses in France and the Netherlands (NL) were estimated using a QMRA model for serotypes of higher virulence (HV), e.g. Typhimurium and Dublin, which are dominant cattle serotypes in these countries. Two modelled scenarios compared risk from EU vs US beef: 1) HV NTS illnesses in these countries from currently available beef and 2) predicted illnesses if all beef consumed in both countries was US beef. Comparisons were made for HV NTS overall, and HV NTS resistant to ciprofloxacin (CIP), third-generation cephalosporins (3GC), ampicillin (AMP), and tetracycline (TET).

Results: Model-estimated annual beef-attributable HV NTS were 1,571 (95% CrI: 503-4,169) in NL and 3,002 (643-8,737) in France. If all beef were sourced from US, illnesses (regardless of AMR) in NL would be equivalent (RR=0.65 (0.34-1.27)), but US beef would reduce illness in France (RR=0.62, (0.48-0.98)). US beef resulted in equivalent AMR RR for quinolones in France (0.9 (0.16-3.00)) and NL (1.2 (0.69-2.20)), reduced AMR RR for TET (France: 0.28 (0.10-0.66); NL: (0.75 (0.42-0.96)) and AMP (France: 0.32 (0.11-0.76)) and NL: 0.78 (0.47-0.99)), equivalent RR for 3GC in France (1.0 (0.88-46)), and increased RR for 3GC in NL (14.7 (3.0-52)).

Significance: Although the EU restricted certain AMU 11 years earlier than the US, our analysis incorporating all available evidence shows no consistent difference in direction or magnitude of risk were predicted in France or the Netherlands.

T7-06: SALMONELLA ENTERITIDIS WITH DECREASED SUSCEPTIBILITY TO CIPROFLOXACIN

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: The Food Safety and inspection Service (FSIS) monitors *Salmonella* serotype distribution and related antimicrobial resistance (AMR) trends. While the top serotypes in chicken samples have remained consistent, a notable increase in *Salmonella* Enteritidis with decreased susceptibility to ciprofloxacin (DSC) from chicken samples has been observed since 2017.

Purpose: Continuous monitoring of serotype and AMR trends allows the timely dissemination of information of public health concern to stakeholders and research partners.

Methods: Samples were collected from establishments nationwide and analyzed for microbial targets including *Salmonella*. Whole Genome Sequencing was performed on bacterial isolates as described in FSIS' Microbiology Laboratory Guidebook Chapter 42. *Salmonella* serotypes were derived using SeqSero2 and sequences were published to the National Center for Biotechnology information from which AMR genotypes were obtained. Results described are for *Salmonella* from chicken product and cecal samples since 2017.

Results: infantis, Kentucky, Typhimurium, Enteritidis and Schwarzengrund have consistently been among the top serotypes among both chicken product and cecal samples. While there were no major variations year to year, an increase in DSC among Enteritidis has been observed. DSC was first noted in 2% (n=13) in 2017 and has increased to 64% (n=229) in 2024 (preliminary data). DSC in *Salmonella* Enteritidis is attributed to a single point mutation in the gyrase A gene (gyrA). Occurrence of DSC is observed across geographic locations, corporations, and establishment sizes and findings correlate with those from retail meat and human sources.

Significance: While serotypes have remained stable, decreased efficacy of ciprofloxacin is of concern as it is commonly used to treat severe human cases of *Salmonella* infection. Additionally, fluoroquinolone use has been prohibited in poultry in the United States since 2005 after initial increases of resistance in

poultry *Campylobacter* isolates, hence, origin and potential drivers remain unknown.

T8-01: INFLUENCE OF SURFACE ROUGHNESS ON BACTERIAL SPORE SURVIVAL DURING ASEPTIC PACKAGE STERILIZATION WITH VAPOR HYDROGEN PEROXIDE

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Presentation type/Format: Technical

Category: Packaging

Abstract:

Introduction: Surface roughness of packaging materials plays a critical role in microbial adhesion and biofilm formation, affecting sterilization efficacy during aseptic processing. This study evaluates how varying surface roughness levels of high-density polyethylene (HDPE) impact bacterial spore inactivation during vaporized hydrogen peroxide (VHP) sterilization.

Purpose: To investigate the influence of surface roughness and hydrophobicity on *Bacillus atrophaeus* spore survival during VHP sterilization, providing insights to enhance aseptic packaging processes.

Methods: HDPE sheets were roughened using sandpaper (P-36 to P-220) to create different surface roughness levels. Surface roughness was measured using a profilometer with a 20 mN load, 50 $\mu\text{m/s}$ scanning speed, and 200 Hz sampling rate, complemented by optical micrographs. *Bacillus atrophaeus* spores (2×10^9 CFU/mL) were applied to each sample and subjected to a VHP cycle at 4450 ppm, 60°C, and 0.5% relative humidity for 9 seconds. Spore recovery and enumeration were performed post-treatment to assess survival. Contact angle measurements were conducted to quantify hydrophobicity. Scanning electron microscopy (SEM) was used to visualize spore adhesion and surface morphology. Data were analyzed using one-way ANOVA (Tukey's) and two-way ANOVA (Sidak's), with significance levels set at $P < 0.01$, and non-significant ($p > 0.05$).

Results: Spore survival was significantly influenced by surface roughness, with roughness values increasing from 0.338 μm (smooth control) to 3.747 μm (roughest). This increase corresponded to a drop in contact angles from 149.7° (smooth) to 105.4° (rough), indicating greater hydrophilicity. The roughest surface

(P-36 grit) showed a 2.75-log reduction, while the smoothest (P-220 grit) had a 4.42-log reduction. SEM images revealed that spores adhered within surface crevices, shielding them from sterilant exposure and enhancing survival.

Significance: The Findings demonstrate that rough surfaces protect spores, reducing VHP efficacy. Optimizing surface topography and hydrophobicity is crucial to enhance sterilization protocols and ensure the safety of aseptic packaging processes

Award Application: Developing Scientist Award

T8-02: SURVIVAL OF LISTERIA MONOCYTOGENES ON D'ANJOU PEARS CO-INOCULATED WITH BACILLUS THURINGIENSIS, AUREOBASIDIUM PULLULANS, AND PENICILLIUM EXPANSUM DURING LONG-TERM COLD STORAGE

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Pears may be stored for up to nine months under controlled atmosphere (CA) conditions before consumption. However, the impact of storage practices and pear commensal organisms on food safety risks remains unclear.

Purpose: To determine the survival of *Listeria monocytogenes* (LM) on pears co-inoculated with *Bacillus thuringiensis* (BT), *Aureobasidium pullulans* (AP), and *Penicillium expansum* (PE) during 9 months of CA cold storage.

Methods: D'Anjou pears (n = 1620) were spot-inoculated with LM alone or with LM and one of the co-inoculums (BT, AP, or PE) on intact or wounded pears. Pears were stored in bulk or wrapped with commercially available tissue paper containing 1.3% copper carbonate and 0.1% ethoxyquin for 9 months in refrigerated CA storage (3 °C; 2% O₂ and 1% CO₂). LM and co-inoculum concentrations were enumerated using nine pears from each of the 12 treatment combinations at 0, 7, 14, 21, 28, 42, 56, 70, 84, 120, 150, 180, 210, 240, and 270 days of storage by spiral-plating on selective and differential agar plates. When LM was below the limit of detection (LOD) (<2.67 log CFU/pear), a most probable number (MPN) method was used. Data were analyzed using a mixed-model for

repeated measures.

Results: None of the treatments supported the growth of LM regardless of co-inoculation or wounding. However, under some conditions, LM populations remained constant (~ 7.5 log CFU/pear) for up to 210 days. Pears wrapped with copper and ethoxyquin-treated paper showed significantly lower populations of LM, BT, and PE compared to intact unwrapped pears ($p < 0.001$). On wounded pears, a significant increase in PE caused LM to fall below the LOD after 84 days of storage ($p < 0.001$). BT and AP persisted at ~ 6 log CFU/pear throughout storage but had no effect on LM growth.

Significance: Tissue paper containing copper and ethoxyquin effectively reduced both LM and PE, improving the safety and shelf life of pears.

Award Application: Developing Scientist Award

T8-03: TRANSCRIPTOMIC RESPONSE OF LISTERIA MONOCYTOGENES BIOFILMS TO COMMERCIALY AVAILABLE SANITIZERS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Exposure to environmental stress often leads to cross-protection against subsequent challenges. Evidence of cross-protection has been observed under oxidative, acidic, and low-temperature stresses, common antimicrobial strategies used in the food industry. Furthermore, biofilm-associated cells exhibit increased persistence and tolerance to these stresses, complicating sanitation efforts.

Purpose: Characterize transcriptional response and survival of *L. monocytogenes* biofilms exposed to sublethal concentrations of various commercial sanitizers and elucidate mechanisms that may contribute to biofilm adaptation, survival, and tolerance.

Methods: A *L. monocytogenes* multi-strain cocktail was used to grow mature biofilms for 96 h at 25°C. Biofilms were treated with sublethal concentrations of lactic acid (0.4%), chlorine (10 ppm), peracetic acid (10 ppm), and silver dihydrogen citrate (5%), with untreated samples as controls. Total RNA was extracted from harvested cells following a 15-second exposure. RNA sequencing was performed using the Illumina NextSeq 2000 platform. Read counts were generated with Salmon v1.9.0. Downstream analysis was conducted in R.

Results: Short exposure to sanitizers elicited distinct transcriptomic responses biofilms, with differentially expressed genes (DEGs, $P_{adj} < 0.05$, $|\log_2\text{FoldChange}| \geq 0.5$) identified. Chlorine and peracetic acid induced the most significant changes in gene expression. Chlorine exposure resulted in 100 DEGs, including 92 upregulated and 8 downregulated genes. Similarly, peracetic acid exposure led to 65 DEGs. In contrast, lactic acid and silver dihydrogen citrate had minimal effects on gene expression. Functional analysis of upregulated DEGs revealed significant ($P_{adj} < 0.05$) enrichment of genes involved in transcription and transcriptional regulation in response to chlorine, while genes associated with ABC transporters were significantly enriched in response to peracetic acid.

Significance: This research highlights how the type and composition of sanitizers influence gene expression profiles, reflecting variation in the kinetics of their antimicrobial actions. Understanding transcriptomic response of *L. monocytogenes* to commonly used sanitizers is crucial for developing more effective control strategies in the food industry.

Award Application: Developing Scientist Award

T8-04: LONGITUDINAL ANALYSIS OF NON-CLINICAL LISTERIA MONOCYTOGENES ISOLATES OBTAINED BETWEEN 2000 AND 2021 IN NEW YORK STATE REVEALS COMMON CLONAL COMPLEXES AND THEIR FOOD-SOURCE ASSOCIATIONS

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: *Listeria monocytogenes* (Lm) can persist for long periods in the food processing environment where it can re-contaminate ready-to-eat food products. Deli meat, soft cheese, produce and smoked fish are among the food types where Lm contamination have resulted in recalls and outbreaks in the past.

Purpose: This study aims to identify clonal complexes (CCs) that are commonly found in non-clinical Lm isolates in New York State (NYS), determine their associations with certain food types, and assess their clustering with clinical isolates.

Methods: Non-clinical Lm isolates collected from processing environments and retailers (n=622) and foods (n=343) as well as clinical isolates (n=1,046) in NYS between 2000 and 2021 underwent whole-genome sequencing. Sequencing data were analyzed to determine multilocus sequence type (MLST), lineage

identification, and virulence and resistance gene presence. Association between CCs and food types was statistically evaluated at a

Significance level of 0.05. Non-clinical isolates were grouped with clinical isolates based on single nucleotide polymorphism (SNP) analysis with a threshold of ≤ 50 SNPs.

Results: Overall, CC5, CC321 and CC2 were found to be more common CCs among non-clinical isolates, while CC1, CC6 and CC5 were more common among clinical isolates. CC6 was associated with dairy products, CC9 was associated with processed meat, and CC121 and CC88 were associated with processed seafood. Clustering based on SNP distances resulted in 41 NY-groups (i.e., isolate groups identified in NYS containing at least one clinical isolate) that spanned at least 3 months with 3 NY-groups spanning isolation dates that ranged over nearly 20 years.

Significance: This study highlights that in NYS (i) most Lm CCs commonly found in foods and food environments are not associated with human illnesses (CC5 is one exception as it was commonly found in foods, food environments, and human cases) and (ii) certain Lm CCs are more commonly found in certain food types, which could aid on source tracking during outbreak investigations.

Award Application: Developing Scientist Award

T8-05: COMPARISON OF METHODS TO DETERMINE ASSOCIATIONS BETWEEN GENETIC VARIATION AND ENVIRONMENTAL STRESS TOLERANCE IN LISTERIA MONOCYTOGENES

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* strains exhibit robust adaptive capabilities to various stresses, partly attributed to genetic variability among strains. These genetic variations can contribute to the bacterium's ability to proliferate under diverse environmental conditions.

Purpose: Quantifying the associations of genetic variation with growth parameters under various environmental stresses using different methods to analyze large datasets with biological complexity.

Methods: A total of 317 *L. monocytogenes* strains, representing 4 lineages and 80 sublineages, were subjected to growth in BHI broth under multiple stress conditions (varying pH, salt concentrations, and temperatures), with OD600 monitored over time in triplicate. The gcplyr R package was employed to fit growth parameters. Both Genome-wide association analysis (Treewas) and machine learning methods (Random Forest, RF and K-Nearest Neighbors, KNN), were applied and compared to identify accessory genetic variations significantly associated with growth capabilities under each of the conditions.

Results: A number of accessory genes, including 20 tRNA and 16 protein encoding genes, were significantly ($p < 0.05$) associated with stress-condition growth parameters, and some of them were repeatedly presented as significant loci under various stress conditions, such as lmo0149. In Lineage 1 strains, 526 core genome single nucleotide polymorphism (SNPs) were significantly associated with stress-condition growth parameters, while only 6 in Lineage 2 strains. After splitting the dataset into 70% training and 30% testing, the prediction accuracy for growth rate was 0.78 using RF and 0.75 using KNN. For lag phase, the prediction accuracy was 0.82 with RF and 0.78 with KNN.

Significance: The identification of important genetic loci through diverse

methodologies deepens our understanding of the genetic factors contributing to *L. monocytogenes*'s resilience in varying environments. This insight provides valuable information for predictive models for bacterial growth in complex settings, such as food processing, and may explain the prevalence of specific subtypes in certain ecological niches.

Award Application: Developing Scientist Award

T8-06: PAN-GENOMIC ANALYSIS UNCOVERS THE GLOBAL DIVERSITY OF CRONOBACTER SAKAZAKII ACROSS THE FOOD PRODUCTION CONTINUUM

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Presentation type/Format: Technical

Category: Data Management and Analytics

Abstract:

Introduction: *Cronobacter sakazakii* is an opportunistic foodborne pathogen linked to outbreaks associated with powdered infant formula. It is known to exhibit high genomic diversity, but the genetic and functional signatures across diverse sources are not well known.

Purpose: This study aimed to develop a reproducible and automated workflow to characterize genomic features associated with different sources at the global scale.

Methods: We retrieved metadata and genome assemblies from the National Center for Biotechnology Information (n=2,089). A fine-tuned model trained with Generative Pre-trained Transformer (GPT-3.5) was employed to assign specific categories based on reported isolation sources in an automated way. Assemblies were processed for quality control using CheckM and annotated with Prokka. A pangenome was built for high-quality assemblies (n=748) with Panaroo, and the encoded metabolic features were identified using eggNOG-mapper2. Machine learning algorithms were applied to identify accessory gene enrichments by sources and continents.

Results: The fine-tuned model achieved near-perfect classification accuracy. Genomic profiles were significantly associated with continent of isolation and source type (PERMANOVA, $P=0.001$). Notably, genes involved in replication, recombination, and repair were enriched in genomes derived from (A) clinical samples across all source types, (B) powdered food among all food sources, and (C) Europe compared to Asia and North America (Fisher's test, adjusted $P<0.05$). Our random forest model predicted sources and geography with good performance, demonstrating that type VI secretion system and heavy metal resistance (e.g., *cusR*, *cusC*) were major contributors to biogeography. Antimicrobial resistance homologous gene clusters enriched across continents and sources were primarily involved in efflux (*arlR*, *OprZ*) and carbapenems resistance (*Kpne_OmpK37*).

Significance: This study uncovered key accessory genes and features associated with isolation sources and geography of *C. sakazakii*, revealing putative mechanisms involved in adaptation and transmission throughout the food system.

Award Application: Developing Scientist Award

T9-01: EFFICACY OF EUGENOL SOLUTIONS AS SEED TREATMENTS FOR CONTROLLING ESCHERICHIA COLI O157:H7 AND SALMONELLA TYPHIMURIUM IN BASIL (OCIMUM BASILICUM L.) UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Pathogens such as *Escherichia coli* O157:H7 (*E. coli*) and *Salmonella* Typhimurium are major concerns in basil production, including controlled environmental production, posing significant public health risks through contaminated seeds. Recent outbreaks of basil within controlled environmental agricultural systems have increased concerns for this industry sector.

Purpose: This study aimed to evaluate the antimicrobial efficacy of eugenol in reducing pathogen levels on basil seeds and its impact on seed germination and biomass production.<

Methods: Basil seeds were inoculated with a final concentration of 7 log CFU/g of *E. coli* O157:H7 and 5 log CFU/g of *Salmonella* Typhimurium, then treated with eugenol solutions at 0.5%, 0.75%, and 1%, while control seeds were treated with water. Samples (2 g) were collected at 0, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-treatment. Bacterial counts were determined on MacConkey Agar for *E. coli* and XLT4 Agar for *Salmonella*. Seedling rates and biomass production were recorded by counting fully germinated seedlings and weighing vegetative growth on day 14. Data was analyzed using ANOVA and Tukey post hoc test, with correlation analysis to assess the relationship between eugenol concentration, germination rates, and biomass production.

Results: Eugenol achieved up to a 4-log reduction in *E. coli* O157:H7 and *Salmonella* Typhimurium within the first 12 hours regardless of the concentration, with 1% eugenol showing the highest antimicrobial efficacy. However, by 24

hours, both pathogens grew to similar levels as the control samples. Germination rates decreased with higher eugenol concentrations for a possible germination delay, showing a negative correlation (-0.3441), and biomass production was reduced at higher eugenol concentrations, with a negative correlation of (-0.6728).

Significance: Eugenol effectively reduces surface contamination on basil seeds but is less effective against internalized bacteria. These findings highlight the need for strategies to address internalization while maintaining seed viability, offering valuable insights for improving basil seed safety and public health protection.

Award Application: Developing Scientist Award

T9-02: BACTERIOPHAGE FOR ESCHERICHIA COLI DECONTAMINATION IN MICROGREENS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Bacteriophage as a biocontrol agent for microgreens remains unexplored despite the lack of safety controls to prevent contamination during pre-harvest.

Purpose: This study evaluated the antimicrobial efficacy of T7 phage against *E. coli* BL21 on microgreen leaf surfaces for up to 4 hours and assessed its stability in growth water and leaf surfaces under a controlled greenhouse over 7 days.

Methods: Sanitized soil and hydroponic-based radish and pea microgreen leaf surfaces in control and treatment groups were spot-inoculated with 8-log *E. coli* BL21 and incubated for 30 minutes for cell attachment. The treatment group was inoculated with 9-log T7 phage (MOI 5), while the control group received sterile saline. Both groups were incubated for 0, 2, and 4 hours at 25°C and 37°C, and sterile silica beads were used for cell detachment before bacterial enumeration. For stability testing, microgreens were cultivated with a hydroponic system in a greenhouse at 25°C, and 9-log T7 phage was spot inoculated into the growing water and onto the leaf surface. The treatment group was exposed to 105 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ of LED light while the control group remained in the dark, with phage enumeration conducted at 0, 24, 72, 120, and 168 hours.

Results: Antimicrobial experiments showed a consistent above 2.5-log reduction (T-test, $P < 0.05$) of BL21 within 2 hours and persisted up to 4 hours, with optimal effects in soil-based microgreens at 25°C and 37°C. Stability experiments highlighted that T7 phage remained stable in growth water for up to 120 hours under LED exposure and 168 hours without LED exposure, maintaining final populations above 6-log. on leaf surfaces, T7 phage stability was weaker, with significant reduction after 24 hours under LED exposure and up to 72 hours without LED exposure, while final populations remained above 4-log.

Significance: T7 phage is an effective antimicrobial agent to prevent bacterial contamination in microgreens.

Award Application: Developing Scientist Award

T9-03: AGGREGATIVE SOIL SAMPLING USING DRAGS AND BOOTIES HYDRATED WITH ALTERNATIVE WETTING AGENTS SHOWS PROMISING RECOVERY OF INDICATOR ORGANISMS ACROSS DIVERSE SOILS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Drags and booties are of interest for produce soil sampling, however the common hydration buffer in animal production, skim milk, is inappropriate for produce soil sampling use.

Purpose: This study tested alternative wetting agents to replace skim milk for hydrating drags and booties and validated their performance across diverse soil types. Methods: Tryptic soy broth, buffered peptone water (BPW), phosphate-buffered saline (PBS), and deionized water were compared to skim milk by sampling in fallow fields with untreated subsurface-injected swine manure and surface-spread dairy manure applied. Aerobic plate counts (APCs), total coliforms, and generic *E. coli* were enumerated from 220 paired drags and booties collected along 100m paths. BPW and PBS were selected for validation in a melon farm, a mixed vegetable farm (leafy greens, peppers, apples), and an apple orchard. Additional 96 paired drags and booties analyzed for the same indicators, with enrichment for generic *E. coli* and 16S V4-V5 microbiome analysis.

Results: For wetting agent selection, mean recovery differences across the five wetting agents ranged from 0.0-0.2log(CFU/g) for APCs, 0.1-0.6log(CFU/g) for total coliforms, and 0.1-0.4log(CFU/g) for generic *E. coli*, across fields with both swine and dairy manure application. For validation in produce fields, mean difference of paired samples (BPW-PBS) were 0.14 ± 0.11 and -0.02 ± 0.09 log(CFU/g) for drags and booties, respectively, for APCs, and 0.26 ± 0.07 and -0.02 ± 0.09 log(CFU/g) for drags and booties, respectively, for total coliforms. Enrichment data showed 88-94% of drags and booties hydrated with PBS or BPW tested positive for generic *E. coli* (no significant differences). Microbiome analysis revealed no major differences in alpha or beta diversity between PBS and BPW hydrated samples.

Significance: Differences between wetting agents were minor and biologically insignificant, suggesting that wetting agent selection can be guided compliance requirements and ease of use.

T9-04: MICROBIOME VARIATION ACROSS DIFFERENT URBAN FARMS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Urban farm plays an increasingly important role in enhancing food sustainability and security. However, the diverse microbial communities in urban farm soils and plants, which may have critical implications on produce safety, have been largely underexplored.

Purpose: To characterize the microbial communities associated with leafy greens in urban farm environments as a factor of site-specific conditions such as soil amendments use, irrigation sources, and plant cultivar.

Methods: We conducted a molecular survey of leafy greens and soils from 7 urban farms in greater Washington, D.C. Samples collected included plant tissue (PT; n=92; i.e., kale, lettuce, chard, and cabbage cultivars), root zone soil (RZS; n=92), and bulk soil (BS; n=39). DNA was extracted from each sample and processed for 16S rRNA gene sequencing on Illumina NextSeq 1000. Sequences were analyzed via QIIME2 and R 4.3.2. Shannon diversity was compared using Kruskal-Wallis test, and microbial community structure was investigated using PERMANOVA. Shared and unique taxa were analyzed within and across sites.

Results: The soil and leaf microbiomes were diverse, though the former had higher richness and evenness ($p < 0.05$) and more conserved genera (i.e., present in > 50% of samples). As the scope of the study did not include enrichment, foodborne pathogens were not detected in the respective microbiomes. Nevertheless, microbial community structure of BS, RZS and PT samples significantly varied across different farms ($p = 0.001$) and correlated with water resource use and plant cultivar, among other factors ($p < 0.05$). Within each site, RZS has the largest number of unique genera compared to PT and BS. Longitudinal sampling further indicated that there were limited shared taxa in BS, though abundant shared taxa in RZS, demonstrating stability of core taxa in RZS over time.

Significance: This study characterized microbial communities of different urban farm systems, which can help inform strategies to optimize urban agriculture practices via enhancing productivity, sustainability and food safety.

Award Application: Developing Scientist Award

T9-05: MODELING TO ASSESS TRADEOFFS IN SAMPLING PLAN ATTRIBUTES AND REJECTION RULES FOR PREHARVEST PRODUCE SAFETY

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Existing tools to assess food safety sampling plans do not model different rejection rules (after a positive test result), such as adjacent lot rejection.

Purpose: Develop a food safety sampling and rejection (FSSR) model and use it to evaluate the impact of produce industry-relevant preharvest sampling plans and rejection rules.

Methods: The FSSR model was programmed in R 4.4.2. We simulated 10-acre fields with 1-acre subplots (over 1000 iterations) for three possible contamination scenarios: uniform (100% at 1 CFU/lb), large cluster (10% at 10 CFU/lb), and small cluster (1% at 100 CFU/lb). We compared a default plan (375 g composite mass from 60 grabs, per acre) to an alternative plan (750 g composite mass from 30 grabs, per acre). The rejection rules were to reject all subplots that test positive, and optionally all subplots adjacent to positive plots.

Results: Under uniform contamination, the alternative plan detected more contaminated subplots through testing [mean of 81% (5th to 95th percentiles from 60-100%)] than the default plan [56% (30-80%)]. With adjacent subplot rejection, the alternative plan more reliably rejected contamination [99% (90-100%)] than the default plan [89% (60-100%)]. Under large cluster contamination, the alternative plan more reliably detected contaminated subplots through testing [99% (93-100%)] than the default plan [97% (81-100%)]. With adjacent subplot rejection, the alternative plan similarly rejected contamination [100% (100-100%)] as the default plan [100% (100-100%)]. Under small cluster contamination, the default plan more reliably detected contaminated subplots through testing [96% (75-100%)] than the alternative plan [91% (25-100%)]. With adjacent subplot rejection, the default plan similarly rejected contamination [99% (100-100%), rare failures lowered the mean] as the alternative plan [95% (100-100%)].

Significance: Compared to rejecting only lots with positive tests, an adjacent lot rejection rule reduced performance differences between the sampling plans and

rejected meaningfully more total contamination.

Award Application: Developing Scientist Award

T9-06: PAENIBACILLUS ALVEI TS-15 EFFICACY IN REDUCING SALMONELLA ON TOMATOES

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: A field study protocol was developed in collaboration with EPA to evaluate the antimicrobial efficacy of *Paenibacillus alvei* strain TS-15 on tomato plants and fruit prior to harvest, against *Salmonella*, known to cause multiple outbreaks associated with the consumption of fresh-cut tomatoes.

Objective: To field verify the protocol and to evaluate TS-15 for the reduction of *Salmonella* in the pre-harvest tomato environment.

Methods: The protocol was optimized in a high-tunnel setting using an attenuated *Salmonella enterica* PY Δ tolC::aph strain and two tomato varieties, Scarlet Red and Red Bounty. Tomato leaves at three canopy stages and blossoms were inoculated with 10 μ L of *Salmonella* (10^8 CFU/mL). Half of the samples were treated with $\sim$ 100 μ L of aerosolized TS-15. Persistence trials were conducted on leaves and contaminated blossoms at 0- and 3-days post-inoculation (dpi). The remaining blossoms were used to assess TS-15's effect on blossom-to-fruit transmission after harvest.

Results: *Salmonella* contamination of leaves was significantly affected by tomato cultivar, canopy position, sampling day, and TS-15 treatment. Upper canopy leaves had fewer positives (28) compared to mid (37) and lower canopy leaves (43). *Salmonella* survival decreased by 40% from 0 dpi to 3 dpi, and TS-15 treatment reduced *Salmonella* incidence by approximately 7%. TS-15 was not a significant factor in the incidence of *Salmonella* from blossoms, but incidence did decrease from 100% recovery to 84% from 0 dpi to 3 dpi. Of the inoculated blossoms that reached maturity (n=252), only 15 were positive for *Salmonella*: 14 from *Salmonella*-only treatments, and 1 from the *Salmonella* with TS-15 treatment

($p = 0.033$).

Significance: The high tunnel experiment optimized and verified the protocol in the field. Results showed that *Paenibacillus alvei* strain TS-15 has potential to reduce *Salmonella* contamination in the pre-harvest tomato environment, particularly for tomato fruits.

T10-01: CONSUMER UNDERSTANDING OF ANTIBIOTIC-RESISTANT BACTERIA AND THEIR OCCURRENCE IN FOOD

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: Antibiotic resistance is a global health threat. The CDC estimates that extended-spectrum beta-lactamase-producing *Enterobacterales* causes 197,400 hospitalizations and 9,100 deaths annually. Consumers can be exposed to antibiotic-resistant bacteria (ARB), either directly from animal products or indirectly from produce where animal agriculture may contribute to environmental contamination with these organisms. It is unclear if consumers consider food as a potential source of ARB.

Purpose: Conduct a nationwide survey to assess consumer perceptions of ARB and their occurrence in food.

Methods: The questionnaire was developed and reviewed by a team of microbiologists and was validated and administered through Qualtrics in February 2024. The survey consisted of 9 questions which focused on general knowledge of antibiotics and ARB, as well as their occurrence in foods. Two screening questions were embedded to exclude participants who lacked awareness of ARB and vegetables being potential sources of these bacteria.

Results: A total of 2,476 participants were included in the survey. Only 35% (n=869) correctly identified bacterial infections as the only treatable infection with antibiotics. Of the respondents, 78.76% (n=1950) were familiar with ARB, while the remaining 21.24% (n=526) were not and were excluded from further analyses. Participants identified beef (n=1201), pork (n=1261), poultry (n=1280), dairy (n=906), and eggs (n=1014) most frequently for having the potential to harbor ARB. Only 542 individuals selected vegetables as a potential source of ARB, and these participants completed the final block of questions. Participants were asked whether production practices and processes influenced their concerns of ARB in vegetables. Results indicate that imported vegetables, non-organic vegetables,

and minimally processed vegetables increased the level of concern among the participants.

Significance: The survey highlights knowledge gaps regarding antibiotics, ARB, and their occurrence in vegetables. These findings provide valuable insight for the development of future educational and extension materials.

T10-02: MICRO AGRICULTURAL SYSTEMS: A COMPREHENSIVE OVERVIEW OF EMERGING RISKS AND SAFETY PRACTICES IN HOME, ON-FARM, INSTITUTIONAL, AND SMALL-SCALE PRODUCTION SYSTEMS

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: Growth of small-scale and home-based agricultural production systems presents new concerns regarding food safety and regulatory compliance. While these micro agricultural systems offer innovative opportunities, they also introduce risks related to contamination, sanitation, and food handling. Many producers operate with limited oversight and resources, which increases the likelihood of unsafe practices, especially as they transition from small-scale to commercial production.

Purpose: An Arkansas-based needs assessment is being conducted to evaluate the food safety challenges faced by non-traditional producers, including home kitchens, small farms, and institutional settings such as schools. This assessment will inform the Food Safety Extension Network's efforts and help land-grant universities address the increasing complexity of technical assistance requests. These requests often blur the lines between cottage food, food freedom laws, FSMA, PCQI, and retail/manufacturing regulations. The goal is to identify risks and develop educational frameworks to equip these producers with the tools and knowledge to address food safety while fostering innovation.

Methods: Interviews with Extension faculty, staff, county agents, technical service providers, and producers have informed the creation of workshops and outreach programs. These data informed programs focused on regulatory requirements, food safety practices, and providing technical assistance for small-scale producers. This includes hands-on demonstrations and guidance on transitioning to commercial production while ensuring food safety.

Results: A comprehensive outreach program is being developed to help producers understand critical food safety aspects, such as product labeling, sanitation, allergen control, and cold-chain management. These educational initiatives will

provide producers with strategies to mitigate foodborne risks, maintain product quality, and navigate regulatory requirements, while also accessing federal funding to support development.

Significance: This initiative supports regional food systems by reducing food insecurity, improving food safety, and creating sustainable economic opportunities for small-scale farmers and producers. It fosters a safer, more resilient food supply while supporting federally funded initiatives.

T10-03: EXPLORING CONSUMER ATTENTION TO FLOUR SAFETY MESSAGES ON COMMERCIALLY AVAILABLE BAKE MIX PACKAGES USING EYE-TRACKING TECHNOLOGY

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: *Lactobacillus rhamnosus* is a widely used probiotic, recognized for its health benefits and safety. However, to support robust quality control, more efficient methods are needed to differentiate between strains of the same species.

Purpose: This study aims to use Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry to rapidly and accurately differentiate *L. rhamnosus* strains.

Methods: Twenty strains of *L. rhamnosus* from diverse sources were analyzed using MALDI-TOF MS. Spectra were acquired and processed using standard protocols and software to identify unique protein mass fingerprints. Principal Component Analysis (PCA) and dendrogram construction were performed to visualize differences among strains and assess clustering patterns.

Results: MALDI-TOF MS successfully generated distinct spectral profiles for each *L. rhamnosus* strain. PCA showed clustering that corresponded with the known phylogenetic relationships among the strains. Dendrograms revealed clear separation, enabling differentiation at the strain level. Strain-specific biomarker peaks were identified and validated across multiple runs.

Significance: This study demonstrates that MALDI-TOF MS can serve as a rapid, reliable tool for strain-level differentiation of *L. rhamnosus*, offering valuable support for probiotic product verification and quality assurance.

Award Application: Developing Scientist Award

T10-04: PERCEIVED EFFECTIVENESS OF FLOUR SAFETY MESSAGES ON FLOUR PACKAGES: AN EVALUATION OF THREE DIFFERENT FRAMED MESSAGES

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: Flour safety messages on packages have been used as a strategy in communicating to consumers. However, their effectiveness in achieving the intended behavioral outcomes remains uncertain, warranting further investigation.

Purpose: This study evaluates the effectiveness of safety messages on flour packaging by assessing consumer perceptions of three distinct message framings and identifying strategies to optimize their impact.

Methods: A nationally representative sample of U.S. wheat flour consumers (N = 1,218) participated in an online survey. We employed the Perceived Message Effectiveness (PME) scale to evaluate three framed flour safety messages (Control, Loss, and Non-Loss) in order to assess their potential to influence behavior. Participants were presented with various attributes of flour safety labels and asked to select their preferences. Additionally, they answered a series of questions related to flour handling practices and the optimal placement of flour safety messages on packaging.

Results: The Loss-framed message (PME = 4.09) and the non-Loss-framed message (PME = 4.04) were perceived as significantly more effective than the Control message (PME = 3.91) ($p < 0.01$). Our findings indicate that the Loss-framed message was the most preferred flour safety message, selected by 56.5% of participants. Additionally, 55.7% of participants preferred placement of flour safety messages on the front of the package. Notably, 55% of participants reported that they had not observed safety messages on wheat flour or baking mix packages. When evaluating the attributes of flour safety labels, the terms "Warning" and "Caution" emerged as the most preferred prefixes for food safety labels on flour packaging.

Significance: This study underscores the importance of message framing in enhancing the effectiveness of food safety communications. The findings provide valuable insights that can guide policymakers and the flour industry in developing more impactful and consumer-centered flour safety messages.

Award Application: Developing Scientist Award

T10-05: CONSUMER RESPONSE TO ALTERNATIVE DESIGNS FOR A REVISED SAFE HANDLING INSTRUCTIONS LABEL: FINDINGS FROM AN EXPERIMENTAL WEB-BASED SURVEY

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: Consumer groups and other stakeholders have continued to advocate updating the Safe Handling Instructions (SHI) label.

Purpose: We used an experimental web-based survey to determine if alternative designs for a revised SHI label would garner more visual attention, change risk perceptions, or impact other outcomes compared with the current SHI label.

Methods: Respondents were randomly assigned to 1 of 10 conditions: 9 test labels and the current label (control) (n=2,400). The 9 test labels had the same instructions and icons (e.g., thermometer for cook message), but varied in terms of shape, title, and risk message. To measure visual attention, a limited time exposure experiment was used where respondents were briefly shown a mock ground beef package with the assigned SHI condition then asked to recall what they saw. Other outcomes were measured using responses to questions that used a 5-point Likert scale.

Results: The rate of unaided recall was 15%–33% for the nine test labels, compared to 14% for the current SHI. For six of the nine test labels, the rate was significantly higher compared with the current SHI ($p < .05$). For aided recall, none of the test labels performed better relative to the current SHI. For the outcome “grabbed attention,” two test labels (mean scores = 3.94, $p = .0006$ and 3.80, $p = .0003$) performed better relative to the current SHI (mean score = 3.49). These

two labels were shaped like an octagon, stated “IMPORTANT! AVOID ILLNESS” at the top, and stated, “This raw product may contain bacteria, follow these instructions to avoid illness.” For the other outcomes examined, none of the test labels performed better relative to the current SHI.

Significance: The study suggests that a revised SHI label may do a better job of attracting consumers’ attention, thus potentially increasing the likelihood that consumers will read and pay attention to the label’s instructions.

T10-06: USING MIXED METHODS COMMUNICATION RESEARCH TO PREVENT CRONOBACTER IN THE U.S.

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: CDC used mixed research methods in summer 2024 to study *Cronobacter* infection prevention knowledge and behavior among pregnant women, infant caregivers, and healthcare providers. *Cronobacter* infections are rare, and most often occur in infants younger than 2 months, often causing death in this age group. *Cronobacter* infection can be prevented by following specific steps to properly prepare and store infant formula.

Purpose: This study aimed understand knowledge and behavior gaps related to *Cronobacter* infections among key US populations. The findings will be used to develop a science-based communication strategy that aims to prevent *Cronobacter* infections in infants.

Methods: Attitudes, knowledge, and behaviors regarding *Cronobacter* were tested among 4,371 US adults with demographic predictors analyzed via logistic regression. in-depth interviews were conducted with 33 healthcare providers and coded for foodborne and waterborne illness priorities including *Cronobacter*. Knowledge and education behaviors related to *Cronobacter* were surveyed among 100 US healthcare providers and analyzed with descriptive statistics.

Results: *Cronobacter* knowledge and awareness is low; 7.5% of respondents were familiar with the pathogen and 25.1% were aware that infant formula is a potential source of infection. Lower-income participants were more aware of risks from harmful germs in powdered formula (OR=1.64, CI=1.19-2.26) and more likely to have some *Cronobacter* knowledge (OR=1.64, CI=1.30-2.04) compared with higher-income respondents. Black participants were more aware that formula can

make babies sick (OR=1.76, CI=1.41-2.19) and more likely to have some *Cronobacter* knowledge (OR=1.69, CI=1.37-2.08) than White participants. Healthcare providers lacked knowledge about *Cronobacter* infections and cited time constraints as barriers to discussing foodborne illness risks, ranking *Cronobacter* as a low priority for patient education, including those who saw pregnant patients or parents of young children.

Significance: These findings highlight the need for communication interventions to increase awareness among the infant caregivers and healthcare providers, such as educational videos, which are under development. Further exploration is required to understand findings regarding race and income.

T11-01: DETECTION, PERSISTENCE AND ANTIMICROBIAL RESISTANCE PROFILES OF FOODBORNE PATHOGENS IN NUT ORCHARDS WITH INTEGRATED GRAZING ANIMALS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Integrating grazing animals into tree nut orchards can enhance farm sustainability and soil quality. However, this practice may also serve as a pathway for the introduction of foodborne pathogens including *Salmonella enterica* and Shiga toxin-producing *Escherichia coli* (STEC).

Purpose: This study investigated the role of livestock in orchard systems as potential sources of pathogenic bacterial contamination by examining pathogen transfer and persistence in the environment.

Methods: Soil and fecal samples from two almond and one walnut orchard in California were collected periodically for 120 days following grazing events with sheep or chickens. Samples were enriched and confirmed via PCR for the presence of STEC and *Salmonella enterica*. Antimicrobial resistance (AMR) profiles were assessed using microdilution assays to determine minimum inhibitory concentrations (MICs) for relevant veterinary antimicrobials. Whole genome sequencing (WGS) was employed to investigate AMR genes and to analyze population structures.

Results: Of the 287 soil and 83 composite fecal samples collected, all tested negative for *Salmonella enterica* and *E. coli* O157. Non-O157 STEC was detected in 3.5% (n=10) of the soil samples, and 33.7% (n=28) of fecal samples. Four fecal and one soil isolate exhibited phenotypic resistance to at least one antibiotic (ceftriaxone, tetracycline, chloramphenicol, or ceftiofur). Phylogenetic analysis of WGS data demonstrated isolates partially clustering by farm and sample type.

Genomic analysis of AMR genes showed the presence of genes conferring singular and multiple drug resistance in a number of the STEC isolates.

Significance: While pathogens were detected in animal feces, their transfer and persistence in orchard environments was rare, with low prevalence overall. These results provide evidence to inform food safety guidelines on nut crops integrated with livestock, while prioritizing sustainability.

T11-02: INVESTIGATING BIOAEROSOL TRANSFER OF ESCHERICHIA COLI AND OTHER COLIFORMS FROM COMMERCIAL POULTRY OPERATIONS IN THE SOUTHEASTERN UNITED STATES USING A PASSIVE SAMPLING APPROACH

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Bioaerosol contamination has been linked to foodborne illness outbreaks in fresh produce near animal operations, but data on bacterial populations disseminated via bioaerosols are limited.

Purpose: This study aimed to quantify airborne indicator microorganisms emitted from concentrated poultry houses at various setback distances.

Methods: Four commercial poultry farms in the Southeastern United States were each visited three times over one flock cycle. Microbial populations deposited from the air were measured using a passive sampling approach. Grade 90 mesh cheesecloths (91.44 cm × 45.72 cm) were secured on wooden stakes at a height of 0.4–0.9 m above the ground at distances of 0, 50, 100, 400, and 1000 m from poultry house exhaust fans. At 0, 50, and 100 m, cheesecloths were accompanied by weather stations measuring air temperature, relative humidity, wind speed, gust speed, wind direction, and particulate matter concentration. After 12 hours of exposure (overnight), cheesecloths were collected, placed into Whirl Pak bags with

100 mL of buffered peptone water containing 0.2% (v/v) Tween 80, and stomached for 1 minute at 240 rpm. Rinsates were plated on Brilliance coliforms/*E. coli* agar in duplicate and incubated at 37°C for 48 hours before enumeration.

Results: The highest concentrations of total coliforms and *E. coli* were found at the exhaust fan location, with counts of 6.51 ± 0.50 and 5.88 ± 0.51 log CFU/sample, respectively. Significant reductions in *E. coli* were observed at 400 m (2.99 log CFU/sample; $p = 0.0003$), with no further reductions beyond this distance.

Significance: Indicator microorganism concentrations decreased significantly with distance from poultry houses and can inform models of bioaerosol pathogen spread, particularly when accounting for environmental conditions.

Award Application: Developing Scientist Award

T11-03: PREVALENCE, CHARACTERIZATION, AND ANTIMICROBIAL RESISTIVITY PROFILING OF SALMONELLA FROM PRE-HARVEST BROILER CHICKENS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Rising concerns of *Salmonella* prevalence even after PAA treatment, a widely used intervention in poultry, suggest potential resistance development, which might contribute to antibiotic resistance and raise health concerns.

Purpose: To examine the prevalence, characterization, and antimicrobial (biocide and antibiotic) resistance of *Salmonella* in preharvest broiler chickens.

Methods: Samples were collected as cloacal swabs (n=40), environmental (n=45), feed (n=48), litter (n=43), and water (n=45) from farm A (poultry house) and aerosol (n=16), cloacal swabs (n=60), and environmental (n=83) from farm B (after leaving the farm but before entering the processing plant) across 3 seasons. The samples were pre-enriched in buffered peptone water, enriched in Rappaport Vassiliadis broth, and plated onto XLT4 agar. Isolates were confirmed via PCR targeting the *invA* gene and serotyped. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of PAA were determined for 20 isolates, while antibiotic sensitivity tests were conducted on 22 isolates using 16 antibiotics. Tests were triplicated, and data analysis using R-Studio and IBM-SPSS.

Results: *Salmonella* prevalence in farm A was highest in litter (72%), followed by feed (26%) and environmental sponges (18%). Farm B showed a significantly higher prevalence: environmental sponges (95%), cloacal swabs (53%), and aerosols (56%). The predominant serotypes isolated were infantis (65%), Typhimurium (13.5%), and Kentucky (9.2%). All isolates exhibited resistance (when compared to the ATCC strain) to PAA, with MICs ranging from 87.5–137.5 ppm and MBCs from 125–175 ppm. All isolates were multidrug-resistant (MDR), showing resistance to >3 antibiotic classes, including antibiotics like colistin. There were no differences in antibiotic resistivity when the serotypes were compared to different antibiotics ($p > 0.05$).

Significance: The persistence of MDR *Salmonella* infantis and potential PAA resistance in poultry highlights significant health risks. The higher *Salmonella* prevalence in birds before entering the plant could be due to travel/handling stress, as indicated by other studies.

Award Application: Developing Scientist Award

T11-04: TEMPORAL AND ENVIRONMENTAL DRIVERS FOR SURVIVAL OF ESCHERICHIA COLI IN FLORIDA SOILS AMENDED WITH HEAT-TREATED POULTRY PELLETS AND COMPOSTED POULTRY LITTER

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Biological soil amendment(s) of animal origin (BSAAO) add nutrients to soils however, previous studies have shown that it can extend the survival of *E. coli* in the amended soils. Quantifying the weather and environmental effects on the survival of *E. coli* in amended soils is important but limited.

Purpose: To quantify the impact of BSAAO type and dynamic environmental conditions on the fate of *E. coli* in soils

Methods: A two-year field study, using a completely randomized design in Florida, evaluated the survival of inoculated *E. coli* TVS 353 in soils amended with Heat-Treated Poultry Pellets (HTPP), composted Poultry Litter (PL) or left unamended (UN) alongside a negative control (uninoculated, unamended). Vidalia onion bulbs were transplanted into selected HTPP plots. Soil samples were collected for up to 140 days for *E. coli* levels. The survival data along with weather variables (rainfall, air temperature, relative humidity, wind speed, and soil temperature) were fitted using a linear mixed effect model (LME).

Results: Overall, *E. coli* levels were 1.8 and 1.6 log CFU/g higher in HTPP than UN-

amended plots in years 1 and 2, respectively. *E. coli* levels in soils significantly decreased over the 140 days in both years, irrespective of soil amendment treatment ($p < 0.05$). The decline in *E. coli* levels was explained by different weather variables. The LME for soil amendment treatment plots identified weather variables that significantly influenced *E. coli* survival ($p < 0.01$), specifically cumulative rainfall (+0.69), and wind speed (-0.71) in Year 1, due to high rainfall events and wind speed during the initial days of the experiment (0-14). For HTPP plots with and without onions, wind speed (+0.50) (Year 1) was significant ($p < 0.05$).

Significance: The LME model developed in this study may help relate weather variables and their timing, to patterns of *E. coli* survival in soils containing BSAAO

Award Application: Developing Scientist Award

T11-05: COMPARATIVE ANALYSIS OF NOROVIRUS-LIKE ILLNESS REPORTING PATTERNS ACROSS FOOD SERVICE SECTORS

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Presentation type/Format: Technical

Category: Data Management and Analytics

Abstract:

Introduction: Human norovirus is the leading cause of foodborne illness in the U.S., with foodservice-related outbreaks prevailing. Aside from investigated outbreaks linked to food service, the complete disease burden is unclear. Crowdsourced data offers a novel approach to characterize sector-specific risk factors, aiding targeted prevention strategies.

Purpose: To analyze, using crowd-sourced data, temporal patterns of norovirus-like illness reporting between chain and independent foodservice establishments.

Methods: Self-reported illness data (N=109,599) were extracted from the crowdsourcing platform [iwaspoisoned.com](https://www.iwaspoisoned.com) from January 1, 2021, to December 31, 2024. inclusion criteria required U.S.-based reports containing symptoms and consumer attribution to a foodservice establishment. Reports were categorized as “norovirus-proxy” when both vomiting and diarrhea were reported, and “All Other” for the remainder. Two cohorts were analyzed: (i) 20 national foodservice chains; and (ii) over 15,000 independent establishments. Reports identified as spam or duplicates were excluded. Year-over-year patterns were analyzed using Chi-square tests of independence to assess temporal relationships between establishment type and norovirus-proxy reporting rates.

Results: Statistical analysis demonstrated that norovirus-proxy reporting patterns differed significantly between chain and independent establishments over the study period ($\chi^2 = 245.41$, $df = 3$, $p < 6.43 \times 10^{-53}$). We observed that independent restaurants consistently exhibited higher proportions of norovirus-proxy reports, increasing from 45.1% in 2021 to 49.3% in 2024. in contrast, chain establishments showed more stable reporting patterns, ranging from 41.0% to 44.0%. Systematic variation between establishment types persisted across all years.

Significance: This study provides statistical evidence of systematic differences in norovirus-proxy reporting patterns between chain and independent food service establishments, with independents showing consistently higher rates. This suggests fundamental differences in food safety practices between establishment types. Such insights can inform targeted intervention strategies and highlight potential areas for enhanced food safety training, particularly in independent establishments.

T11-06: PSYCHROTROPHIC LACTIC ACID BACTERIA AS STARTER CULTURES: THEIR EFFICACY IN CONTROLLING FOODBORNE PATHOGENS IN KIMCHI

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Presentation type/Format: Technical

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Kimchi is a traditional Korean fermented vegetable dish that undergoes fermentation at low temperatures without pasteurization. Despite its acidic environment, which generally inhibits microbial growth, kimchi can be susceptible to contamination by acid-tolerant foodborne pathogens.

Purpose: We investigated the antimicrobial effects of psychrotrophic lactic acid bacteria (LAB) as starter cultures on the survival of foodborne pathogens during kimchi fermentation.

Methods: The psychrotrophic LAB starters (*Dellaglistia algida* and *Leuconostoc gelidum* subsp. *aenigmaticum*) and foodborne pathogens (*Escherichia coli* O157:H7 and *Listeria monocytogenes*) were inoculated into the kimchi at final concentrations of approximately 7 and 6 log CFU/g, respectively. Control samples were divided into three groups: one without LAB starter culture, one with a mesophilic LAB starter (*Leuconostoc mesenteroides*), and one without any inoculation. All samples were stored at 4 °C for 42 days, and microbial and physicochemical changes were monitored during fermentation. All experiments were performed in triplicate, and statistical significance was analyzed by Dunnett's multiple-range test ($p < 0.05$).

Results: During the entire fermentation period, the introduced starter cultures consistently dominated the total LAB populations, comprising more than 70%. Compared to kimchi inoculated with the mesophilic LAB starter, those with psychrotrophic LAB showed significantly greater reductions in populations of *E. coli* O157:H7 and *L. monocytogenes*, achieving an additional decrease of 1.8 and 1.0 log CFU/g, respectively, after 42 days. There was no significant difference in pH and acidity between the starter and non-starter kimchi.

Significance: These results demonstrate that psychrotrophic LAB can enhance the safety and quality of kimchi by effectively reducing foodborne pathogens during fermentation without adverse effects on product quality.

Award Application: Developing Scientist Award

T12-01: BUILDING A DECISION SUPPORT SYSTEM TO ADDRESS FOOD SAFETY AND DISPARITIES THROUGH A MULTI-METHOD, INTERDISCIPLINARY APPROACH: SALMONELLOSIS IN THE POULTRY INDUSTRY AS A USE-CASE

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: Transformative efforts are needed to significantly reduce salmonellosis in the United States, which has been persistent for over three decades, costing \$4.1 billion annually.

Purpose: This project develops a Decision Support System (DSS) by integrating interdisciplinary methods into a technical solution aimed at addressing food safety challenges through use-inspired research.

Methods: We engaged in three-phase prototype development through interviews with subject matter experts and end-users. Here we triangulate interdisciplinary methods to offer a technical solution as part of our SENS-D (Sensor enabled Decision Support System) DSS powered by one Health data, including descriptive information about food disparities, predictive models for pathogen growth and shelf-life, and optimization models for sensor placement and intelligent distribution.

Results: Using Missouri as a use-case in 2023, 10.5% of Missouri counties (12/114) demonstrated both the highest rates of salmonellosis and food insecurity. of the highest-risk counties, all twelve were rural and located south of the interstate 70 corridor, with seven forming a cluster in southeast Missouri. Food disparity maps guide food safety Extension staff in targeted outreach, while predictive models

using sensors track temperature fluctuations in the supply chain through retail to assess pathogen growth and shelf-life. These models provide data-driven decisions on product disposition, improving food safety and reducing waste. Results indicate raw poultry risks increase with temperature abuse and long transportation times. Optimized sensor placement with our models ensures cost-effective monitoring across the supply chain, while an intelligent distribution model uses pathogen sensing/testing and prediction data to optimize product diversion and distribution decisions for safety, efficiency, and equity.

Significance: Our SENS-D DSS uses convergence science to optimize pathogen testing and sensing along the supply chain with goals to improve public health and reduce disparities. This interdisciplinary approach delivers actionable insights for food safety stakeholders and health partners with measurable impacts.

T12-02: FOOD SAFETY INTERVENTION IN REDUCING ESCHERICHIA COLI CONTAMINATION

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: Foodborne diseases impose substantial public health and socio-economic burdens worldwide, with the greatest health burden falling on low- and middle-income countries (LMICs). These regions need food safety interventions that are context-specific, go beyond training and information provision, and integrate intensive engagement of participants to mitigate the problem.

Purpose: To estimate the effect of the intervention on *Escherichia coli* reduction in butcher shops in Addis Ababa, Ethiopia.

Methods: This study was a parallel randomized controlled trial (RCT) of a food safety intervention implemented in butcher shops. The shops were individually randomly assigned to an intervention arm or control group, with 100 shops in each group. Intervention 1 received training and hygiene materials. Intervention 2 ('scores on doors'), in addition to the above, involved shop inspections, public posting of ratings, and hygiene evaluations. Control shops received no intervention. Surface swab samples from shop workers' hands, meat cutting boards, meat hanging boards, meat surfaces, and water samples were collected pre- and post-intervention using Hygiena swabs. Samples were transported in a portable fridge to the laboratory, incubated, and analyzed for the presence of *E. coli*. Mixed-effects logistic regression was used for data analysis.

Results: Samples from the 'scores on doors' shops were 43% (OR: 0.57, 95% CI: 0.37, 0.89) less likely to be contaminated with *E. coli* compared with controls. Samples from intervention 1 shops were 32% (OR: 0.68, 95% CI: 0.44, 1.06) less likely to be contaminated than controls, though this result was not statistically

significant. The ‘scores on doors’ intervention showed a significant reduction of *E. coli* on workers’ hands and hanging boards, but reductions on meat surfaces, cutting boards, and water were not statistically significant.

Significance: Food safety interventions that combine education, hygiene materials, and public rating systems like ‘scores on doors’—while also incorporating intensive participant engagement—can effectively reduce microbial contamination in LMICs.

T12-03: FROM PAST TO PRESENT: THE EVOLUTION OF FOOD SAFETY MANAGEMENT AND FOOD SAFETY CULTURE IN THE CALIFORNIA ALMOND INDUSTRY

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: The California almond industry experienced a remarkable transformation in food safety management and culture after outbreaks of salmonellosis associated with raw almonds in 2001 and 2004.

Purpose: This study aims to showcase the evolution of food safety management and culture in the California almond industry and identify key factors contributing to these changes.

Methods: Multifaceted methods were used: (1) Content analysis of minutes from 99 food safety committee meetings and of 35 action plan updates from the Almond Board of California (ABC); (2) Content analysis of 100 news articles covering the outbreaks and the almond mandatory pasteurization program; (3) Semi-structured interviews with 11 experienced individuals involved with the almond industry during the early 2000s; and (4) Semi-structured interviews with 15 current food safety managers from almond processors.

Results: The study identified two factors for improvements in food safety management, which were (1) repeated outbreaks (with 11% more news coverage in the second outbreak than the first), which catalyzed changes toward food safety, and (2) ABC's proactive leadership in driving these changes. The participants highlighted ABC's efforts in crisis management, risk assessment, and the development of a mandatory pasteurization program, establishing a model for industry self-regulation and long-term food safety control. The evolution of food safety culture was marked by increased prioritization, stronger management commitment, and reduced resistance to change. A combination of external factors, such as food safety regulations and third-party certification requirements, along with internal factors, such as the company's commitment to food safety and ongoing communication, were identified as influencing advancements in food safety culture.

Significance: Positioning the almond industry's approach as a replicable framework, this research provides actionable lessons for other sectors seeking proactive, industry-driven food safety improvements and self-regulation to enhance food safety. This research also offers valuable insights into the dynamics of cultural transformation.

Award Application: Developing Scientist Award

T12-04: LONGITUDINAL IMPACT OF FOOD SAFETY TRAINING ON AUDIT RESULTS

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: The adoption of digital training has enabled the food industry to deliver more food safety training at a lower price and greater convenience. However, the design of digital training often affects the transfer of learning into real settings, as it tends to focus on measuring learning effectiveness without considering the impact on food safety management system performance.

Purpose: This study aims to understand which instructional design and training management practices influence the transfer of food safety training and its impact on audit outcomes.

Methods: Instructional design and training management data — including count of training completed, total duration of training, average time lag from date of training to date of audit, count of unique learners, and completion of knowledge assessments for HACCP training — were collected from food manufacturing facilities. Longitudinal multi-level regression models were used to predict internal audit performance.

Results: Data from 5,561 individual training records was used to predict the results of 212 audit events at 95 manufacturing facilities in the United States, Canada, and Mexico. The only statistically significant variable for predicting audit findings was the average time lag between training and audit. A 1.00 ($e^{-0.0011797}$) day increase in time lag was associated with a one-unit increase in audit findings on average ($p = 0.002$), suggesting that more recent training correlates with better audit performance. Other variables — including number of trainings completed since the previous audit, training duration, number of knowledge assessments completed, and number of unique learners — were not significant predictors of audit outcomes.

Significance: This study suggests that the timing of training (specifically, more frequent or recent training) has a greater effect on food safety management system performance than instructional design alone.

T12-05: FOOD SAFETY SUPERVISOR TRAINING PROMPTS CHANGES IN PROCEDURES AND BEHAVIORS ON PRODUCE FARMS

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: The Food Safety Modernization Act Produce Safety Rule and buyer requirements mandate produce farms to implement food safety programs. Food safety supervisors often play a primary role in creating and managing food safety programs that must be tailored to the farm's practices.

Purpose: The objective of this training is to provide food safety supervisors with education, experiences, and resources to support implementation and management of food safety on the farm and support cultivation of a food safety culture.

Methods: A one-day training was created to share information and resources to support food safety managers with implementation and management of food safety requirements. Presentations and activities were given on five topic areas: Managing a Worker Training Program, Pre-Harvest Water, Water Treatment, Harvest and Post-Harvest Cleaning and Sanitation, and Food Defense and Traceability. Two industry panels with question-and-answer sessions facilitated discussion among participants, and a breakout activity allowed attendees to realize the importance of well-written SOPs. Evaluations were administered via a Qualtrics survey to capture program quality, skills, and intended behavior changes. A six-month follow-up survey was sent to attendees to capture on-farm changes as a result of participating in the training.

Results: Twenty-five food safety supervisors representing thirteen farms attended the program. Post-training evaluations (n=15) indicated attendees learned concepts or lessons and shared resources that will result in changed food safety practices or behaviors. These practices include changing SOPs, modifying worker training programs, setting a good example, and increasing documentation of food safety practices. Attendees reported they realized the importance of creating a food safety culture and indicated the discussion among participants was a beneficial part of participating.

Significance: Food safety supervisors benefited from attending the training by receiving more in-depth information and reported implementing changes to farm policies and behaviors.

T12-06: TEMPERATURE VARIABILITY IN POWDERED INFANT FORMULA RECONSTITUTION: IMPLICATIONS FOR CRONOBACTER INACTIVATION AND PUBLIC HEALTH GUIDELINES

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: *Cronobacter spp* can be found in powdered infant formula (PIF), and can cause infections in high-risk infants. Public health guidelines recommend reconstituting PIF with water heated to 70°C (158°F) to inactivate *C. sakazakii*. However, the consumer guidance might not specify a target temperature but instead give general instructions (e.g., “bring water to a boil, wait 5 min”).

Purpose: To quantify the differences in PIF reconstitution temperatures achieved under different preparation conditions, and identify conditions that ensure a PIF reconstitution temperature of 70°C (158°F).

Methods: We evaluated three heating vessels (stainless-steel pot, Pyrex measuring cup, electric kettle) under various preparation conditions (i.e., lid on/off, removal from heating surface, different water volumes) that may impact cooling. Additionally, four types of baby bottles (4 oz and 8 oz, BPA-free polypropylene and glass) were tested. We quantified temperature drops that occurred during the “wait 5 min” hold time, following water transfer to the bottle, after PIF addition and the “hot shot” method. Statistical analysis was conducted using ANOVA and Tukey’s HSD tests.

Results: Significant differences were observed based on vessel volume ($p < 0.05$), lid condition ($p < 0.001$), and removal from heating surface ($p < 0.01$). Water transfer to the bottle also showed significant variation by bottle material and volume ($p < 0.01$, $p < 0.001$). The greatest temperature retention occurred with an 8 oz polypropylene bottle (final temperature 81.9 ± 0.9 after PIF addition). In contrast, the lowest temperature was observed in a 4 oz glass bottle (final temperature 58.9 ± 0.7 after PIF addition). The consumer “hack” using the “hot shot” method, starting at 70°C with 1 oz volume of water, resulted in even lower temperature ($49.2^\circ\text{C} \pm 2.9$ to $60.2^\circ\text{C} \pm 0.6$).

Significance: These

Results suggest that PIF heating and preparation methods, following public health guidance, may render water temperatures too high at the end of the 5 min waiting period, or not achieve sufficient conditions for microbial safety when PIF is added, highlighting the need for more reliable guidelines and consumer education to prevent *Cronobacter* infections.

Award Application: Developing Scientist Award

T13-01: EVALUATION OF TOOLS AND CRITERIA FOR *SALMONELLA* SEROTYPE DETERMINATION BY NANOPORE SEQUENCING

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Traditional serology for *Salmonella* is a time-consuming process which requires a large number of antisera. The process is highly dependent on the analyst performing the testing. The need for rapid and accurate serotype determination has been an ongoing goal for food manufacturers and producers. Molecular serology provides an accurate alternative to traditional serology.

Purpose: The purpose of this work is to establish the criteria needed for accurate determination of serovar from sequencing *Salmonella* isolates.

Methods: Twenty-four *Salmonella* isolates obtained from food and environmental samples (and identified by traditional serology) underwent DNA extraction by two different methods (bead-based vs. chemical lysis). The resulting DNA was then used for Oxford Nanopore Technologies (ONT) sequencing with the ONT Rapid Barcoding kit. Samples were sequenced for twelve hours, and results were analyzed every two hours for the full time course. Results analysis was done using three tools (Epi2ME, SeqSero2, and SISTR), and results were compared based on analysis tool, sequencing time, and sequencing yield.

Results: Marked differences were observed between the three analysis tools. SISTR returned correct serotype results in 130/144 analyses, Epi2ME returned correct serotype results in 78/144 analyses, and SeqSero2 returned correct serotype results in 104/144 analyses. Results were compared based on criteria such as quality score, yield, and coverage. When applying a per-sample sequence yield minimum of 10 megabases (Mb), serotype calls greatly improved. For this 10 Mb criteria, correct serotype calls were made 119/119 times, 78/119 times, and 96/119 times, by SISTR, Epi2ME, and SeqSero2, respectively. Two hours of sequencing time yields, on average, 55.8 Mb of sequence, well above the 10 Mb criteria.

Significance: This work established a methodology using bead-based extraction and long read sequencing for accurate *Salmonella* serotype determination from an isolate within 4 hours.

T13-02: GENOMIC EVALUATION OF SALMONELLA ENTERICA SER. DUBLIN IN CATTLE AND HUMANS IN THE UNITED STATES

Primary Author:

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Increasingly antimicrobial resistant (AMR) *Salmonella* Dublin are a threat to human and animal health, therefore requiring a One Health approach to comprehensively understand pathogen evolution. Moreover, *S. Dublin* dissemination throughout the United States and the food supply chain is a concern for food safety and security.

Purpose: This study leverages multi-agency biosurveillance data and genomic sequencing of *S. Dublin* strains to provide a robust analysis of its evolution across human, animal, and environmental reservoirs. Furthermore, factors driving AMR emergence are investigated to inform interventions to protect public health.

Methods: A total of 2,150 strains collected between 2002–2023 throughout the United States from clinical bovine (N=581), clinical human (N=664), and environmental (N=905) sources were identified. After uniform quality control, raw reads were assembled de novo followed by genome annotation and characterization of plasmids, antimicrobial resistance genes, and virulence factors. Strain relatedness was calculated using a core genome maximum-likelihood phylogeny, and pairwise core genome single nucleotide polymorphism (SNP) analyses were performed to assess genetic relatedness between strains.

Results: Strain source substantially influenced genomic characteristics. Bovine clinical strains demonstrated the highest prevalence of drug-specific antimicrobial resistance genes and of multidrug resistance plasmid, *incA/C2* ($p < 0.001$). The pangenome of bovine strains was the most open, indicating greater genetic diversity, while environmental strains displayed the most closed pangenome. Strains were highly related, with 72% of *S. Dublin* strains in our study differing from at least one other strain by 20 or fewer SNPs.

Significance: The high degree of genomic similarity highlights the potential for cross-transmission between humans, animals, and the environment and underscores the importance of considering strain source when assessing and monitoring antimicrobial resistance.

T13-03: A NEW STEC METHOD IN LINE WITH THE USDA-FSIS MLG5C.04 TO IMPROVE FOOD BUSINESS OPERATORS' SCREENING OF PRIORITY STEC IN BEEF MEAT

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) carrying the eae gene of serogroups O157, O26, O111, O103, O145, O121, O45 and more recently O80 (top 8 serogroups), are usually monitored worldwide, whereas eae-negative STEC strains that are less clinically significant are not surveyed. Screening beef with stx and eae is challenging, overestimating the presence of highly virulent STEC, causing disruption and costs for food producers.

Purpose: This study aims to evaluate new genetic markers recently introduced in the updated MLG5C.04 reference method to improve specificity and effectiveness of priority STEC.

Methods: 5,357 beef samples (25g enrichment each) were tested by PCR targeting virulence genetic markers, the top 8 serogroups plus O91, O171, O174, O148, O146, O128, O113, and O104.

Results: We showed that 92% of the 2,121 stx-positives ($p < 0.05$) carry stx2 alone or in association with stx1. Among the stx2-positive samples, stx2a and/or stx2d subtypes dominate. Introduction of espK, espV markers on 868 stx+/eae+ beef enrichment broths reduced the number of presumptive positives by 31% ($p < 0.05$), compared to the ISO/TS 13136:2012 reference method. Subsequent analysis of the presumptive positives combining the O-group and the eae-subtype provided a significant reduction of the number of the presumptive positives for the top 8 eae-positive STEC serogroups; and showed that O26, O103 and O157 were the most prevalent ones. Regarding the stx+/eae- samples, which are proportionally predominant in beef as compared with the stx+/eae+ samples, 65% ($p < 0.05$) of them were positive for the serogroups monitored in this study (O91, O171, O174, O148, O146, O128 O113 and O104).

Significance: Routine testing of beef samples should be revised to prioritize a hierarchical surveillance system based only on high risk STEC (STEC carrying the eae gene) and not on all STEC. This approach would provide Food Business

Operators a significant improvement, saving time and costs while maintaining a high level of product safety.

T13-04: UNLOCKING GENOMICS FOR FOOD SAFETY: ADVANCING WGS APPLICATIONS AND OVERCOMING ADOPTION BARRIERS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Purpose: Globalization of food production has significantly extended supply chains, increasing the complexity of foodborne pathogen tracking and outbreak investigations. Our genomics program aims to evaluate and demonstrate the potential of Whole Genome Sequencing (WGS) in identifying the root causes of microbial food safety issues. By addressing technical and practical barriers, this program strives to advance the adoption of WGS and pave the way for transitioning food safety management from reactive to predictive and preventative systems.

Methods: Over the past decade, we have developed and evaluated workflows that integrate WGS into food safety practices. Leveraging third-generation sequencing technologies, the program enhanced pathogen detection and characterization. one key achievement is the development of an internal bioinformatics platform with a user-friendly interface, designed to simplify data analysis and make WGS more accessible for non-specialists. This platform reduces reliance on external expertise, enabling faster and practical implementation of genomics for food safety.

Results: The program's advancements reduced *Salmonella* serotyping time from over seven days to eight hours, improving incident response and contamination control. For *Listeria*, species-level identification was achieved within 24 hours, while *Listeria monocytogenes* subtyping was completed within 48 hours. These achievements illustrate the practical potential of WGS for accelerating pathogen characterization. Additionally, by addressing hurdles such as data analysis complexity and resource demands, our bioinformatics platform has made progress toward lowering barriers to WGS adoption. However, challenges such as high implementation costs and scalability remain, and this program represents an important step in paving the way for broader industry adoption.

Significance: This demonstrates how genomics can support the evolution of food safety management by addressing real-world challenges and showcasing practical applications of WGS. While significant hurdles remain, it highlights the potential of

WGS in microbial risk management and foster a predictive, preventative approach to food safety. Sharing our experiences aims to encourage collaboration across the industry and contribute to a more resilient global food supply chain.

T13-05: PARALLEL DETECTION OF CARBAPENEM RESISTANCE GENES BLANDM-1 AND BLAOXA-1 USING A PLASMONIC NANO-BIOSENSOR AND FIELD PORTABLE DNA EXTRACTION METHOD

Primary Author:

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Antimicrobial resistance (AMR) is a rapidly growing global concern resulting from the overuse of antibiotics in agricultural and clinical settings. Carbapenems are an important class of antibiotics due to their broad-spectrum effectiveness for infections, and increasing resistance to carbapenems is a major concern in the healthcare community.

Purpose: This study is to develop a rapid, carbapenem-resistant gene detection method utilizing a plasmonic nano-biosensor.

Methods: In this work, a DNA-based biosensor was developed to detect carbapenem resistance genes blaNDM-1 and blaOXA-1 using gold nanoparticles (GNPs) as a signal reporter that enables the visible differentiation between target and non-target DNA. This biosensor produces optical results due to the unique properties of GNPs. When GNPs aggregate, they become blue, which signifies the absence of target DNA in the sample. A red sample signifies the presence of target DNA. In addition to qualitative results, absorbance spectra can quantitatively determine whether the sample contains target DNA by comparing the ratio of absorbance values at 520 nm and 620 nm (A_{520}/A_{620}) for target and non-target samples. A high A_{520}/A_{620} indicates a redder target sample, and a lower A_{520}/A_{620} indicates a bluer, non-target sample. Bacterial DNA is extracted through a simple boiling procedure, and samples are run using a portable thermal cycling device.

Results: Preliminary data from pure bacterial cultures and clinical isolates show, with a sample size of five ($n=5$) and 95% confidence ($p=0.05$), successful differentiation between target DNA containing AMR genes blaNDM-1 and blaOXA-1 and non-target DNA from susceptible strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Staphylococcus aureus*.

Significance: With a 45-minute assay time, this biosensor has the potential to be more accessible, affordable, and rapid than current testing methods. The rapid detection of carbapenem resistance genes is useful to screen for AMR in food

products, crops, and animals to prevent the spread of AMR between these potential sources and humans and the environment.

T13-06: POPULATION STRUCTURE ANALYSIS OF SALMONELLA SEROVAR MUENCHEN TO REDEFINE GENO-SEROTYPING USING GENOME INDEXING APPROACHES.

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Precise identification of *Salmonella* serovars from food matrices is critical for source attribution of illness outbreaks. Validated genomics-based methods like SeqSero2 have proved successful for serotype determination. Advances in bioinformatic methods using genome indexing can be used to update the serovar calling scheme by incorporating genome proximity calls while preserving the historical context of serotyping nomenclature.

Purpose: This study will help elucidate genomic lineages of confounding serovars that are polyphyletic serovars—serovars with the same antigen scheme but are genomically disparate.

Methods: *Salmonella* Muenchen was selected as an example of a polyphyletic serovar. Sequences attributed to *Salmonella* Muenchen (1,940 - Short Read Archive (SRRs)) were downloaded from NCBI Pathogen Detection. Serovar calls based on genome indexing were made with bettercallsal (v 0.7.0) while geno-serotyping was performed using SeqSero2. SRRs with conflicting results between SeqSero2 and bettercallsal were analyzed further using SISTR to obtain cgMLST serovar calls. For cgMLST serovar calls that agreed with bettercallsal, SRRs were assembled using SPAdes. Prokka was used to annotate the assembled contigs and a pangenome was subsequently built using Roary.

Results: Of 1,940 *Salmonella* Muenchen (8.d.1.2, ST-83) SRRs, 153 exhibited conflicts between bettercallsal and SeqSero2. There were 135 conflicting serovar calls between cgMLST and SeqSero2, including Valdosta (n=93, 8.a.1.2, ST-112), Manhattan (n=22, 8.d.1.5, ST-81), and Newport (n=20, 8.e.h.1.2, STs-31, 5, 2424). The pangenome and core genome analysis revealed distinct lineages potentially resulting from niche adaptation. This method offers precise identification of novel STs with a genome indexing approach and genome proximity calls identify the

serovar genomically closest to the confounding serovar.

Significance: Genome indexing provides calls that can distinguish serovars that are genomically disparate. Along with SeqSero, the concomitant use of both tools seems best suited for characterization to obtain the utmost information and a robust prediction which is critical for source attribution in food outbreaks.

T14-01: SANITIZERS AND ANTIMICROBIALS: CONCENTRATION TESTING SYSTEM REVIEW AND ADDITION OF A TOOL FOR SYSTEM VERIFICATION

Primary Author:

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Marina Tschida, Ecolab

Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Test kits are ubiquitous in sanitizer and process water antimicrobial monitoring, yet very little data is provided with kits with regard to measurement accuracy and ongoing verification, and improvements to address these shortcomings will be presented.

Purpose: Assessment of test kit performance for oxidizer-based antimicrobials is presented, addressing volume delivery of the critical titrant component, and an overall assessment of test kit accuracy was verified with an equivalency standard.

Methods: Titrant volume delivery methodology was measured gravimetrically, with assessment of controlled variations in the delivery method with regard to rate of titrant addition and methodology of dropper bottle operation, or constant pressure versus pulsed pressure to obtain titrant drop outputs. The use of a reference standard for the

Purpose of assessing the test kit on a systematic basis was also used to verify the accuracy of the test kit.

Results: Outcomes determined that a variable drop rate application of at least 1 drop/second with constant pressure resulted in the most accurate delivery with the lowest variance. Methods where constant bottle pressure was applied resulted in 10-drop averages greater than a 0.50 g target output, and those averages were more precise than the methods where pressure was fully released between drops. Methods where bottle pressure was fully released between drops resulted in 10-drop averages lower than the 0.50 g target output. Using a single factor ANOVA test, all methods were statistically different from each other. Accuracy assessments further reduced test kit variance from >20% to within 5% of the stated equivalence value.

Significance: The assessment of the test kits results in the identification of best practice methodology for operating the test kits, and utilization of an oxidizer equivalence standard provides routine users the ability to demonstrate system suitability on a routine basis, a glaring omission in current monitoring programs.

T14-02: BEHAVIOR OF SALMONELLA ON FROZEN CANTALOUPE UNDER FREEZE-THAW CONDITIONS

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Presentation type/Format: Technical

Category: Retail and Food Service Safety

Abstract:

Introduction: Produce-associated outbreaks have been extended due to consumers freezing products that were purchased fresh. For example, the US FDA recommended that consumers who saved frozen cantaloupe for later use be urged to throw away recalled products during the 2023 *Salmonella* outbreak linked to cantaloupe.

Purpose: The objective was to quantify the single and synergistic effect of repeated freeze-thaw cycles on *Salmonella* in frozen cantaloupe.

Methods: Ten-gram samples of fresh cantaloupe were spot inoculated with 10 μ L (8-10 drops) of a five-strain rifampicin-resistant *Salmonella* cocktail at ca. 4 log₁₀ CFU/g. Samples were exposed to seven consecutive freeze/thaw cycles over a week. Each cycle consisted of 24 h of freezing and 15 min, 2 h, or 4 h of thawing at 23 or 35°C. Two trials were conducted with five replicates per treatment. Samples were mixed with 20mL of peptone water and macerated for 90 s at low speed. Serial dilutions were surface plated in duplicate on tryptic soy agar + rifampicin (80ppm) + 0.1% sodium pyruvate. *Salmonella* concentrations across treatments were analyzed using linear models in R-Studio (V.4.3.3).

Results: Based on linear models, *Salmonella* concentrations did not significantly increase in samples thawed for 15 min, 2 h, and 4 h at 23°C and 15 min and 2 h at 35°C and during any of the seven freeze/thaw cycles. For samples thawed for 4 h at 35°C, an upward trend was observed after the second cycle, and a 1 log₁₀ CFU/g increase was detected at cycle 5. By cycle 7, *Salmonella* concentrations increased 2.09 log₁₀ CFU/g.

Significance: This study shows that *Salmonella* can persist and grow during repeated freeze-thaw cycles, highlighting the need for follow-on research to inform recommendations for better managing salmonellosis risks associated with consumption of thawed frozen produce.

Award Application: Developing Scientist Award

T14-03: CONSUMER ACCEPTANCE AND BUTCHER EVALUATIONS OF HYPOCHLOROUS ACID

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Presentation type/Format: Technical

Category: Retail and Food Service Safety

Abstract:

Introduction: Food sanitizers offer a novel and potentially game-changing solution for reducing microbial contamination and enhancing food safety, but affordable, safe, and accessible options for low- and middle-income countries (LMICs) remain underexplored. We conducted experiments to assess the potential of citric acid and hypochlorous acids in butcher shops in Addis Ababa, Ethiopia.

Methods: Red meat samples were collected from butcher shops (n=3) and assigned to hypochlorous acid, citric acid and control, measuring coliform count before and after treatment. Opinions of meat consumers (n=48) about use of food sanitizers on red meat was assessed at butcher shops. In addition, consumers (n=45) evaluated different attributes of meat treated with hypochlorous acid, citric acid and untreated meat. Similarly, butchers (n=10) assessed different attributes of meat (specifically related to shelf life) treated with hypochlorous acid, citric acid and untreated meat stored at their shops at three time intervals (1st day at around 10:00, 1st day at around 15:00 and 2nd day at around 10:00).

Results: Hypochlorous acid and citric acid reduced coliform counts in meat samples almost equally, by 98% and 97%, respectively. About 75% of meat consumers eat meat treated with natural food sanitizers such as lemon and believe that such products improve safety of food. Consumers preferred meat treated with hypochlorous acid (mean acceptance: 0.13, 95% CI: -0.12, 0.38) over controls but rated meat treated with citric acid lower (-0.70, 95% CI: -0.94, -0.45). Butchers rated meat treated with hypochlorous acid higher for improving meat shelf life (mean: 1.2, 95% CI: 0.74, 1.67) compared to controls, while citric acid-treated meat was rated lower (mean: -0.01, 95% CI: -0.34, 0.32).

Significance: Hypochlorous acid's ability to reduce microbial contamination, gain high consumer acceptance, and receive strong butcher ratings for improving meat shelf life makes it a promising and innovative food safety solution for resource-limited settings.

T14-04: FSIS HAZARD IDENTIFICATION PROCESS TO ADDRESS POTENTIAL FOOD SAFETY RISKS

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: The U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) protects public health by preventing illness from meat, poultry, and egg products. Over the last decade, One Health has been incorporated as a strategic approach to address complex challenges by bridging multiple disciplines at local, national, and global levels. One Health is a concept that recognizes that the health of people is closely connected to the health of animals and our shared environment.

Purpose: FSIS uses a variety of tools and resources to help identify hazards in the food industry that could pose a risk to consumers. FSIS has adopted the internationally accepted definition and evaluation criteria for emerging food safety risks. These risks are defined as "a risk that results from a newly identified hazard to which a significant exposure may occur, or from an unexpected new or increased significant exposure or susceptibility to a known hazard." FSIS established the Hazard Identification Team (HIT) to track, evaluate, and prioritize potential emerging or evolving food safety risks related to meat, poultry, and egg products, and bring identified food safety risks to FSIS leadership via the Agency's enterprise governance.

Methods: The HIT uses a structured, science-based evaluation process that incorporates literature reviews, surveillance data, stakeholder input, and expert consultation to identify and assess food safety risks. Risks are scored based on severity, likelihood, and potential public health impact. Evaluated risks are presented to FSIS leadership through formal briefings, and recommendations are generated for follow-up action or further assessment as needed.

Results: This process provides a systematic and proactive approach to evaluate emerging or evolving food safety issues and make science-based recommendations on whether the Agency should focus additional resources to further assess or address the issues. The HIT serves as a primary pathway through which risk evaluation findings and scientific recommendations are brought to the attention of the agency leadership.

Significance: The information and recommendations HIT provides are central to the Agency's One Health decision-making on emerging food safety issues. This

presentation will provide an overview of the HIT process and how it contributes to One Health.

T14-05: BUSINESS AND PUBLIC HEALTH IMPACTS OF A FOOD SAFETY RATING PROGRAM AMONG PORK VENDORS IN VIETNAM

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Presentation type/Format: Technical

Category: Retail and Food Service Safety

Abstract:

Introduction: Despite significant demand for food safety, consumers typically lack credible information about the safety of foods available in traditional and informal markets in LMICs, where microbial contamination is often high.

Purpose: This study tests whether a food safety training and vendor rating intervention can improve microbial safety of pork in traditional Vietnamese markets, where meat is typically stored at ambient temperatures and access to running water is often limited.

Methods: A randomized controlled trial was implemented in 68 traditional pork markets across five provinces in Vietnam, with half of markets assigned to the treatment group. Vendors in treatment markets received a one-hour hygiene training and were invited to enroll in a food safety rating proGram. Food hygiene practices of enrolled vendors were assessed during unannounced inspections, and these vendors were assigned a rating accordingly. Consumers were informed of the meaning of the rating system through an awareness campaign that included distribution of promotional materials and public signage. A subset of randomly selected treatment market vendors were given equipment to enable adoption of recommended practices.

Results: Regression analysis controlling pre-intervention outcomes and additional LASSO-selected controls indicates that food hygiene practices improved in treatment markets (8%, $p < 0.1$), especially for vendors who received equipment

(13.3%, $p < 0.05$) but that contamination of pork was not significantly affected. Pork prices (2.6%, $p < 0.05$) and sales volume (8%, $p < 0.1$) increased in treatment markets, as well as vendor perceptions of consumer demand for cleanliness and food safety (89%, $p < 0.01$). Business outcomes for highly-rated vendors were not statistically different from those of vendors with lower ratings, suggesting that consumers may perceive vendor food safety practices as binary.

Significance: Impacts on business outcomes suggest the potential for food safety rating programs to improve vendor incentives to follow good food safety practices, but more significant improvements in market infrastructure may be necessary to reduce the high levels of contamination observed in traditional meat markets.

T15-01: IMPEDANCE BASED MICROFLUIDIC BIOSENSOR FOR ACCURATE AND QUANTITATIVE DETECTION OF SALMONELLA IN RAW TURKEY PRODUCTS

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella* causes 1.35 million infections, 26,500 hospitalizations, and 420 deaths annually in the U.S., disproportionately affecting lower socioeconomic and underrepresented groups. Despite national prevention efforts, infection rates have remained unchanged for three decades.

Purpose: This study introduces impedance biosensor that leverages microfluidic technology for the rapid and sensitive detection of multiple *Salmonella* serotypes including Typhimurium, infantis, Enteritidis, and Heidelberg, in raw turkey products.

Methods: The biosensor features a unique architecture, integrating microfluidic channels and multiple electrodes and leveraging positive dielectrophoresis to concentrate, guide, and trap *Salmonella* cells onto the sensing electrode. The sensing region features two interdigitated electrode arrays. one coated with a crosslinked *Salmonella*-specific antibody and the other serves as a negative control. Antigen-antibody binding triggers an impedance change, revealing the sample's status. This advanced design ensures efficient cell concentration to detectable levels, even at low concentrations, enabling sensitive, rapid and reliable detection of *Salmonella* with high specificity.

Results: The biosensor exhibited high selectivity, specificity, and a detection limit of 1 CFU/ml, identifying *Salmonella* serotypes within 70 minutes (45 for sample delivery, 10 for binding, 15 for washing). Its impedance-concentration curve

followed a logarithmic trend, crucial for quantitative analysis. The impedance changes were distinguishable between viable *Salmonella* at a low concentration (1 CFU/ml) and non-viable *Salmonella* at a high concentration (1,395 cells/ml). Each sample was tested three times with disposable sensors for accuracy. Fluorescence labeled *Salmonella* were used to validate the sample concentration mechanism and confirm effective focusing and trapping of target cells.

Significance: This project develops a portable, cost-effective, and easy-to-use biosensor for rapid *Salmonella* detection in poultry, enabling prompt interventions of potential *Salmonella* contamination. The technology aligns with USDA FSIS's proposed regulatory requirement for specific *Salmonella* serotypes in raw poultry products. Additionally, the biosensor is adaptable for detection of other foodborne pathogens in products like beef, dairy, leafy greens, and water.

Award Application: Developing Scientist Award

T15-02: QUICK DETECTION OF BIOFILM SPECIES AND CELL COUNTS BY SENSOR ARRAY

Primary Author:

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Biofilm formation by foodborne pathogens on food processing surfaces poses significant risks to food safety and public health. Current methods for its detection are time-consuming and labor-intensive by plating counting.

Purpose: Develop a rapid method for detecting and predicting biofilm species and viable cell counts using a sensor array and machine learning models.

Methods: Biofilms of *Salmonella* Enteritidis, *Escherichia coli* (K12), and *Listeria monocytogenes* were cultivated on stainless steel surfaces and sampled at 12, 24, 48, 72, and 96 hours under room temperature. Biofilm samples were collected by cotton swabs, and after vortexing they were then added to the sensor array composed of 12 single-stranded DNA elements with nanoparticles as quenchers. Fluorescence data were recorded using a plate reader, with 24 replicates per timepoint for each species (total: 360 samples). Viable cell counts were determined by plate counting. Data preprocessing included normalization, train-test split, and principal component analysis (PCA). Machine learning models, including support vector machines (SVM), random forests (RF), and convolutional neural networks (CNN), were employed to classify species and predict viable cell counts.

Results: Cell counts increased significantly between 12 and 72 hours across all species. The sensor array exhibited distinct fluorescence patterns for specific species and timepoints, with PCA revealing that five components accounted for 93% of variance. The first three components demonstrated good separation for most biofilms, though overlap was observed at 12 hours. Model accuracies were 82.3%, 88.5%, and 91.2% for SVM, RF, and CNN, respectively. The detection limit was a 12-hour-old biofilm with $\sim 3 \log \text{ CFU/cm}^2$.

Significance: This approach enables rapid detection of biofilms with straightforward sampling procedures, providing species and cell count information to guide immediate intervention strategies and prevent biofilm-associated contamination in food processing environments.

Award Application: Developing Scientist Award

T15-03: SINGLE-CELL IDENTIFICATION OF VIABLE BUT NONCULTURABLE CAMPYLOBACTER JEJUNI

Primary Author:

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Pierre-Luc Longchamps, McGill University

Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Campylobacter jejuni* is a leading foodborne pathogen that may enter a viable but non-culturable (VBNC) state to survive under environmental stresses, posing a significant health concern. However, there are limited studies analyzing VBNC cells at the single-cell level for accurate detection and understanding of their unique behavior.

Purpose: This study aimed to develop a culture-independent approach to identify and characterize VBNC *C. jejuni* using single-cell Raman spectra and machine learning.

Methods: Two *C. jejuni* strains were induced into the VBNC state under osmotic pressure (7% w/v NaCl solution) and aerobic stress (atmospheric condition), respectively. Raman spectra were collected from single bacterial cells using a homebuilt Raman optical tweezers system integrated with a microfluidic device. A convolutional neural network (CNN) was used to distinguish the culturable and VBNC cells. Furthermore, we conducted a gradient-weighted class activation mapping (Grad-CAM) to identify the spectral regions that contribute most to the classification.

Results: Using single-cell Raman spectra and CNN, VBNC *C. jejuni* cells were identified from their culturable counterparts with an accuracy of 91.7% to 93.4% at the single-cell resolution. There were no significant spectral differences between the VBNC cells formed under different stressors. The Grad-CAM highlighted the top 10 spectral regions that are most responsible for the CNN-based VBNC identification. Most of the contributing spectral features were attributed to proteins, nucleic acids, lipids and peptidoglycan, indicating the molecular changes in VBNC *C. jejuni* cells.

Significance: Raman optical tweezers combined with CNN represents a promising tool for identifying VBNC bacteria in heterogeneous real-world samples at the single-cell resolution. The knowledge from this study also provided valuable insights to the molecular mechanisms of VBNC formation.

Award Application: Developing Scientist Award

T15-04: ENHANCING AI MICROSCOPY FOR FOODBORNE BACTERIAL CLASSIFICATION VIA ADVERSARIAL DOMAIN ADAPTATION ACROSS OPTICAL AND BIOLOGICAL VARIABILITY

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: AI-enabled microscopy is emerging for rapid bacterial classification, yet its utility remains limited in dynamic or resource-limited settings that demand robustness to variable data quality.

Purpose: This study aims to enhance the generalizability of AI-enabled microscopy for bacterial classification by addressing imaging variability using domain adaptation techniques.

Methods: Six bacterial species, including three Gram-positive (*Bacillus coagulans*, *Bacillus subtilis*, *Listeria innocua*) and three Gram-negative (*E. coli*, *Salmonella* Enteritidis, *Salmonella* Typhimurium), were studied. Bacterial microcolonies were grown on soft tryptic soy agar plates (0.7% agarose) at 37°C for 3–5 h. Images were acquired using an Olympus IX71 microscope under varying modalities and magnifications. Domain adaptation techniques, including domain-adversarial neural networks (DANNs) for single-target domains and multi-DANNs (MDANNs) for multiple domains, were employed to address imaging variability. EfficientNetV2 served as the backbone architecture, leveraging fine-grained feature extraction for small targets, with few-shot learning enabling scalability in data-limited domains. The source domain included 105 images per species ($n = 630$) collected under optimal conditions (phase contrast, 60× magnification, 3-h incubation). After reserving 15% for testing, the rest were split 70/30 for training/validation. Target domains involved variations in modality (brightfield, BF), magnification (20×), and extended incubation for lower resolution (20×-5h), with <5 labeled training images per species ($n \leq 30$) and test datasets of 60–90 images. Model performance was evaluated by classification accuracy across domains.

Results: DANNs improved target domain classification accuracy by up to 54.45% (20×, n = 90), 43.33% (20×-5h, n = 60), and 31.67% (BF, n = 60), with minimal accuracy loss (<4.44%) in the source domain. MDANNs further improved accuracy in BF and 20× conditions, demonstrating the ability to generalize across multiple imaging conditions simultaneously. Feature visualizations validated the model's ability to learn domain-invariant features across conditions.

Significance: This study presents a scalable AI framework for bacterial classification, extending the utility of microscopy to decentralized and resource-limited settings where imaging variability often challenges performance.

T15-05: SOLVENT-FREE SCREENING OF MICROBIAL CONTAMINATION IN FOODS USING VOCs

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Previous research on microbial detection using metabolomics has focused on identifying polar biomarker metabolites via GC-MS. However, extracting polar metabolites often involves hazardous solvents and complex workflows, which are unsuitable for high-throughput food testing.

Purpose: This study aims to explore a rapid, solvent-free method for screening microbial contamination by identifying non-polar volatile organic compounds (VOCs) released in the headspace of contaminated samples. This method could provide a quick screening tool for detecting microbial presence in foods, followed by confirmatory testing.

Methods: *Salmonella enterica* and *Escherichia coli* were spiked into buffered peptone water and UHT milk, respectively, at a standardized inoculum (1 cfu/mL) and enriched at 37°C for 15-24 hours. VOCs from spiked and control samples (n=3) were extracted using static headspace and solid-phase microextraction (SPME). Extracted VOCs were analyzed using unidimensional and multidimensional GC, followed by identification with MS and TOF-MS. Univariate and multivariate statistical analyses were performed to identify potential biomarkers.

Results: After 24 hours of enrichment, HS-SPME GC-TOF-MS analysis showed that in *Salmonella*-spiked samples, 4-propylbenzaldehyde was downregulated, while 2-nonanone and 2-decanone were upregulated compared to the control. In *E. coli*-spiked UHT milk, GC×GC-MS with an 8-minute run-time identified ethanol (MDL 44.66 mg/mL), 1-propanol (MDL 37.53 mg/mL), and acetaldehyde (MDL 23.47 mg/mL) as significant biomarkers ($p < 0.05$) after 15 hours of enrichment.

Significance: Unlike methods focusing on polar metabolites of primary metabolism, this research highlights the rapid detection of non-polar secondary metabolites, which vary across microbial species. The solvent-free workflow offers a promising alternative for high-throughput, automated microbial screening, complementing traditional pathogen confirmation methods in food testing.

T15-06: RELIABLE DETECTION AND ENUMERATION OF CONTAMINANTS IN PROBIOTICS

Primary Author:

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Benjamin Diep, Nestle

Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Probiotics can be present in high concentrations, which may have a negative impact on microbiological contaminant testing.

Purpose: We have validated two alternative sample preparation protocols needed to ensure reliable detection and enumeration of contaminants in probiotics and products containing probiotics.

Methods: LOD₅₀ studies based on ISO 16140-2 were performed for *Salmonella*, *Enterobacteriaceae*, *Cronobacter*, *Listeria* and *Staphylococcus* coagulase positive. Accuracy profiles, also according to ISO 16140-2, were performed for *Enterobacteriaceae*, *Staphylococcus* coagulase positive, *Bacillus cereus*, contaminating microorganisms (sugar free agar), yeast and mould and *E. coli*.

Results: For qualitative methods, we demonstrated that using an increased buffering capacity (6sBPW) in a standard 1/10 dilution is sufficient for all tested probiotics in finished products (probiotic concentration <10⁸ CFU / g). In contrast, all probiotic but *L. johnsonii* in culture powder (probiotic concentration > 10⁸ CFU /g) required a 1/100 dilution. In total, over 100 LOD₅₀ were performed to validate this approach for *Salmonella*, *Listeria*, *Enterobacteriaceae* and *Cronobacter*; including a larger test portions (100 g) for powdered infant formula and some supplements.

For quantitative methods, 65 accuracy profiles (methods comparison between products without probiotic (reference method) and the same products with probiotic (alternative method)) for *Enterobacteriaceae*, *Staphylococcus*, *Bacillus cereus* and contaminating microorganisms showed that for the majority of combinations the current standard sample preparation instructions (1/10 initial dilution) are sufficient. Only one contaminant method (*E. coli*) in a mix of probiotics combined with a complex supplement required a 1/100 initial dilution.

Significance: These results demonstrate that without adapting the standard sample preparation protocols, there may be a negative impact on the detection and enumeration of contaminants. We describe for the first time an uniform

approach that can be applied across probiotic strains and methods.

T16-01: COMPREHENSIVE ANALYSES OF CARBAPENEM-RESISTANT AND ESBL-PRODUCING BACTERIA IN FRESH VEGETABLES AND THEIR RESISTOME

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Historically associated with hospitals, carbapenem-resistant and extended-spectrum β -lactamase (ESBL)-producing bacteria have been increasingly detected in community settings. Food and the environment may serve as reservoirs for antibiotic-resistant bacteria.

Purpose: A multistate prevalence study of antibiotic resistant bacteria in retail fresh vegetables was conducted in three U.S. states-- Arkansas, Wisconsin, and California--between 2022 and 2023.

Methods: A total of 1,218 samples of five types of vegetables (lettuce, spinach, carrots, sprouts/microgreens, and salads) were analyzed for carbapenem-resistant bacteria and ESBL-producing *Enterobacterales*. Shotgun metagenomic sequencing and culture-based methods were used. Culture-based methods included screening on CHROMagar, antibiotic susceptibility testing, ESBL production and carbapenem resistance evaluation, carbapenemase typing, and whole genome sequencing to examine the presence of β -lactamase genes in phenotypically positive isolates.

Results: From the 1,218 vegetables, 62 carbapenem-resistant isolates (5.09%) and 70 ESBL-producing *Enterobacterales* isolates (5.74%) were identified. Carbapenemase-producing *Enterobacterales* included 31 *Enterobacter* strains and 1 *Kluyvera* strain. The majority of these *Enterobacterales* strains (n=19) were isolated from microgreens and sprouts, representing a significantly higher prevalence ($p<0.01$) compared to other vegetable types. Genera of ESBL-producing *Enterobacterales* isolates included *Serratia* (39 isolates), *Enterobacter* (20 isolates), *Klebsiella* (6 isolates), *Raoultella* (3 isolates), and *Rahnella* (2

isolates). Comparative genomic analysis revealed that the antibiotic-resistant *Enterobacterales* isolates from vegetables exhibited close genomic similarity to clinical isolates from humans. The carbapenemase gene bla_{IMI-6} in an *Enterobacter asburiae* strain from microgreens collected in Arkansas can be transferred to *E. coli* Dh5 α by conjugation. Shotgun metagenomic analyses of vegetable samples also indicated the presence of various antibiotic-resistant bacteria and resistance genes.

Significance: This prevalence study suggested that vegetables harbored clinically relevant antibiotic-resistant bacteria, underscoring the importance of monitoring antibiotic resistance in vegetables and their production environments.

T16-02: EVALUATING EFFECTS OF BENEFICIAL ROOT-ASSOCIATED BACTERIA ON LETTUCE STOMATAL CONDUCTANCE AND SALMONELLA ASSOCIATION

Primary Author:

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Persons:

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Diksha Klair, University of Maryland

Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Stomatal conductance is a measure of gas exchange required for photosynthesis, also regulating water loss by transpiration. Beneficial root-associated (probiotic) bacteria can influence plant modulation of leaf conductance.

Purpose: Evaluate the effect of beneficial root-associated bacteria on 1) Romaine lettuce leaf stomatal conductance and 2) *Salmonella enterica* association with lettuce grown under water restriction.

Methods: Rhizobacteria, and root and leaf bacterial endophyte isolates (n=35), recovered from Maryland-grown bok choy were applied to roots of romaine lettuce cv. 'Rouge d'Hiver' seedlings 3- and 6-days post-germination with single-strain suspensions of 7 log CFU/plant (n=6/strain). Control plants were root-inoculated with water only. Plants were subjected to regular or restricted watering. Stomatal conductance (gsw) was measured using a LI-COR600. Root-inoculated plants were also screened for their potential to restrict *Salmonella* 4,[5],12:i:-, a monophasic variant of *Salmonella* Typhimurium, applied at 5 log CFU/plant. *Salmonella* populations were enumerated by direct plate counting on Tryptic Soy Agar 24 h after leaf inoculation.

Results: Overall, gsw was lower ($0.03 \text{ mol m}^{-2} \text{ s}^{-1}$) in lettuce grown under water stress compared to fully watered plants ($0.14 \text{ mol m}^{-2} \text{ s}^{-1}$; $p < 0.001$). Under regular watering, plants colonized with root and leaf endophytes had higher ($p < 0.001$, Steel Method) and lower ($p < 0.05$) gsw, respectively, relative to control. Under water stress, plants in the rhizobacteria group had lower gsw ($p < 0.001$) than control. Root colonization with 3/35 isolates (BC28, 36, 35) increased gsw under regular watering ($p < 0.05$). In contrast, 4 root-inoculated isolates reduced gsw ($p < 0.1$). No significant effects were seen in plants under water stress. Under regular watering, 3 leaf endophytes (BC1, 3, 8) and rhizobacterium BC17 restricted *Salmonella* by 0.7–1.5 log ($p < 0.05$), compared to control. Under stress, 3 root (BC35–37) and 3 leaf (BC5–7) endophytes reduced *Salmonella* by 1.7–2.2 log ($p < 0.05$ for all). Stomatal conductance associated negatively with *Salmonella* log reduction ($r = -0.2$, $p < 0.001$)

Significance: Beneficial root bacteria may improve water-use efficiency and modulate *Salmonella* colonization of lettuce.

T16-03: PERSISTENCE OF INTERNALIZED ENTEROPATHOGENS IN BABY LEAF KALE AS INFLUENCED BY PRE-HARVEST WATER STRESS AND POST-HARVEST STORAGE CONDITIONS

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Data on internalized enteropathogens in baby kale leaves are lacking. Pre- and post-harvest factors during kale production and handling could impact enteropathogen persistence.

Purpose: To investigate how soil moisture during kale cultivation influences persistence of internalized enteropathogens in harvested baby kale and during storage at 4 °C.

Methods: Kale 'Red Russian' was planted in a high tunnel at the Central Maryland Research and Education Centre, Upper Marlboro Facility. Two irrigation regimes were set up in a randomized design based on regular (High, n=3) and restricted (Low, n=4) watering. Volumetric soil water content was recorded continuously up to harvesting at 26 days post-germination. On harvest day, three sets of two leaves per treatment replicate were placed into bags and transported to the lab on ice for inoculation with three pathogens. Inocula of 5–6 log CFU/mL were prepared for rifampicin-adapted *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes*, and leaves were infiltrated with 100 µL of inoculum using a needleless syringe. Leaves were incubated for 1, 2, and 4 days at 4°C, then homogenized in 0.1% peptone water and analyzed by plate counting.

Results: Overall, counts varied by pathogen ($p < 0.001$) – in the order *Listeria*, *E. coli* O157:H7, and *Salmonella* (5.0, 4.4, and 3.6 log CFU/mL, respectively). Regression analysis identified soil moisture (F-ratio = 6.3, $p < 0.05$) and storage time (F-ratio = 5.2, $p < 0.05$) as factors in *Salmonella* persistence. *Salmonella* in low-irrigation kale after 4 days of storage was most affected by soil moisture as compared to high-irrigation kale ($p < 0.05$). Storage time was a factor for *Listeria* (F-ratio = 13.8, $p < 0.001$). An interaction between storage time and soil moisture was detected (F-ratio = 7.9, $p < 0.01$), whereby *Listeria* counts decreased after 4

days of storage compared to 1 and 2 days ($p < 0.05$) within high-irrigation kale only. *E. coli* O157:H7 was unaffected by soil moisture or storage time.

Significance: Internalized pathogen persistence in kale differed by species and varied by storage time and field soil moisture in a pathogen-dependent manner.

Award Application: Developing Scientist Award

T16-04: IMPACT OF WASTEWATER FROM INDUSTRIALIZED AGRICULTURE ON NUTRIENTS LEVELS IN WATER RESOURCES IN CENTRAL NORTH CAROLINA

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Presentation type/Format: Technical

Category: Water

Abstract:

Introduction: North Carolina is a major producer of pork products in the United States, with swine raised at concentrated animal feeding operations (CAFOs). Swine CAFOs generate large volumes of wastewater stored in lagoons until land application to spray fields occurs. Wastewater contains elevated nutrient concentrations that may leach into groundwater or runoff to streams.

Purpose: This study aimed to analyze nutrient concentrations in wastewater and water resources downgradient from spray fields at a swine farm.

Methods: Groundwater samples were collected from piezometers installed upgradient, beneath, and downgradient of spray fields, and surface water samples were taken from the upgradient and downgradient of the farm. Monthly samples (July 2023–February 2024) were analyzed for total dissolved nitrogen (TDN) and phosphate.

Results: Wastewater irrigation significantly elevated nitrogen levels in downgradient water resources. Lagoon wastewater had a median TDN concentration of 1651.12 mg/L, significantly greater than groundwater beneath spray fields (13.82 mg/L) and riparian buffer (2.34 mg/L). Surface water downstream of the farm had TDN concentrations of 15.17 mg/L, significantly higher than the upstream location (1.25 mg/L). Groundwater and surface water located hydraulically downgradient from spray fields contained elevated TDN concentrations relative to water resources upgradient from wastewater application sites.

Significance: High nitrogen concentrations in water resources have been linked to public health challenges such as blue baby syndrome and cancer resulting from ingestion of contaminated food and/or water. These findings suggest that CAFOs are a significant source of nitrogen pollution to nearby water resources, highlighting the need for improved nitrogen management strategies.

Award Application: Developing Scientist Award

T16-05: EFFICACY OF PERACETIC ACID AND CHLORINE IN MANAGING SALMONELLA BIOFILMS IN A SIMULATED IRRIGATION SYSTEM

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Justin Daniel, University of Georgia

Ruben Vinueza, The University of Georgia

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Presentation type/Format: Technical

Category: Water

Abstract:

Introduction: Biofilms in water distribution systems can disperse pathogens to irrigated crops; therefore, evaluating mitigation measures is essential to prevent this occurrence.

Purpose: To evaluate the effectiveness of peracetic acid (PAA) and chlorine (Cl) at 20 ppm against *Salmonella* biofilms in irrigation lines with or without fertilizer.

Methods: Pond water (PW) or PW with 0.1% (v/v) organic fish emulsion (2-4-1) fertilizer (PWO) (N=144) was inoculated with ~2 log CFU/mL of a rifampicin-resistant *Salmonella* cocktail, and then circulated through polyethylene irrigation lines connected to a pump for 1 h. Water was then flushed and replaced with non-inoculated PW only or containing PAA or Cl on days 3, 10, and 17. Non-inoculated PW with or without fertilizer was replaced on days 7 and 14. *Salmonella* populations both in the water and attached to the tubing line were determined [limit of detection (LOD): ≤ -1 log CFU/mL; ≤ -0.22 log CFU/line].

Results: From day 0 to 3, *Salmonella* population in water significantly increased by 3 log CFU/mL in PWO (P=0.0001), then declined to ~4 log CFU/mL until day 17. On days 7 and 14, ~2 and 0.5 log CFU/mL in PWO+PAA and PWO+Cl, respectively, were recovered from water. PAA or Cl efficacy significantly differed for both PWO (P=0.0001) and PW (P=0.02) in water. Populations on the PWO lines significantly increased by 8 log CFU/line from day 0 to 14 (P=0.0001). For PWO+Cl and

PWO+PAA, the population increased and then remained constant (~ 2 log CFU/line). For PW, the population was ~ 1 log CFU/line and

Significance: The use of fish emulsion fertilizer in-line may support the establishment of *Salmonella* biofilms and subsequent cross-contamination of irrigation water if not fully flushed from the system. PAA and CI effectively reduced *Salmonella* contamination in water but did not eliminate populations in-line once biofilms were formed.

Award Application: Developing Scientist Award

T16-06: ESCHERICHIA COLI AND SALMONELLA FROM IRRIGATION PONDS: TYPING AND ANTIMICROBIAL RESISTANCE.

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Presentation type/Format: Technical

Category: Water

Abstract:

Introduction: Whole-genome sequencing of *Escherichia coli* and *Salmonella* over time may reveal sources of contamination or a reservoir in irrigation water.

Purpose: To characterize *E. coli* and *Salmonella* found in irrigation ponds to better evaluate possible their sources, pathogenicity, and resistance to antimicrobials.

Methods: *E. coli* and *Salmonella* isolates collected during a 28-month sampling effort at two irrigation ponds in Georgia, USA were sequenced. Two ponds 14.7km apart in the Little River Watershed were analyzed. Pond 1 had wildlife and cattle access, while Pond 2 only had wildlife access. Most probable number of *E. coli* (mpnEC) was measured for each sample. Isolates were assigned sequence types (ST) using the 7-gene Achtman scheme in addition to phylogroup determination for *E. coli* and serotype for *Salmonella*. Antimicrobial resistance (AMR) genes were determined using AMRFinderPlus 3.12.8.

Results: Phylogroups of *E. coli* (n=151) differed significantly between ponds (Fisher's exact test, p=0.033) and by month (p=0.003), but not by sample area (perimeter, surface, subsurface; p>0.05). pHylogroup B1 was most prevalent among samples (63%). Sample mpnEC differed significantly by phylogroup at pond 2 ($\chi^2=12.8$, df=5, p=0.025), but not pond 1 (p>0.05). of 93 isolates assigned an ST, 68 were unique. *Salmonella* serotypes (n = 19) differed significantly by pond (p=0.016) and month. of the 18 isolates assigned a ST, 9 were unique. AMR gene distribution in *E. coli* (n=125) did not differ significantly between ponds ($\chi^2= 80.7$, df=72, p=0.23) and 17/19 *Salmonella* carried AMR genes. Shiga-toxin genes were found in 10 *E. coli* isolates.

Significance: A diverse set of *E. coli* was recovered, possibly indicating a variety of sources in these ponds that change with season, while *Salmonella* may have more limited and episodic sources. These findings may help better understand when water sources are at increased risk of harboring foodborne pathogens.

Award Application: Developing Scientist Award

T17-01: MICROBIAL ASSESSMENT OF COMMERCIALY AVAILABLE SEAMOSS PRODUCTS

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Randy Worobo, Cornell University

Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Seamoss is an edible sea vegetable in the algae family that is rich in nutrients. Social media posts suggested that seamoss can boost immune system and aid in weight loss, lead into a spike in seamoss consumption during the COVID pandemic. Cornell University extension program have observed similar trends over the last two years as large number of companies reaching out for guidance on seamoss production and processing. While its health benefits remained to be proven, seamoss product specifically the gels can represent a food safety concern when not processed correctly.

Purpose: The goal of this study is to survey commercially available dried seamoss and gels and microbial quality.

Methods: Dried seamoss samples and seamoss gels were purchased online from various producers. The dried seamoss was stored at room temperature (20-22C), and the gels were refrigerated (2-4C) upon delivery. Samples were analyzed for total aerobic plate count, yeast and mold count, *E. coli* O157:H7, *S. aureus*, *B. cereus*, *Vibrio* spp., *L. monocytogenes*, *Salmonella* spp. Products were serially diluted in sterile 0.1% peptone and enumerated on selective media for each tested organism. Presumptive colonies were plated on ChromAgar for secondary screening and confirmed with sequencing.

Results: Overall, 53 dried seamoss, and 13 seamoss gels were tested for microbial enumeration. The dried seamoss total aerobic plate counts ranging from 2.18 to 6.48 log CFU/g whereas the gels ranged from undetected to 6.26 log CFU/g (Detection Limit is 2 log). Approximately 75% of the dried seamoss tested positive for presumptive *S. aureus*, and 96.23 % for *B. cereus*, 42.31% for *Vibrio* spp. All the gel samples were positive for presumptive *B. cereus*, and *S. aureus*, and one was for *Vibrio* spp.

Significance: This project provides a preliminary framework to identify the biological hazards associated with seamoss, as there is limited information available. Results warrants future studies to improve microbial safety of seamoss

and seamoss products.

T17-02: CHARACTERIZATION OF BIOFILMS PRODUCED BY *SALMONELLA* SPP. IN HYDROPONIC LEAFY GREEN NUTRIENT SOLUTION

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Sanja Ilic, The Ohio State University

Presentation type/Format: Technical

Category: General Microbiology

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 11 *Salmonella* strains (serovars Albany, Anatum, Braenderup, Enteritidis, Heidelberg, Javiana, Muenchen, Montevideo, Newport, Typhimurium) 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 invitro (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 on XLD, and RDAR (red-dry-and-rough) morphotype analysis assessed exopolysaccharide production.

Results: Most strains (9/11) did not form biofilms in LG invitro (25°C/28°C/37°C). *Salmonella* Anatum and Newport formed weak biofilms at 25°C. Under controlled environment conditions, weak biofilms were produced by 5/11 strains (S. Albany, S. Anatum, S. Enteritidis, S. Heidelberg, S. Newport). in the presence of lettuce exudates, all strains formed moderate biofilms at 25°C. At 28°C, 6/11 strains formed moderate biofilms, while 5/11 formed weak biofilms. S. Enteritidis and S. Heidelberg formed strong biofilms at 37°C. in the presence of sucrose, glucose, and inositol, >50% of the strains formed moderate-strong biofilms under all test conditions. Five strains exhibited RDAR morphology in LG, while nine had RDAR morphology in LGE at 28°C.

Conclusion: *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.

Award Application: Developing Scientist Award

T17-03: GENOMIC BASES OF THE ECOLOGICAL SUCCESS OF MOTILE AND NON-MOTILE LISTERIA

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Shannon Hepp, Virginia Tech

Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Addressing the ecological success of foodborne pathogens is essential to understanding their persistence and transmission within diverse environments, which directly impacts food safety. Motility can influence the strategies pathogens adopt to survive and spread in the environment, posing different challenges for controlling their presence. However, the genetic mechanisms underlying the ecological success of motile and non-motile species remain largely unknown.

Purpose: This study aims to identify the genomic bases underlying the ecological success of motile and non-motile bacteria using *Listeria* as a model.

Methods: We analyzed genomes of 141 *L. welshimeri* (motile) and 90 *L. booriae* (non-motile) isolates, obtained from 1,004 soil samples collected across the United States. Phylogeographic analysis of genetic dissimilarity and geographic distance was performed to infer dispersal frequency. BUSTED models followed by functional enrichment analysis were conducted to identify genes and functions targeted by positive selection (PS). Comparative genomics of soil and wildlife isolates of *L. welshimeri* was performed to investigate wildlife as a potential dispersal vector.

Results: Both *L. welshimeri* and *L. booriae* are highly prevalent in soils. *L. welshimeri* appears to disperse frequently across locations, likely facilitated by wildlife. Motility genes of *L. welshimeri* show evidence of strong PS, potentially offering fitness advantages in niche expansion. In contrast, *L. booriae* exhibits strong dispersal limitations. A large number of *L. booriae* genes, particularly those involved in inorganic ion, amino acid, and coenzyme transport and metabolism, are under positive selection, suggesting a role for metabolic versatility in adapting to a broad range of niches.

Significance: Our study reveals substantial differences in the strategies used by motile and non-motile *Listeria* species to achieve ecological success in the environment. These findings underscore the importance of developing targeted biocontrol interventions that account for motility in foodborne pathogens.

T17-04: CYTOTOXICITY, TRANSCRIPTION, AND STABILITY OF VIRULENCE FACTORS PRODUCED BY PSYCHROTOLERANT BACILLUS CEREUS GROUP ISOLATES

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Psychrotolerant *Bacillus cereus* strains are typically associated with food spoilage. However, some carry virulence genes that can be expressed at lower temperatures, causing cytotoxicity.

Purpose: This study aimed to evaluate variations in virulence gene transcription, cytotoxicity, and stability of virulence factors produced at 32°C and 37°C by psychrotolerant *B. cereus* isolates from two phylogenetic groups.

Methods: Isolates from phylogenetic groups II (n=33) and VI (n=37) were grown in brain heart infusion (BHI) broth at 32°C and 37°C, and skim milk broth (SMB) at 32°C to extract cell-free supernatants and RNA. Cytotoxicity was assessed using a WST-1 assay with Caco-2 cells. Virulence gene (hblC, nheA, cytK, sph, plc) transcription was measured using RT-qPCR. Supernatants were exposed to high temperature, low pH, and trypsin/mucin to assess the stability of virulence factors using a WST-1 assay. Differences in cytotoxicity between isolates grown at 32°C and 37°C, as well as before and after treatments, were analyzed using one-way ANOVA with a Tukey post-hoc test. Virulence gene relative expression was calculated using the 2- $\Delta\Delta C_t$ method with *gatB* as the reference gene and compared using one-way ANOVA.

Results: Culturing at 32°C versus 37°C had an insignificant effect on overall cytotoxicity, although some isolates (n=10) were cytotoxic at 32°C but not at 37°C. Relative expression of *hblC* and *plc* was significantly higher in group II

($p=0.019$) and group VI ($p=0.048$) isolates, respectively, when grown in BHI at 32°C. Relative expression was significantly lower for all tested genes for both groups except *nheA* (II and VI) and *hblC* (VI) when grown in SMB. Exposure to pH 5 did not significantly reduce cytotoxicity, while all other treatments resulted in significant decreases ($p<0.05$) compared to untreated controls.

Significance: *B. cereus* isolates exhibited temperature- and substrate-dependent variations in virulence gene expression and cytotoxicity, highlighting possible food safety risks of psychrotolerant isolates.

Award Application: Developing Scientist Award

T17-05: HEAVY METAL TOLERANCE IN BACKGROUND MICROORGANISMS ASSOCIATED WITH FOOD PROCESSING ENVIRONMENTS

Primary Author:

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Heavy metals can enter the food supply through various pathways, potentially contributing to metal tolerance in microorganisms and ultimately exerting selective pressure on microbial populations, raising concerns about the impact of heavy metal tolerance on the effectiveness of biocides and other interventions in food processing.

Purpose: To determine the phenotypical heavy metal tolerance properties of specific background microorganisms found in meat and poultry process environments.

Methods: *Citrobacter rodentium* ATCC51459, *Enterococcus faecium*, *Escherichia coli* ATCC 25922, *Klebsiella aerogenes* ATCC 13048, *Lactobacillus acidophilus*, *Lactococcus lactis*, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 were grown in Muller Hinton (MH) broth medium (105 CFU/ml), exposed to varying concentrations of Zn^{2+} , Cu^{2+} , and Cd^{2+} , and incubated at 35 °C for 24 h. The metal solutions were prepared as a two-fold dilution series, ranging from 32 mM to 0.01525 mM. The minimum inhibitory concentration (MIC) for each bacterium was determined as the lowest concentration of metal that inhibited the growth of the corresponding bacterial strain and confirmed with two trials.

Results: MICs (mM) resulted as follows for Zn, Cu, and Cd, respectively. *C. rodentium*: 2.0, 1.0, and 0.5; *E. coli*: 4.0, 4.0, and 1.0; *K. aerogenes*: 4.0, 4.0, and 1.0; *P. aeruginosa*: 16.0, 8.0, and 4.0; *S. aureus*: 0.5, 4.0, and 0.5. *L. lactis* showed 0.03125 mM for Zn; *L. acidophilus* had 0.0625 mM for Zn and 0.03125 mM for Cu; and *E. faecium* demonstrated 8 mM for Zn and 4 mM for Cu.

Significance: These results can be used to identify potential environmental stresses that can influence resistance to biocides and other antimicrobial interventions in food processing. Further research is being conducted to evaluate the heavy metal tolerance of pathogenic and non-pathogenic *Listeria* spp.

Award Application: Developing Scientist Award

T17-06: SYNERGISTIC EFFECTS OF ELEVATED HYDROSTATIC PRESSURE, MALIC ACID, AND CITRICIDAL AGAINST WILD-TYPE, RIFAMPICIN-RESISTANT, AND PRESSURE-STRESSED O157 AND NON-O157 SHIGA TOXIN-PRODUCING ESCHERICHIA COLI

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Various serogroups of Shiga toxin-producing *Escherichia coli* are important foodborne pathogens of public health concern in various commodities including apple cider. Adaption of high-pressure processing (HPP) continues to gain importance and momentum in the food industry.

Purpose: The current study investigated impact of malic acid, and citricidal for augmenting the pressure-based inactivation of O157 and non-O157 serogroups of wild-type, rifampicin-resistant, and pressure-stressed Shiga toxin-producing *E. coli* in apple cider.

Methods: A six-strain mixture of *E. coli* O157: H7 and six-strain mixture of non-O157 serogroups of the pathogen (O26, O45, O103, O111, O121, and O145) were used in this study. Additionally, three phenotypes of each strain mixture (wild-type, rifampicin-resistant, and pressure-stressed) were utilized for pressure-based (300 and 600 MPa) treatments with and without 3.5% malic acid and citricidal. Temperatures of trials were adjusted at 4.4 and 60.0 °C using a stainless-steel jacket surrounding Hub880 Barocycler chamber connected to a circulating water bath. Results were statistically analyzed using Tukey and Dunnett's-adjusted ANOVA. Studies were conducted in two biologically independent blocks, each consisting of two replications, with two microbiological repetitions per replication.

Results: While 300 MPa treatments at 4.4 °C resulted in 0.45 log reduction ($P < 0.05$) after five minutes, same treatment resulted in 4.26 and 6.02 log reductions ($P < 0.05$) with addition of malic acid and citricidal, respectively. O157 and non-O157 serogroups and all three tested phenotypes illustrated similar sensitivity to elevated hydrostatic pressure, mild heat, and the two antimicrobials.

Significance: Results illustrate that application of selected antimicrobials could augment the decontamination capability of a pressure-based treatments against Shiga toxigenic *E. coli*. Additionally, we observed O157 and non-O157 serogroups of the pathogen have comparable sensitivity to the elevated hydrostatic pressure and the antimicrobials. Thus, a validated treatment plan against O157 serogroups is most probably equally effective against the non-O157 serogroups. A validated HPP plan against wild-type cells could similarly be efficacious against rifampicin-resistant and pressure-stressed phenotypes of the pathogen as well.

Award Application: Developing Scientist Award

T18-01: NATURAL OCCURRENCE OF FUMONISINS IN PRE-HARVEST AND POST-HARVEST MAIZE IN SOUTH AFRICA: A MAIZE TRUST PROJECT.

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Mycotoxins, particularly fumonisins, are toxic secondary metabolites produced by toxigenic fungi that frequently contaminate crops, contributing to food insecurity and economic losses. Fumonisins in maize have been strongly associated with the high prevalence of esophageal cancer in certain regions of South Africa. This connection is primarily due to the dietary reliance on maize, a staple food, and the frequent contamination of maize with fumonisins, particularly fumonisin B1 (FB1), a mycotoxin produced by *Fusarium* species.

Purpose: This study aimed to assess fumonisin contamination levels in maize from Gauteng Province, South Africa, and compare levels between pre-harvest and post-harvest samples to identify contributing factors.

Methods: Eighty maize samples (40 pre-harvest and 40 post-harvest) were collected from maize farmers in Gauteng Province, South Africa. Fumonisin levels were quantified using Ultra-High-Performance Liquid Chromatography Tandem Mass Spectrometry (UHPLC-MS/MS).

Results: Contamination was detected in 35%, 28.75%, and 13.75% of total samples with FB1, FB2, and FB3, respectively. FB1 concentrations were higher in post-harvest maize (

Significance: This study emphasizes the urgent need for effective post-harvest management, robust monitoring programs, improved agricultural practices, and targeted mitigation strategies to reduce fumonisin contamination in South African maize, particularly during storage.

Award Application: Developing Scientist Award

T18-02: EVALUATION OF AIR PLASMA AND PLASMA-ACTIVATED CHEMICALS FOR MICROBIAL REDUCTION ON RAW CHICKEN BREAST FILLETS

Primary Author:

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Microbial spoilage of raw poultry contributes significantly to food waste and loss, and reducing spoilage microorganisms can extend shelf life and lower costs.

Purpose: This study aimed to evaluate the efficacy of an air plasma-generating device and plasma-activated hydrogen peroxide (H₂O₂) in reducing microbial load on fresh, raw chicken breast fillets.

Methods: Two trials were conducted with six different treatments and three repetitions each (n=36): a control, H₂O₂ alone, 5 and 10 minutes of direct plasma exposure, and 5 and 10 minutes of plasma-activated H₂O₂. Chicken samples (2.5 cm²) were exposed to the treatments, diluted in 9 mL of buffer peptone water (BPW), plated on trypticase soy agar (TSA), and incubated for 24 hours at 35°C. Viable colonies were then counted for each treatment. Data was analyzed using analysis of variance (ANOVA) ($P \leq 0.05$) to determine statistical differences between treatments.

Results: In the first trial, H₂O₂ alone achieved a 0.42 log CFU/mL reduction, while 5 and 10 minutes of direct plasma exposure reduced counts by 0.98 and 1.29 log CFU/mL, respectively. Plasma-activated H₂O₂ for 5 and 10 minutes reduced counts by 0.63 and 1.4 log CFU/mL, respectively ($P \leq 0.05$). The second trial showed greater efficacy, with H₂O₂ achieving a 0.67 log CFU/mL reduction. Five and 10 minutes of direct plasma exposure reduced counts by 0.63 and 1.19 log CFU/mL, while plasma-activated H₂O₂ reduced counts by 1.6 and 2.85 log CFU/mL ($P \leq 0.05$).

Significance: The findings suggest that the air plasma-generating device effectively reduces microbial levels, potentially extending the shelf life of chicken products while minimizing chemical use and water waste in processing plants, thus addressing consumer concerns.

Award Application: Developing Scientist Award

T18-03: UV-C TREATMENT FOR THE INACTIVATION OF LISTERIA SPECIES FROM THE SURFACE OF GALA APPLE

Primary Author:

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: The incidence of *Listeria* spp. in apple packing houses, reported at rates as high as 4.8%, highlights the need for alternative or supplementary sanitation strategies, such as Ultraviolet-C light (UV-C), a surface disinfectant already utilized in the food industry.

Purpose: This study aims to 1) determine feasible UV-C doses for decontaminating *Listeria* spp. on whole gala apples and 2) evaluate the suitability of *Listeria innocua* as a surrogate for inactivating *Listeria monocytogenes* by UV-C.

Methods: *L. innocua* was harvested from bacterial lawns grown on Trypticase Soy Agar with 0.6% Yeast Extract, diluted, and spot-inoculated onto the equatorial surface of pre-sterilized gala apples, achieving approximately 8.6 log bacteria per apple. Inoculated locations were exposed to UV-C doses of 6136 $\mu\text{J}/\text{cm}^2$, 63620 $\mu\text{J}/\text{cm}^2$, and 706418 $\mu\text{J}/\text{cm}^2$ in a benchtop chamber. Later, apples were hand massaged in a stomacher bag containing 100 mL of 0.85% saline to recover bacteria. The recovered solution was plated onto harlequin agar for enumeration. The same treatments were performed on *L. monocytogenes* cocktails to compare reductions with *L. innocua*. Statistical significance was determined at $p < 0.05$. Antimicrobial treatment in conjunction with UV-C treatment is also being investigated for enhanced efficacy.

Results: Six replicate treatments with UV-C dosages of 6136 $\mu\text{J}/\text{cm}^2$, 63620 $\mu\text{J}/\text{cm}^2$, and 706418 $\mu\text{J}/\text{cm}^2$ achieved log reductions of 2.6 ± 0.63 , 3.1 ± 0.65 , and 4.2 ± 0.31 , respectively. Lower treatment dosages (6000–60000 $\mu\text{J}/\text{cm}^2$) did not show significantly different reductions from each other at $p=0.05$, suggesting a non-linear efficacy of UV dose. No statistical difference was observed between reductions of *L. monocytogenes* and *L. innocua* under the same treatment conditions, although further validation is required to confirm *L. innocua* as a suitable surrogate.

Significance: This study demonstrates a non-damaging and residue-free approach to inactivate *Listeria* spp. on apples, suggesting a practical solution for apple growers and processors to enhance food safety.

Award Application: Developing Scientist Award

T18-04: GREEN TEA ALGINATE COATING INACTIVATES *LISTERIA MONOCYTOGENES* ON SHIITAKE

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Fresh mushrooms are highly perishable and susceptible to microbial contamination. *Listeria monocytogenes* is a pathogen of concern in fresh mushrooms since these food products have been involved in listeriosis outbreaks. Edible coatings incorporated with natural extracts have emerged as a promising strategy to ensure the safety of fresh mushrooms during storage.

Purpose: To evaluate the effectiveness of an edible alginate coating with green tea (*Camelia sinensis*) extract against *L. monocytogenes* on shiitake (*Lentinula edodes*) during refrigerated storage.

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), and sterile distilled water. Green tea extract (w/v; GTE) was incorporated into the coating solution at the minimum inhibitory concentration (MIC) determined against *L. monocytogenes* using microdilution in nutrient broth. Shiitake (20 g) were immersed (2 min) into the coating solution and further into the crosslink solution (2% CaCl₂; w/v; 1 min). Mushrooms were spot inoculated with *L. monocytogenes* (6 log CFU/g). Non-coated mushrooms were evaluated as controls. Samples were stored at 4 °C and 95% relative humidity. Analyses were done in triplicate. *L. monocytogenes* was enumerated at 0, 2, 4, 6, 8, and 10 days with a detection limit of 1.5 log CFU/g. Statistical analyses considered $p < 0.05$.

Results: The MIC of GTE against *L. monocytogenes* incorporated into the coating solution corresponded to 0.125 mg of catechins per g of GTE. In non-coated shiitake, *L. monocytogenes* counts increased by 1.58 log CFU/g over 10 days of storage. In coated shiitake, the pathogen decreased by 2.74 log CFU/g after 5 days of storage, with a total reduction of 4.5 log CFU/g after 10 days.

Significance: GTE coating showed activity against *L. monocytogenes* in shiitake during refrigerated storage. The GTE coating could be a sustainable alternative to

conventional preservation methods for fresh mushrooms.

T18-05: PLANT-BASED PRINTABLE EDIBLE INK FOR FOOD SAFETY AND SUSTAINABLE ENGINEERING APPLICATIONS

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Developing edible ink from natural components is an eco-friendly, sustainable alternative to synthetic printing inks. It promotes the direct printing of food substrates and thus reduces the need for synthetic chemical-based ink and labels.

Objective: To develop sustainable and vibrant hibiscus-based edible ink using hibiscus, citric acid, and guar gum.

Methods: The ink formulation involved the natural colourant hibiscus flowers (Hb), the binder guar gum (GG), and the additive citric acid (CA). The process begins with the drying and grinding of hibiscus flowers in a ball mill. After this, the ink was prepared by adding citric acid in varying proportions (1%, 3%, or 5% w/w) to the powder. The ink was formed with a slow addition of guar gum to the mixture, followed by continuous stirring at 40°C for 1 hour. The developed edible ink was screen-printed onto edible substrates.

Results: The study highlighted the impact of additives on the physicochemical characteristics of the ink. The formulation Hb/GG/CA-3, containing hibiscus (2 g), citric acid (0.10 g), and guar gum (0.05 g), exhibited excellent properties. It includes an overall colour property (ΔE of 54.96 ± 0.82) attributed to citric acid. The ink demonstrated lower colour change after rub resistance due to the adhesive nature of guar gum. Its rheological properties showed shear-thinning behaviour, facilitating smooth application during screen printing. The ink also revealed the highest antioxidant activity ($62.23 \pm 0.53\%$) and a low pH (2.26 ± 0.08), which helps reduce food oxidation and enhance shelf life. This screen-printed ink showed vibrant colour printing and was found safe for consumption, reassuring its cost-effectiveness as an alternative to synthetic ink.

Significance: This investigation not only enables the direct printing of food substrates but also opens up employment opportunities for local communities on a small scale, demonstrating the practical benefits of our research.

Award Application: Developing Scientist Award

T18-06: ENHANCING INACTIVATION OF ESCHERICHIA COLI, PSEUDOMONAS, AND VIBRIO SPP. IN VITRO BY SEQUENTIAL APPLICATION OF ALKALINE AND ACIDIC ELECTROLYZED WATER

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Microorganisms, such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Vibrio parahaemolyticus* are significant threats to food safety, contributing to contamination and foodborne illnesses. Electrolyzed water (EW) is a safe and cost-effective disinfection technology, with two key forms: acidic EW (AEW) and alkaline EW (ALEW). Their antimicrobial activity may be enhanced with sequential application.

Purpose: This study evaluated the antimicrobial efficacy of AEW, ALEW, and their sequential combination against microbial strains relevant to food safety in vitro.

Methods: AEW and ALEW were generated using 0.5% sodium chloride and potassium chloride solutions in an Eco one HOCL generator. Stock cultures (1 mL) of *E. coli* ATCC 33625 (10^8 CFU/mL), *Pseudomonas aeruginosa* ATCC 23993 (10^8 CFU/mL), and *Vibrio parahaemolyticus* EB101 (10^9 CFU/mL) were exposed to 9 mL of each EW solution for 1 min, followed by 1 mL transfer to 9 mL of the same solution for an additional 1 min. Sequential treatments applied ALEW followed by AEW under the same conditions. Controls included deionized water (*E. coli* and *Pseudomonas*) or phosphate-buffered saline (*Vibrio*). Treated samples were neutralized with 0.5% sodium thiosulfate, serially diluted, and analyzed via pour plating (*E. coli* and *Pseudomonas*) or the MPN method (*Vibrio*).

Results: The pH values were 2.54 ± 0.01 for AEW, 11.65 ± 0.02 for ALEW, and 3.51 ± 0.02 for sequential EW. ORP values were -143 ± 52.26 mV (ALEW), $1336 \pm$

4.79 mV (AEW), and 1262 ± 19.44 mV (sequential EW). Available chlorine content ranged from 15.50 ± 0.55 mg/L (ALEW) to 36.60 ± 0.48 mg/L (sequential EW). Sequential EW achieved significantly higher bacterial reductions than single treatments ($P < 0.05$): 7.20 ± 0.24 log CFU/mL (*E. coli*), 5.20 ± 0.20 log CFU/mL (*Pseudomonas*), and 8.72 ± 0.21 log MPN/mL (*Vibrio*)

Significance: This study highlights the enhanced inactivation of foodborne microbial strains using sequential EW technology, which has potential for use in diverse food applications.

Award Application: Developing Scientist Award

T19-01: INGREDIENT IDENTIFIERS: A SIMPLISTIC APPROACH TO GLOBAL FOOD SAFETY AND REGULATORY COMPLIANCE

Primary Author:

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Presentation type/Format: Technical

Category: Food Law and Regulation

Abstract:

Introduction: Food manufacturers are faced with myriads of regulations to protect public health. Cognizant of the complexities of food safety and regulatory frameworks—and notwithstanding the emerging regulatory landscape—food manufacturers are seeking simplified processes to ensure the utilization of safe and compliant ingredients (including food additives).

Purpose: Using ingredient identifiers, product development teams could readily identify ingredients in their pantries and develop food safety and country-specific formulations to meet statutory acceptance criteria.

Methods: The ingredient identifier methodology consisted of four phases. (1) Phase I included categorization of ingredients by applying regulated common names or food categories. (2) In Phase II, ingredients were evaluated and verified against specific food manufacturing and statutory requirements, including allergens, food labeling, claims, biotechnology, food contact substances of concern, rules of origin, and other requirements. (3) Phase III addressed exceptions and ambiguous cases, including risk assessments, testing and validation, and regulatory agency approvals for novel ingredients and advanced tariff classifications. (4) The final phase, pantry labeling, involved assigning ingredient identifiers and corresponding legislative classifications. Web-based tools, product life cycle management platforms, and Microsoft Office digital solutions were effective in delivering these objectives.

Results: The findings revealed that when pantry labeling was implemented, ingredient identifiers and their corresponding legislative classifications streamlined product development processes. This phased approach also proactively facilitated the inclusion of emerging sound science. This session will summarize this model approach and present the benefits of ingredient identifiers.

Significance: The invention of ingredient identifiers within the ingredients' pantry simplified formula development, assured food safety and regulatory acceptability, proactively addressed evolving regulations, facilitated market access, and prevented country border food consignment detentions.

T19-02: ROLE OF MICROBIOLOGICAL CRITERIA GSO 1016/2015 TO CONTROL E. COLI IN MEAT

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Presentation type/Format: Technical

Category: Food Law and Regulation

Abstract:

Introduction: STEC causes hemolytic uremic syndrome (HUS) to human and normally associated with consumed food, particularly undercooked meat. Many countries established microbiological criteria applied by food law to test and control the consumption of food matrix against foodborne pathogens especially the most well-known strain of STEC, *E. coli* O157. However, some other countries did not include *E. coli* O157 and other important pathogens in their microbiological criteria which may lead to serious outbreaks.

Purpose: Identify a relationship between food outbreaks and lack in food microbiological criteria.

Methods: Twenty microbiological criteria were intensively investigated (Table 1). Those criteria are currently applied in fifty-three counties. According to this study approximately only one third of food worldwide is controlled by microbiological criteria. A number of foodborne outbreaks worldwide were positively or negatively linked to microbiological criteria.

Results: Out of the microbiological criteria tested in this study, fifteen (75%) applied *E. coli* O157 test to at least one food matrix related to meat products but not the rest. All the evaluated microbiological criteria (100%) accepted prevalence of *E. coli* in two out of five replicates of tested food samples with maximum of 5×10 colony forming unit (CFU) (with not further serotype identification). For example, Brazil and India's microbiological criteria do not require testing *E. coli* O157 in food matrix. Brazil and India are one of the biggest meat importers to Saudi Arabia market. According to Saudi Food and Drug Authority (SFDA), at least 6 % of tested meat imported from Brazil and India are infected with *E. coli* O157 (Table 2 and 3). This is probably due to the lack of Brazil and India's microbiological criteria in testing *E. coli* O157 in food matrix before export (Table 1).

Significance: There is a possible link between absence of testing *E. coli* O157 in microbiological criteria and existence of the same pathogen in food matrix that publicly available for consumption.

T19-03: RHEOLOGICAL PERFORMANCE OF BIOFILM FORMATION AT OIL-WATER INTERFACES

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Persons:

Yi Wang, University of Connecticut

Yangchao Luo, University of Connecticut

Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Bacterial adsorption and biofilm formation at oil-water interfaces can significantly impact food safety and industrial processes. However, the mechanical properties and hydrophobicity of bacterial biofilms in such systems remain poorly understood.

Purpose: This study investigates bacterial hydrophobicity and biofilm formation at oil-water interfaces using interfacial rheology and microscopy techniques.

Methods: Vegetable oil and medium-chain triglyceride (MCT) oil were used as the oil phases. *Escherichia coli* (K12) and *Salmonella* Enteritidis were suspended in PBS at OD600 = 0.6, and their zeta potentials were measured using a Zetasizer. The bacterial adhesion to hydrocarbons (BATH) test was conducted by measuring the light absorbance of the aqueous phase after vortexing with oil. Biofilm formation and elasticity at the oil-water interface were monitored using a rheometer (MCR 302, Anton Paar) with biconical disk geometry, at 28°C. Dynamic interfacial storage (G') and loss (G'') moduli were recorded over time. Light microscopy was employed to visualize changes in bacterial adsorption and biofilm structure at the interface.

Results: *Salmonella* formed more elastic biofilms than *E. coli*, with a maximum elasticity of 0.08 Pa·m against vegetable oil at 28 hours. The dynamic crossover of G' and G'' extended to 100% strain for both species compared to LB medium. Zeta potential measurements revealed net negative charges for *E. coli* (-17 mV) and *S. Enteritidis* (-6 mV) at pH 7. *E. coli* exhibited higher hydrophobicity, with values of $30.9 \pm 8.1\%$ and $38.8 \pm 1.1\%$ against vegetable oil and MCT oil, respectively. Microscopy confirmed three-dimensional biofilm structures at the interface.

Significance: These findings provide insights into bacterial interactions at oil-water interfaces. Such knowledge could inform strategies to manage bacterial contamination and biofouling in food processing environment.

Award Application: Developing Scientist Award

T19-04: SECONDARY COLONIZATION AND MICROBIAL COMMUNITY DYNAMICS OF *LISTERIA MONOCYTOGENES* IN RETAIL DELI BIOFILMS

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: The persistence of *Listeria monocytogenes* in food processing environments poses a significant challenge to food safety, due to its colonization of multi-species biofilms, which complicates sanitation efforts.

Purpose: This study aimed to investigate the role of *L. monocytogenes* as a secondary colonizer in biofilms, its cooccurrence with other genera, and the relationship between microbial diversity and its detection.

Methods: Stainless steel coupons were installed in retail deli environments and sampled over a 12-week period. Biofilm samples were collected from 10 stainless steel coupons, resuspended, and subjected to 16S rRNA amplicon sequencing. Aerobic plate counts and *L. monocytogenes* detection were performed. Beta diversity analyses (Qiime2) and differential abundance tests (DESeq2) were used to evaluate microbial community composition and identify genera associated with *L. monocytogenes* presence.

Results: *L. monocytogenes* was not detected within biofilms until later sampling dates, supporting a role as a secondary colonizer within biofilms dominated by *Pseudomonas*, *Psychrobacter*, and *Acinetobacter*. PERMANOVA

Results demonstrated significant clustering of microbial communities associated with drain surface swab results collected during coupon retrieval ($p < 0.05$). In contrast, no clustering was observed in microbial communities derived from within-biofilm samples. Differential abundance analyses revealed elevated levels of cobalamin-dependent bacteria in *L. monocytogenes*-positive samples, suggesting metabolic interactions that support its persistence.

Significance: This study confirmed the secondary colonization of biofilms by *L.*

monocytogenes, demonstrating its reliance on pre-existing biofilm communities for establishment and persistence. Additionally, the observed clustering of microbial communities by drain surface swab results, rather than within-biofilm results, suggests that biofilm community composition is instrumental in facilitating the propagation and spread of *L. monocytogenes*. This finding suggests that the biofilm community is more influential in the pathogen's environmental spread and detection outside the drain than in its presence within biofilms themselves.

T19-05: REDOX HOMEOSTASIS AND STRESS RESPONSES IN *CAMPYLOBACTER JEJUNI* FACILITATE SURVIVAL UNDER STRESSES IN THE AGRI-FOOD ENVIRONMENT AND ANTIBIOTIC TREATMENT

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: *Campylobacter jejuni* is a leading cause of bacterial gastroenteritis globally, with transmission primarily linked to the agri-food system, including poultry, dairy, and livestock. Its ability to survive in hostile environments is critical to its role as a foodborne pathogen. Beyond rising antibiotic resistance, *C. jejuni* exhibits persistence, where a subpopulation of cells temporarily survives antibiotic treatment without acquiring genetic mutations, potentially contributing to resistance development. Reactive oxygen species (ROS) generated by bactericidal antibiotics and food-related environments induce oxidative stress, highlighting the importance of redox homeostasis for *C. jejuni* survival. Antioxidants present in food matrices may counteract oxidative stress, further promoting bacterial persistence.

Purpose: This study aimed to investigate the impact of antioxidants on *C. jejuni* survival under oxidative stress and antibiotic treatment. The redox-related alterations induced by different classes of antibiotics were also assessed.

Methods: *C. jejuni* cultures were treated with ampicillin (100 µg/mL) or ciprofloxacin (1 µg/mL) in the presence of dimethyl sulfoxide (DMSO) at 0.1%, 0.5%, 1%, and 5% (v/v) as an antioxidant. After 6 hours, surviving cells were quantified via plating assays. Lipid peroxidation was assessed using a BODIPY™ sensor, and respiratory changes were evaluated via oxygen consumption rate (OCR) measurements using the Seahorse Extracellular Flux Analyzer.

Results: DMSO concentrations of 1% and 5% reduced antibiotic killing by at least 1.5 log ($p < 0.05$), while concentrations above 5% decreased cell viability. DMSO also enhanced *C. jejuni* survival under aerobic conditions. No significant changes were observed in lipid peroxidation, but antibiotics significantly elevated OCR ($p < 0.05$), indicating shifts in *C. jejuni* redox physiology.

Significance: This study reveals that antioxidants, such as DMSO, mitigate oxidative stress caused by antibiotics and aerobic conditions, reducing their killing efficacy and enhancing *C. jejuni* persistence. The presence of antioxidants in food

matrices may further counteract oxidative stress, promoting bacterial persistence and resilience.

Award Application: Developing Scientist Award

T19-06: INVESTIGATING PREVALENCE AND ECOLOGY OF *S. ENTERICA* AND *CAMPYLOBACTER* ON AGRITOURISM OPERATIONS

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Farm visits and on-farm activities (“agritourism”) are an increasingly important revenue source for farmers but have been linked to zoonoses in visitors.

Purpose: Determine prevalence of *Salmonella* and *Campylobacter* on agritourism farms with poultry in Vermont, Oregon, and North Carolina.

Methods: Agritourism farms with poultry were recruited and fecal and environmental samples collected from animal housing and visitor-specific areas. Samples were analyzed for *Campylobacter* and *Salmonella* using standard enrichment and selective isolation procedures. Isolates were confirmed as target species by biochemical (e.g., VITEK) and/or molecular techniques (e.g., PCR or WGS). Chi-squared tests were performed to determine differences in pathogen prevalence by farm, sample type, and animal type.

Results: 18 agritourism operations in VT (9), OR (5) and NC (4) were sampled from January-December 2024. Nine farms (50%) ≥ 1 sample positive for *Campylobacter* spp. (VT: 3/9; NC: 1/4; OR: 5/5). Ten farms (56%) had ≥ 1 sample positive for *Salmonella* (VT: 4/9; NC: 2/4; OR: 4/5). Farms differed significantly in the prevalence of both *Salmonella* and *Campylobacter* (p-values < 0.05). one OR farm had a significantly (p < 0.05) higher prevalence of *Campylobacter* (7/30; 23%). This same OR farm and two VT farms had a significantly higher prevalence of *Salmonella* (24/30; 80%; 7/25; 28%, 4/32, 12.5% respectively). The remaining farms had ≤ 2 samples positive for either pathogen. Pathogens were detected in

fecal samples (20/336; 6%) and booties (11/98; 11.22%) and 2/158 touchpoint (1.26%) samples, but not water, bedding, or feed (0/13). *Campylobacter* was detected in fecal samples from birds (chicken: 16/156; goose: 1/8; and ostrich: 1/5) and horses (1/19), while *Salmonella* was detected in feces from both birds (chicken: 23/193, goose: 1/8) and multiple mammal species (cow: 5/48, dog: 3/6; goat: 1/40; pig: 3/20; sheep 1/24).

Significance: *Salmonella* and *Campylobacter* are prevalent in agrotourism environments with animals onsite but rare on visitor touchpoints.

T20-01: SYNERGETIC EFFECT OF ELEVATED HYDROSTATIC PRESSURE, MILD HEAT, AND CARVACROL FOR INACTIVATION OF NON-TYPHOIDAL SALMONELLA SEROVARS

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: First reported more than a century ago, nontyphoidal *Salmonella* serovars are still the leading cause of foodborne hospitalizations and deaths in the United States.

Purpose: The purpose of current study is to investigate the impact of elevated hydrostatic pressure at 4.4 and 60.0 °C in presence of 0.2% (v/v) carvacrol for inactivation of non-typhoidal *Salmonella* serovars in buffered environment (PBS) and ground poultry.

Methods: A four-strain validated mixture of nontyphoidal *Salmonella* and a strain of S. Tennessee were exposed to elevated hydrostatic pressures of 350 and 650 MPa for 0 (control), 3, 5, and 10 minutes at temperatures of 4.4 and 60 °C with and without 0.2% carvacrol. Treatments were conducted in PULSE tubes inside the chamber of Hub880 Barocycler unit. Temperatures were adjusted using a stainless-steel jacket connected to a circulating water bath. Data were statistically analyzed at type I error level of 5% using Tukey-adjusted ANOVA. Additionally, linear (D-value) and non-linear (kmax) inactivation indices were calculated.

Results: Combination of mild heat (at 60.0 °C), low concentration of carvacrol (0.2%), and mild pressure (350 MPa) resulted in > 5.0 log CFU/mL reduction ($p < 0.05$) of *Salmonella* serovars, surpassing the log reductions obtained by the current high-pressure processing industry standard of 650 MPa. Selected strain mixture and *Salmonella* Tennessee exhibited comparable ($p \geq 0.05$) sensitivity to pressure-based treatments with D-values (350 MPa/4.4 °C) of 9.43 and 8.22 min, respectively. These values were reduced ($p < 0.05$) to 4.37 and 4.15 min, respectively with the addition of 0.2% carvacrol.

Significance: Application of mild heat at 60.0 °C and low concentration of carvacrol (0.2%) showed microbiologically important synergism for augmenting the decontamination efficacy of high-pressure processing. Additionally, *Salmonella* Tennessee, an epidemiologically important serovar, illustrated comparable sensitivity to the 4-strain mixture of nontyphoidal *Salmonella* and thus could be used interchangeably in future public health microbiology validation studies.

Award Application: Developing Scientist Award

T20-02: INTERACTIONS OF FIVE NATURAL BIOACTIVE COMPOUNDS AND ELEVATED HYDROSTATIC PRESSURE FOR INACTIVATION OF TYPHOIDAL AND NON-TYPHOIDAL SALMONELLA SEROVARS IN BUFFERED AND ACIDIC ENVIRONMENTS

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: With recent advancements in engineering of commercially available high-pressure processing (HPP) units and consumers' acceptability of pressure-treated products, the application of this non-thermal processing is gaining widespread adaptation in the food industry.

Purpose: The current study investigated impacts of HPP as augmented by five natural bioactive compounds for decontamination of typhoidal and nontyphoidal *Salmonella* serovars in buffered and acidic environments.

Methods: As a complete randomized block design, this study was statistically analyzed using Tukey- and Dunnett's-Adjusted ANOVA (at type I error level of 5%). To further investigate the impact of treatments, a forest plot of log reductions and linear and non-linear inactivation indices were additionally calculated. The trials were conducted under buffered condition (HEPES buffer) and in acidic environment (pomegranate juice) using pressure intensity level of 400 MPa with addition of 0.2% carvacrol, thymol, lactic acid, citric acid, and malic acid.

Results: Among the tested antimicrobials, lactic acid showed highest capability to augment ($P < 0.05$) the pathogen reduction efficacy of HPP. In pomegranate juice, as an example, a 400 MPa treatment for 6 minutes reduced ($P < 0.05$) nontyphoidal *Salmonella* serovars by 1.63 log CFU/mL while addition of 0.2% lactic acid to the same treatment, resulted in 3.80 log reductions ($P < 0.05$). For nearly all treatment times and used antimicrobials, similar ($P \geq 0.05$) reduction trends were observed for typhoidal and nontyphoidal *Salmonella* serovars in buffered and acidic environments.

Significance: Addition of low concentration of natural bioactive compounds was able to augment the decontamination efficacy of the HPP treatments. Application of mild pressure synergized by the validated antimicrobials could lead to microbiologically safe products while ensuring superior quality and organoleptic properties. Typhoidal and nontyphoidal *Salmonella* serovars illustrated comparable sensitivity to elevated hydrostatic pressure and antimicrobials and thus could be used interchangeably in future public health microbiology validation studies.

Award Application: Developing Scientist Award

T20-03: SALMONELLA MONTEVIDEO SURVIVAL ON CHILI DURING MICROWAVE DRYING: OPTIMAL MODELS AND PRECISION ANALYSIS

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: The World Health Organization ranked chili pepper as the spice most at risk for *Salmonella* contamination, but chili drying processes lack data on *Salmonella* inactivation kinetics.

Purpose: This study investigates the fate of *Salmonella* Montevideo during microwave drying of fresh chili peppers, comparing multiple predictive models for the effect of dynamic temperature and water activity conditions on *Salmonella* survival.

Methods: Fresh red chili peppers were sliced lengthwise, inoculated with *S. Montevideo*, and dried in a microwave oven for 45 min at 120W and 18 min at 240W. Thermal image was captured rapidly at each time point, using an infrared camera and the water activity was measured. *Salmonella* survivors were grown on differential non-selective media and enumerated. We compared four primary models (Weibull, log-linear, Geeraerd, and Cerf models) and incorporated a modified-Bigelow type model into the selected primary model to consider the effect of effect of temperature and water activity. All models were evaluated using the root mean square errors (RMSE) and corrected Akaike information criterion (AICc).

Results: Higher microwave power level resulted in lower *Salmonella* survival over time ($P < 0.05$). Statistical analyses identified the Geeraerd and log-linear models as the preferred primary models. incorporating the modified-Bigelow model into both primary models yielded in similar RMSE (0.501-0.504) and AICc (12-18). But the Geeraerd/modified-Bigelow showed large errors (2.46-139.43%) and presented small, scaled sensitivity coefficients causing higher error in the parameter estimates. The log-linear/modified-Bigelow model better described the *Salmonella* inactivation, and the tailing effect observed during chili microwave drying.

Significance: These findings serve as a basis for the food industry to develop and refine spice drying conditions for enhanced *Salmonella* control while maintaining sensory quality. This study is one of the few works that considers the dynamic

status of product water activity in *Salmonella* inactivation model in food drying applications.

Award Application: Developing Scientist Award

T20-04: SYNERGISTIC ANTIMICROBIAL EFFECT OF PLASMA-ACTIVATED MICROBUBBLE WATER (PAMW) AND ULTRAVIOLET LIGHT (UV) TO ENHANCE ESCHERICHIA COLI INACTIVATION ON FRESH PRODUCE

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: The approach of combining plasma and UV light enhances microbial inactivation through a synergistic effect, where PAMW generates reactive oxygen and nitrogen species that complement UV's DNA-damaging ability, improving surface exposure, reducing microbial stress resistance development, and potentially shortening treatment time.

Purpose: To investigate the synergistic antimicrobial effect of PAMW and UV on the inactivation of *E. coli* on strawberries and blueberries. **Method:** Strawberries (n=108) and blueberries (n=108) were spot inoculated with *E. coli* TVS355 and then suspended in 4L of water under varying treatment conditions, including PAMW and UV combined (PUV), UV with agitation, PAMW, microbubble water (MBW), and distilled water (DW) for 10, 15, and 20 minutes. PAMW was generated by ionizing compressed air using a rod-to-mesh dielectric-barrier discharge (DBD) reactor and injecting plasma gas into water via a microbubble diffuser. The flow rate was 1.5 SCFH, with a 36.4 kVpp power supply and a 500 Hz discharge frequency. Two UV intensities (10 and 18 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were tested, and the agitation speed was 142.8 rpm. *E. coli* recovery from the berry surfaces was quantified using MacConkey agar plates containing rifampicin (50 mg/L). Statistical analysis was conducted using one-way ANOVA ($p=0.05$).

Results: The average initial *E. coli* populations were 6.97 ± 0.27 log CFU/mL on strawberries and 6.05 ± 0.24 log CFU/mL on blueberries. For strawberries, synergistic effects of PAMW and UV were observed for both high and low UV intensity after a 20-minute treatment with reductions of 3.57 ± 0.12 and 3.80 ± 0.25 log CFU/mL, respectively. For blueberries, synergistic effects were observed for low UV intensity after a 10-minute treatment with a 3.87 ± 0.53 log CFU/mL *E. coli* reduction.

Significance: This study shows that combining PAMW and UV light is a promising approach to enhancing microbial safety on fresh produce.

T20-05: QUANTITATIVE ANALYSIS OF AN ENZYME-BASED SURROGATE TO ASSESS THE ANTIMICROBIAL EFFECTIVENESS AT DIFFERENT DOSAGES OF PLASMA-ACTIVATED WATER

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: While plasma treatment has been applied to medical and food industries for sanitization, its antimicrobial effectiveness is hardly compared among the different plasma technologies and equipment as the composition and the number of reactive species may vary for different equipment. Our previous studies have developed rapid assessment methods that correlate plasma antimicrobial capacity with the activity of biochemical surrogates, eliminating the need for traditional, time-consuming bacterial surrogate testing. However, the accuracy of the assessment requires further improvement, particularly since previous work relied solely on a single parameter of foam height.

Purpose: This study aimed to quantitatively analyze a catalase-based surrogate for rapid assessment of the antimicrobial effectiveness of plasma-activated water (PAW).

Methods: Various doses of PAW were generated with different levels of organic matters. The PAW doses were assessed using a visual assay based on the catalase-H₂O₂ reaction, in which the released oxygen was trapped by surfactants to form a visible foam. The foam (n=5) was then analyzed by Dynamic Foam Analyzer for its height, bubble structure, and images. Microbiological experiments (n=5) on *E. coli* reduction were also conducted to correlate the effectiveness. The foam analysis and microbial reduction results were imported in Matlab for correlation analysis.

Results: Multiple statistical models were established between plasma dosages, foam parameters, and actual microbial reduction. *E. coli* reduction of 0.2 to 5.7 log CFU/mL was achieved by the PAW dosages tested in the study. The results confirmed that catalase activity which was quantified by foam height, was

associated with plasma dosages and ultimately correlated with microbial reduction. In addition, plasma dosages were negatively associated with the average bubble radius in foam. The bubble count half-life was also significantly impacted by plasma dosages ($P < 0.05$).

Significance: This study provides a systematic assessment of this enzyme-based surrogate on estimating plasma antimicrobial effectiveness.

Award Application: Developing Scientist Award

T20-06: COLD PLASMA GAS FLOW RATES IMPACT INACTIVATION OF PATHOGENS IN FRESH BASIL

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Fresh produce is often associated with outbreaks of enteric pathogens. The search for inactivation techniques that preserve the quality characteristics of fresh produce has been a challenge. Cold plasma can reduce microbial loads while maintaining food freshness. Studies on effective conditions for the application of cold plasma for inactivating foodborne pathogens are still needed.

Purpose: The

Purpose of this study was to evaluate the effect of different gas flow rates on the reduction of *Salmonella enterica* and *Escherichia coli* O157:H7 in fresh basil leaves using cold plasma.

Methods: Small portions (~3 g) of whole fresh basil (*Ocimum basilicum* L.) leaves were spot inoculated with inocula of *S. enterica* (Saintpaul, Newport, Enteritidis, and Typhimurium) or *E. coli* O157:H7 (final concentration ~7 log CFU/g). inoculated leaves were exposed to glow discharge plasma generated at three gas flow levels (10, 20, and 30 mL/min) at 80 kV for 15 min. Pathogens were enumerated just after the cold plasma application, and after 72 and 120 h of refrigerate storage. Samples not subjected to cold plasma were used as a control. Experiments were performed three times in triplicate.

Results: Cold plasma at 10 and 20 mL/min reduced ($p < 0.05$) *Salmonella* counts by approximately 3.5 log CFU/g and *E. coli* O157:H7 by approximately 3.0 log CFU/g in basil leaves ($p > 0.05$). increasing gas flow to 30 mL/min decreased the efficacy of the cold plasma treatment against both pathogens tested (reductions of 1.5 and 2. log CFU/g for *Salmonella* and *E. coli* O157:H7, respectively).

Significance: Results show the impact of different cold plasma treatments on two important foodborne pathogens associated to outbreaks involving fresh produce. Findings indicate that gas flow rates used when generating cold plasma should be considered when designing inactivation studies.

T20-07: IMPACT OF SHEAR VALVE GEOMETRY AND FLUID VISCOSITY ON BACTERIAL SPORE INACTIVATION IN ULTRA-SHEAR TECHNOLOGY

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Ultra-Shear Technology (UST) is a novel hybrid nonthermal-thermal method for sterilization of low-acid liquids by inactivation of bacterial spores. During UST, fluids are pressurized up to 400 MPa and then rapidly decompressed through a small gap (shear valve). Limited studies have investigated the impact of valve geometry and fluid rheology on the degree of microbial inactivation.

Purpose: This study investigated the impact of shear valve geometry and fluid viscosity on the inactivation of bacterial spores during UST processing.

Methods: *Bacillus cereus* ATCC14579 spores (7.4 ± 0.2 log CFU/mL) were suspended in 0.1M HEPES buffer (pH 7.3, flow consistency index (K) = 0.34 mPa·sn) and in HEPES buffer containing 50% (w/v) polyethylene glycol (PEG) (pH 7.8, K = 41.91 mPa·sn). The spore suspensions were UST processed using either an ultra-shear valve (USV, shear rate up to 10^{10} s⁻¹) or a low-shear annular valve (LSV, shear rate up to 10^8 s⁻¹), applying 400 MPa and exit temperatures of 105 or 120 °C for 2 s holding time. Spore survival was determined through plate counting ($n \geq 3$).

Results: At an exit temperature of 105 °C, UST processing with the USV resulted in 2.0- and 2.5-log reductions in spore counts for HEPES and HEPES + PEG, respectively, while the LSV caused no significant inactivation ($p > 0.05$). Similarly, at 120 °C, the USV achieved 2.9- and 2.5-log reductions, compared to 1.7- and 1.5-log reductions with the LSV. The USV appeared to induce greater spore inactivation, likely due to the higher elongational and shear stresses to which the spores were exposed.

Significance: This study highlights the critical role of shear valve geometry in bacterial spore inactivation during UST processing of liquid foods with varying rheological properties.

T21-01: A PROBABILITY-BASED GROWTH/NON-GROWTH BOUNDARY MODEL FOR BACTERIAL POPULATIONS

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Growth probability near the growth/non-growth boundary depends on initial inoculum size, yet a theoretical model from the single-cell perspective is lacking.

Purpose: We developed a single-hit model to estimate the probability of bacterial population growth at various initial inoculum levels.

Methods: *Escherichia coli* and *Bacillus cereus* were each inoculated into tryptic soy broth supplemented with glycine, sodium chloride, or ethanol in triplicate 96-well microplate experiments. Six initial inoculum levels (0, 1, 2, 3, 4, 5 log CFU/well) were tested at 25°C for up to 7 days. Turbidity was recorded before incubation and on days 3 and 7; wells showing an increase in optical density greater than 0.1 were deemed positive for growth. Growth probability under each condition was the proportion of positive wells. A Bayesian approach was used to fit a single-hit model, yielding estimates of individual cell growth probability that scale with initial inoculum size.

Results: in all test conditions, higher initial inoculum levels corresponded to higher growth probabilities. The single-hit model captured these trends effectively, with root mean squared error below 0.11 on day 3 and below 0.20 on day 7, indicating robust performance across both species and stressors.

Significance: This simple yet interpretable model generalizes the growth probability at any starting cell count from an estimated single-cell growth probability. incorporating this approach into existing boundary models will strengthen risk assessments and food safety predictions by accounting for the influence of initial bacterial load.

T21-02: PREDICTIVE MICROBIAL ECOLOGY IN BROILER PROCESSING ENVIRONMENT USING MACHINE LEARNING AND GENOMICS

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Artificial intelligence (AI) and machine learning (ML) are transforming predictive microbial risk assessment by enabling the integration of complex microbiome data from food processing environments.

Purpose: This study applied 16S rRNA amplicon sequencing, bioinformatics, and ML to profile microbial diversity changes at various stages of broiler chicken processing by analyzing rinsate samples in a case study conducted at an Australian abattoir.

Methods: ML models were employed to identify contamination patterns, predict microbial persistence, and estimate risk hotspots along the processing chain. The research utilized supervised learning to classify bacterial genera across different processing stages and unsupervised learning to cluster microbial communities based on sequencing data. Principal coordinate analysis (PCoA) and non-metric multidimensional scaling (nMDS) revealed distinct clusters of microbial populations at different processing points.

Results: A significant finding was that the defeathering and chilling stages exhibited the highest levels of contamination and microbial redistribution. ML algorithms identified correlations between microbial abundance in processing waters and post-chill carcass contamination, highlighting critical control points for contamination management. The study demonstrated that microbial diversity changes dynamically throughout the processing line, with Firmicutes dominating scalding and chilling stages, while Proteobacteria and Bacteroidota were prevalent during defeathering and evisceration. ML models accurately predicted microbial persistence during critical stages, identifying potential risk factors for contamination spread. Sensitivity analysis revealed that bacterial genera such as *Anoxybacillus*, *Tepidimicrobium*, *Lactobacillus*, and *Acinetobacter* were key contributors to post-chill contamination, emphasizing their importance in microbial risk management strategies.

Significance: This research highlights the utility of combining AI-driven ML with next-generation sequencing for more accurate and dynamic microbial risk

assessments in the food processing environment. These tools can improve hazard analysis critical control point (HACCP) protocols by providing real-time, data-driven insights for managing microbial contamination, thereby enhancing food safety and extending product shelf life.

T21-03: PERACETIC ACID INACTIVATION OF *SALMONELLA* DURING IMMERSION POULTRY CHILLING: MATHEMATICAL MODELING OF FUNDAMENTAL DYNAMICS

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: While the chiller step is an essential HACCP for poultry processors of all sizes, the underlying mechanisms governing the changes in *Salmonella* levels on carcasses and in chiller water are still not well understood.

Purpose: The goal of this study is to quantify the fundamental mechanisms regulating *Salmonella* shed (from carcass surface to water) and peracetic acid (PAA) inactivation rates in chiller water and on carcass surfaces during immersion chilling.

Methods: Under lab-scale simulated immersion chilling conditions, a five-strain *Salmonella* cocktail inoculum (10^5 – 10^7 CFU/mL) was used to inoculate chiller water and chicken thighs (100 ± 20 g). Inactivation and shedding dynamics were measured over 0.5–10 minutes with exposure to 1–50 ppm PAA, with or without organic load, using a neutralizing buffered peptone water rinse. Using ordinary differential equations and chemical reaction theory, novel mathematical forms were built to describe shedding and inactivation rates reflected in the data. Parameters governing characteristic time scales for shedding and inactivation were determined using nonlinear least squares algorithms (Levenberg-Marquardt type).

Results: A best-fit saturation form (Michaelis-Menten type) for *Salmonella* transfer from poultry to chiller water revealed a characteristic time scale for shedding as a decreasing function of time, with 50% shed occurring between the initial 15 to 60 seconds. The PAA inactivation coefficient of *Salmonella* was 0.13 ± 0.01 L/(mg·min) in the absence of organic load. PAA inactivation rates of *Salmonella* on poultry surfaces during simulated chilling ranged from approximately 30%–90% of the PAA inactivation coefficient in water.

Significance: The developed mathematical forms for shedding and PAA inactivation are essential for building and validating accurate cross-contamination models for *Salmonella* during immersion chilling. In turn, results based on elemental features can improve decision making for pathogen control as well as provide tools to ensure processor confidence towards compliance.

T21-04: MICROBIAL EXPOSURE ASSESSMENT OF UROPATHOGENIC E. COLI IN RTE CHICKEN

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Poultry is a significant reservoir for uropathogenic *Escherichia coli* (UPEC), an emerging foodborne pathogen linked to urinary tract infections. Ready-to-eat (RTE) chicken, frequently consumed without reheating, is highly susceptible to contamination during retail. This study is the first in Taiwan to quantitatively assess UPEC exposure risk from RTE chicken and identify intervention strategies for risk mitigation.

Purpose: This study aimed to develop a retail-to-fork quantitative microbial exposure assessment to evaluate UPEC risks from RTE chicken and propose evidence-based interventions to enhance food safety.

Methods: Ninety-six RTE meat samples (chicken, pork, beef, and duck) were collected from wet markets and caterers in Taiwan. *Escherichia coli* enumeration was performed via plate count

Methods, and UPEC was confirmed using PCR for genes C3509, chuA, and C3686. Monte Carlo simulation (10,000 iterations) with the 'mc2d' package in R estimated probabilities of exposure exceeding UPEC thresholds (10^5 – 10^7 CFU/g per serving). Key model parameters included UPEC prevalence, growth during retail-to-consumption stages, time-temperature conditions, and serving sizes. Sensitivity analysis identified critical control points, and scenarios such as reducing retail duration and optimizing storage conditions were evaluated for risk reduction.

Results: Chicken had the highest UPEC prevalence (7/21, 33.3%). The model estimated a high UPEC exposure probability (>7 log CFU/serving) of 11.69% for wet markets and 3.72% for caterers. UPEC concentration at retail and prolonged retail duration were the most significant risk factors. Shortening retail time and maintaining storage at 18°C reduced risks by 0.97 times.

Significance: This study provides a framework for assessing UPEC risks in RTE chicken in Taiwan and highlights practical interventions to reduce contamination and enhance consumer safety through safer retailing and consumption practices.

T21-05: A MONTE CARLO SIMULATION MODEL TO EVALUATE THE EFFECTS OF RETAIL STORAGE CONDITIONS ON CONSUMER RISK FROM ROMAINE LETTUCE

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Maintenance of the cold chain is vital to ensure the microbiological quality of fresh produce; temperature abuse can significantly increase growth of harmful bacteria, leading to foodborne illness. Despite regulatory oversight, the storage conditions of fresh produce in retail vary. Few studies evaluate the significance of retail storage conditions on the safety of fresh produce, making the retail environment the ‘forgotten’ part of the supply chain.

Purpose: Here, we aim to address the microbiological risk associated with Shiga toxin-producing *Escherichia coli* (STEC) in bagged Romain lettuce stored in closed vs. open refrigerated display cases.

Methods: Previously collected data on retail storage across 5 US states to evaluate temperature profiles of produce display cases was used. A full farm-to-fork risk assessment model to evaluate STEC concentrations at the end of the supply chain, and corresponding consumer risk in relation to levels at harvest was developed in R, and effects of variability and uncertainty in the supply chain input parameters were separated using second-order Monte Carlo simulations.

Results: The median STEC concentration of Romaine lettuce at consumption was lower when lettuce was purchased from retailers with closed display cases (-5.39 log₁₀CFU/g), compared to those with open display cases (-4.87 log₁₀CFU/g). Similarly, due to potentially broad ranges of temperature conditions associated with open storage cases the variability in STEC concentration was drastically larger in open (95% CI: -7.15, 1.33 log₁₀CFU/g) vs. closed (95% CI: -7.49, -3.03 log₁₀CFU/g) display cases. Based on these STEC concentration estimates the median public health risk associated with lettuce stored in closed retail displays was 1 STEC case per 100,000 people (95% CI: 0, 42 cases) vs. 32 cases (95% CI: 0, 655 cases) associated with open retail display cases.

Significance: The results of our study demonstrate the need for further evaluation and control of retail storage conditions to reduce consumer risk.

T21-06: HOW DO TEMPERATURE CONTROLS AND PHYSIOLOGICAL CHANGES DURING FORWARD PROCESSING IMPACT SHIGA TOXIN-PRODUCING ESCHERICHIA COLI O157:H7 RISKS IN LETTUCE?

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Temperature controls such as refrigerated conditions are recommended during forward processing, where lettuce is transported under cold storage, potentially over multiple days, prior to further processing, to mitigate microbial risks in lettuce. However, cold stress may induce Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 to enter a viable but nonculturable (VBNC) state, undetectable by conventional plating techniques. These cells can resuscitate under favorable conditions, posing a potential infection risk. This study assessed the effect of cold stress on STEC physiology during forward processing and utilized a probabilistic quantitative microbial risk assessment (QMRA) to evaluate its impact on risks.

Purpose: To determine whether cold stress during forward processing impacts the formation of VBNC *E. coli* O157:H7 and if such changes influence food safety risk in lettuce.

Methods: Mature Romaine lettuce was inoculated with three rifampicin-resistant STEC strains and harvested at 9°C and 17°C. Post-harvest, the produce was stored at 2.2°C for five days, with daily analyses quantifying VBNC cells. A QMRA model quantified the illness risk from consuming field-bagged Romaine hearts and shredded and packaged lettuce. Model parameters were informed by literature and industry data. Scenario analyses evaluated the effect of VBNC switch rates on risk using convolution tests.

Results: Extended cold storage of lettuce did not significantly ($p > 0.05$) affect the VBNC switch rates. Lettuce that was harvested at 17°C rather than 9°C had significantly higher odds (odds = 1.08, $p < 0.001$) of VBNC formation. Median illness risks were 1.24×10^{-8} for field-bagged Romaine hearts and 2.67×10^{-10} for shredded lettuce. The probability of a case in a million was 0.48 and 0.24, respectively. Cold stress-induced physiological changes did not significantly ($p > 0.05$) increase risks in either type of lettuce. Uncertainty factors for shredded

lettuce were dominated by post-processing variables, while for Romaine hearts, pre- and postharvest factors were key.

Significance: Proper temperature controls effectively mitigate risks posed by STEC in lettuce. This study highlights that forward processing does not significantly impact risks, reinforcing the importance of temperature management and offering insights for improving produce safety strategies.

T21-07: EVALUATING THE REUSE OF GREYWATER FOR IRRIGATING GROUND-LEVEL FRESH PRODUCE: A MICROBIOLOGICAL RISK ASSESSMENT OF GENERIC E. COLI CONTAMINATION ON LETTUCE

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Chile's water scarcity crisis, driven by prolonged droughts and increasing agricultural demand, necessitates innovative solutions like reusing treated greywater for irrigation.

Purpose : The primary objectives were to assess the microbial safety of greywater reuse for irrigation and pinpoint key risk factors throughout the production chain.

Methods: This study applied a Quantitative Microbial Risk Assessment (QMRA) model to evaluate the risks of *Escherichia coli* contamination on lettuce, a crop consumed raw and sensitive to irrigation water quality. The QMRA model incorporated modules for microbial dynamics at each stage, spanning from production to retail. Baseline scenarios simulated contamination risks from three irrigation sources: untreated greywater, surface water, and groundwater. Treatment scenarios evaluated the efficacy of wetland-based and microfiltration primary systems combined with secondary disinfection methods (ClO₂, ozone, and ultraviolet light). Additionally, sensitivity analyses identified other key risk factors, from soil contamination to post-harvest practices.

Results: Key findings demonstrated that untreated greywater posed significant risks, with 85.06% and 82.10% of simulated cases exceeding the *E. coli* threshold of 3-logCFU/g in fresh produce during Spring/Summer and Fall/Winter, respectively. However, integrated primary and secondary treatments effectively reduced microbial levels by over 5-log, aligning with international safety criteria. In addition to irrigation water, soil contamination emerged as a dominant risk factor, particularly in scenarios with insufficient manure application intervals. Post-harvest risks were amplified by inadequate refrigeration, as simulated lettuce exceeded safe *E. coli* levels within 30 hours at ambient temperatures, emphasizing the need for cooling systems during transportation and storage.

Significance: This study highlights the feasibility of greywater reuse for irrigation when supported by effective treatment while emphasizing the diverse

contamination risks from irrigation water, soil, and post-harvest practices in lettuce production. The findings provide a comprehensive framework to inform policies for safely reusing greywater and mitigating risks across the supply chain, promoting sustainable water management while ensuring public health.

Award Application: Developing Scientist Award

T21-08: RISKS ASSOCIATED WITH THE USE OF UNTREATED MANURE APPLICATION IN ORGANIC PRODUCTION OF FRESH PRODUCE: A SEMI-QUANTITATIVE RISK ASSESSMENT

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Pathogens in animal manure can transfer to soil and potentially contaminate fresh produce grown from manure-amended soil. To better harmonize organic agriculture with food safety practices, a risk assessment approach can be applied to incorporate such findings into a decision-making tool.

Purpose: To develop a reproducible semi-quantitative risk assessment tool to support decision-making under various ‘what if’ scenarios.

Methods: We synthesized current literature on food safety risks associated manure application and constructed a pathway-based risk assessment model with identified risk factors. Literature-based probability of Shiga Toxin-producing *E. coli* (STEC) contamination on fresh produced were estimated from STEC concentration in manure and survival in manured soil using Pert distribution. Public dataset was used to formulate normal distributions of environmental factors. Expert elicitation supplemented parametrization when data was insufficient. The model was evaluated using Global Sensitivity Analysis (GSA) and Random Forest (RF) to identify influential risk factors and assess model performance.

Results: The risk assessment model represented the current practices in organic production and a total of 26 articles were filtered for parameterization. A scored risk of STEC contamination on fresh produce was formulated while accounting for different association strength. This model identified that surface applying shortly stored dairy or cattle manure in winter, with a rate of under 5 tons per acre per year, to the silty soil, where touching-the-soil produce is harvested 30 days after manure application, resulting in a medium-high risk of STEC contamination. The ranking of risk factors and respective uncertainty was visualized upon GSA and RF outputs.

Significance: This tool will support decision-making among organic growers and food safety professionals to improve manure management and application under various ecological and environmental scenarios across the US. It will also bridge the gap between practical operations and regulatory requirements and help growers practice food safety effectively on organic-certified farms.

Award Application: Developing Scientist Award

Poster Abstracts

P1-02: IN VITRO ANTIMICROBIAL ACTIVITIES OF PELARGONIC ACID AGAINST FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Pelargonic acid (PA) is an antimicrobial food additive that is Generally Recognized as Safe (GRAS). Due to its poor solubility in water, its use is challenging. This study investigates the antimicrobial efficacy of chemically modified PA, combined with a citrus product.

Purpose: The objective is to examine the antimicrobial activity of modified PA and a citrus blend against foodborne pathogens.

Methods: PA at a concentration of 0.43 M was modified to increase its pH to 8.15 using sodium bicarbonate. This modified PA was used to develop two products with lemon juice, with and without buffering. The minimal inhibitory concentration (MIC) of the PA products was determined using a 96-well microtiter plate broth dilution assay with foodborne pathogens. The antimicrobial efficacy of PA products in controlling lactic acid bacteria (LAB) growth in meat (briskets and ready-to-eat [RTE] turkey) was tested with an initial inoculation rate of about 2.6 log cfu/g at room temperature (18-20°C) for 24 hours.

Results: Single-factor ANOVA showed that PA treatments significantly inhibited *Listeria monocytogenes*, *Salmonella* species, *E. coli* species, and LAB species ($p \leq 0.05$). F-Test results indicated that PA treatments significantly controlled LAB in both RTE turkey and briskets ($p \leq 0.05$). PA lemon juice without buffering demonstrated a synergistic and significant effect on LAB growth control.

Significance: The results indicate that PA combined with lemon juice has a promising effect on controlling the growth of pathogens and spoilage microorganisms in processed food.

P1-03: ASSESSING THE POTENTIAL OF SUSTAINABLE PLANT DERIVED INGREDIENTS ON INHIBITING FOODBORNE PATHOGENS AND FOOD SPOILAGE MICROORGANISMS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Citrus fruits by-products after juicing, including peel, seeds, pulp, and segmental residues, are abundant in flavonoids, polyphenols, terpenoids, organic acids (primarily citric acid), amino acids, essential oils (mainly d-limonene), lipids, and vitamins. Bioactive molecules extracted from citrus waste demonstrate antimicrobial, antioxidant, and antiallergic properties.

Purpose: This study aims to explore the potential of developing functional food ingredients from citrus by-products and to evaluate their antimicrobial effectiveness against foodborne pathogens and spoilage microorganisms.

Method: Citrus waste, such as peels, pulpy fiber, and rag materials, were collected from citrus juice processors in the USA. These products were processed individually to develop antimicrobial functional ingredients. Final ingredients developed in dry form were tested to determine the minimal inhibitory concentration (MIC) of each ingredient using the 96-well microtiter plate broth method against foodborne pathogens and spoilage microorganisms, such as lactic acid bacteria (LAB).

Results: Single Factor ANOVA analysis indicated that ingredients derived from citrus fiber, rag, and peel significantly inhibited the growth of various strains of *Listeria monocytogenes* ($p \leq 0.05$). Ingredients developed from peel showed poor efficacy in inhibiting the growth of *Salmonella* species, *E. coli* species, and LAB species ($p \geq 0.05$), comparable to the control. However, rag and fiber ingredients significantly inhibited their growth in broth culture ($p \leq 0.05$).

Significance: Broth test results revealed that antimicrobial ingredients developed from citrus by-products have a promising effect on inhibiting the growth of pathogens and spoilage microorganisms.

P1-04: EFFICACY OF ANTIMICROBIAL WASHES FOR CONTROL OF SALMONELLA ENTERICA ON FRESH-CUT ORCHARD PEACHES

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Category: Antimicrobials

Abstract:

Introduction: Peaches contaminated with *Salmonella* via fugitive dust were implicated in a 2020 outbreak of salmonellosis. Washing of fruit may be an important critical control point for control of *Salmonella* on peaches; however, little is understood regarding the efficacy of various washes when peaches are subsequently prepared as fresh-cut fruit.

Purpose: To determine the efficacy of various produce washes for control of *Salmonella* on inoculated fresh-cut peaches.

Methods: Orchard-acquired fruit were sliced and 100 g was spot-inoculated with a cocktail of rifampicin-resistant *Salmonella enterica* on either the skin or flesh side of the peaches and dried for 1 hour. Initial levels of *Salmonella* were approximately 6 log CFU/100 g peaches. Pieces were washed with either tap water, 40 ppm chlorine, 40 ppm peracetic acid wash, or a commercial cut produce wash prepared as directed for 1 or 3 minutes. All treatments were followed by neutralization with 100 mL Butterfield's Phosphate Buffer and enumeration on Brain Heart Infusion Agar with rifampicin. Population reductions were compared using ANOVA with $p < 0.05$ considered significant.

Results: Overall, there was no significant difference in population reductions based on inoculation placement (skin vs. flesh) or wash time. Washing with tap water and commercial wash resulted in less than 1.5 log CFU/100 g reduction, while the 40 ppm chlorine wash resulted in a 2.4 to 2.7 log CFU/100 g reduction. The highest reductions of 3.4 to 4.1 log CFU/100 g were observed when using 40 ppm peracetic acid. Only the 40 ppm chlorine and 40 ppm peracetic acid washes were significantly different from the water control.

Significance: The results of this study contribute to understanding the efficacy of various washing schemes for control of *Salmonella* on fresh-cut peaches and other stone fruits.

P1-05: COMPARATIVE EFFICACY OF CHEMICAL AND COMMERCIAL WASHES ON CONTROLLING SALMONELLA ENTERICA ON WHOLE FRESH ORCHARD PEACHES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Peaches have recently been implicated in an outbreak of *Salmonella enterica* with fugitive dust being considered a potential novel method of contamination. Little is understood regarding how *Salmonella* on fruit contaminated in this manner may be reduced via chemical or commercial washes.

Purpose: This study looked at the impact of various washes on the reduction or inactivation of *S. enterica* on whole peaches that were contaminated with fugitive dust.

Methods: Fresh whole peaches were acquired from a local orchard in Illinois. Peaches were dry inoculated with a four-strain cocktail of *S. enterica* by blown silica dust resulting in a population of 6.02 ± 0.49 log CFU/peach. Peaches were washed with either tap water, 40 ppm chlorine, 40 ppm peracetic acid, and either a lactic acid-based or organic mineral and oil-based commercial wash for 1 or 3 min followed by drying and enumeration for *S. enterica* on Brain Heart infusion agar. *S. enterica* population reductions were statistically compared using ANOVA with $p < 0.05$ considered significant.

Results: Washing peaches with tap water for 1 and 3 min led to *S. enterica* population reductions of 2.82 ± 1.34 and 2.67 ± 0.09 log CFU/peach, respectively. The highest log reduction was achieved with both chlorine and peracetic acid ($> 4.42 \pm$ log CFU/peach) after 3 min. For commercial washes, the lactic acid-based wash achieved log reduction of 3.02 ± 0.73 and 2.83 ± 0.16 log CFU/peach after 1 and 3 min washing, respectively. The mineral and oil-based wash led to population reductions of 2.67 ± 0.13 and 2.73 ± 0.08 log CFU/peach after 1 and 3 min, respectively. For all washes, additional wash time was not significant for further reduction of *S. enterica* on peaches.

Significance: The result of this study contributes to identifying optimal washing

conditions and assessing their influence on the microbial safety of whole fresh peaches contaminated with *Salmonella*.

P1-06: ANTIMICROBIAL RESISTANCE OCCURRENCE IN BRAZILIAN ANTIBIOTIC-FREE CHICKEN PRODUCTION FARMING

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Chicken meat production has traditionally relied heavily on antibiotic use. However, the rising demand for antibiotic-free production systems has driven efforts to explore alternative practices. The emergence and spread of resistant bacteria are not solely linked to antibiotic use. Tolerance can arise and propagate in antibiotic-free systems due to persistence of environmental resistance genes and horizontal transfer.

Purpose: This study aimed to investigate the microbial composition and profiles of antimicrobial resistance genes (ARGs) in cloacal swabs collected from chickens raised in certified antibiotic-free production systems.

Methods: Five antibiotic-free broiler farms were visited and a total of 5 composite cloacal samples (20 animals from each farm) were collected and examined. DNA extraction was performed using the MagicPure® Stool and Soil Genomic Kit and shotgun metagenomics carried out with high-throughput DNA sequencing on NextSeq platform (Illumina). Raw data were evaluated using FASTQC and Trimmomatic tools. For the resistome abundance analysis, ARGS-OAP pipeline was used. Abundance of each type and subtype of ARGs was calculated and normalized. Resistome of a given experiment was analyzed in terms of gene abundance (number of gene copies/16S rRNA) and diversity.

Results: Across all collected samples 22 type and 410 ARG families were detected. The co-resistome comprised 79 ARGs shared across samples, including genes conferring resistance to beta-lactams (*Escherichia coli* ampC, *Klebsiella pneumoniae* OmpK37, and TEM-1), aminoglycosides (AAC(3)-Via, ANT(6)-Ia, APH(3')-IIIa, and aadA12), bacitracin (bacA and bcrA), tetracyclines (tet 40/44/A/M/O/Q/U and W), polymyxins (arnA, eptA, pmrF, rosA, rosB, and ugd), and other multidrug resistance genes. The most prevalent ARGs identified were bacA (372.27), tet(W) (334.69), mdtL (229.86), lnu(C) (210.41), and mdtK (193.96).

Significance: The detection of genes such as bacA, tet(W), and mdtL highlights that resistance is not confined to a single antibiotic class, underscoring the complex nature of antimicrobial resistance driven by interactions between microorganisms and their environment, even in antibiotic-free systems.

Award Application: Developing Scientist Award

P1-07: GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE AND HEAVY METAL TOLERANCE IN ENTEROCOCCUS, E. COLI AND SALMONELLA ISOLATES FROM BRAZIL BROILER FEED SAMPLES

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: The role of in-feed antimicrobials in the evolution and persistence of bacterial resistance remains poorly understood; and the emergence of antimicrobial resistance, even in systems with limited use, poses a substantial risk to product safety and public health.

Purpose: The study aims to characterize Enterobacterales isolates from broiler feed samples by whole-genome sequencing (WGS) and investigate their genotypic and phenotypic antimicrobial and heavy metal tolerance.

Methods: A total of 16 samples were collected from conventional (8) and antibiotic-free (8) farms in Brazil. The sampling was conducted during winter and another during summer. Samples were analyzed for the presence of *Enterococcus*, *Escherichia coli*, and *Salmonella* following USDA guidelines, and isolates were confirmed by PCR. WGS was carried out on an Illumina MiSeq sequencer. NCBI Pathogen Detection was used to determine antimicrobial resistance genes and SNP (single nucleotide polymorphism) clusters. Antimicrobial and heavy metal susceptibility was assessed also phenotypically (via Sensititre and agar dilution methods).

Results: A total of 24 isolates were obtained: 1 *Salmonella* (from antibiotic-free system), 8 *E. coli* (6 conventional and 2 antibiotic-free systems), and 14 *Enterococcus* (8 conventional and 6 antibiotic-free systems). Phenotypic resistance to at least one antimicrobial and tolerance to heavy metals was observed in 60% of isolates, including cephalosporin, colistin, streptomycin, and tetracycline as well as copper. WGS analysis revealed that resistant isolates carried diverse resistance genes, and the majority of isolates exhibited the copper resistance operon and silver tolerance genes in the *Salmonella* isolate. Mobile genetic elements including class I integron were identified.

Significance: This study highlights the harborage of antimicrobial resistance and heavy metal tolerance in the Brazil broiler production system, even in antibiotic-free farms, emphasizing the need for further investigation into environmental factors that may influence the evolution and spread of resistance and its implications for food safety and public health.

Award Application: Developing Scientist Award

P1-08: A STUDY OF CINNAMALDEHYDE CONTROLLING THE TRANSFER OF LISTERIA MONOCYTOGENES BIOFILM TO MEAT AT DIFFERENT FORMATION PERIODS AND ITS MECHANISM

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Cross-contamination issues with *Listeria monocytogenes* (Lm) biofilms have led to large-scale meat product recalls and serious consumer health risks. Natural essential oils have received widespread attention as promising microbial inhibitors

Purpose: in this study, Lm biofilms were treated with sub-inhibitory concentrations of cinnamaldehyde (CA) and characterized over a 4-day period.

Methods: The study firstly determined the period of biofilm from adhesion to the formation of mature biofilm by crystal violet staining, i.e., adhesion (0-12 h), proliferation / formation of the matured biofilm (12-72 h) and dispersion (72-96 h). Both 1/2 MIC (160 µg/mL) and 1/4 MIC (80 µg/mL) CA delayed the development of Lm biofilm on abiotic surfaces and reduced the maximum biofilm formation. Further scanning electron microscopy and laser confocal microscopy observations revealed that the production of extracellular polymers within the biofilm was significantly reduced and showed a thinner biofilm structure after treatment with sub-MICs of CA.

Results: CA delayed the development of Lm biofilm on abiotic surfaces and

reduced the maximum biofilm formation. Further SEM and CLSM revealed that the production of extracellular polymers within the biofilm was significantly reduced and showed a thinner biofilm structure after treatment with sub-MICs of CA. Then, the study also characterized biofilm transfer to beef in the adhesion phase (24 h) and maturation phase (48 h), respectively. CA significantly reduced the biomass (1-1.5 log CFU/cm²) transferred from biofilm cells to the beef surface and its risk of sustained contamination. Finally, transcriptomics results showed that CA was involved in inhibitory pathways dominated by energy metabolism and peptidoglycan synthesis during the initial adhesion period and the maturation period of the biofilm, respectively.

Significance: This study provided the theoretical basis for the control of Lm biofilm and its cross-contamination in the food industry by natural essential oils.

P1-09: INHIBITING GROWTH OF PENICILLIUM CHRYSOGENUM IN VITRO AND IN BREAD SLICES BY USING COLD PLASMA-MODIFIED POLYPROPENE FILMS CONTAINING TRANS-CINNAMALDEHYDE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Cold plasma technology is an innovative technology for the surface modification of polymers used as food packaging materials, improving the adhesion of antimicrobials to the films and enhancing their controlled release.

Purpose: To assess the in vitro and in situ antifungal effect of cold plasma-modified polypropene films containing trans-cinnamaldehyde against *Penicillium chrysogenum*.

Methods: Aliquots of trans-cinnamaldehyde (1, 2, 5, 10 μ L) were applied: (i) by either spraying on cold plasma-modified (Plasma_PP) or non-modified polypropene films (PP) and (ii) by addition in absorbent pads (PAD). Films and absorbent pads were attached on the lid's internal side of each Petri dish containing either Malt Extract Agar (MEA) or bread slices ($\varnothing$ 6.8 cm) after spot-inoculation (10 μ L) of *P. chrysogenum* (n=14). The in vitro experiment was carried out at 10°C and 25°C, while in situ at 20°C. Non-inoculated MEA plates and bread slices served as controls. Fungal growth was estimated by measuring the colony diameter. Trans-cinnamaldehyde migration in MEA was spectrophotometrically quantified (n=6).

Results: The in vitro antifungal activity of trans-cinnamaldehyde followed the

order: Plasma_PP > PP > PAD. on Plasma_PP samples, the required amount of trans-cinnamaldehyde for total inhibition was significantly lower (5 µL) compared to the respective amount on PP samples (10 µL). The results were enforced by the 2-fold faster migration rate of trans-cinnamaldehyde quantified in MEA when plasma was applied versus PP and PAD samples. PAD was the least effective application, as at 10°C, there was no inhibition, at all studied volumes of trans-cinnamaldehyde. on bread slices, Plasma_PP had the best inhibitory effect against *P. chrysogenum* after 20 days at 20°C, followed by the PP and PAD samples, causing total inhibition when >2 µL of trans-cinnamaldehyde were applied.

Significance: Surface modification of polypropylene via cold plasma is a promising technology that may create significant perspectives for the active antimicrobial packaging.

P1-10: SALMONELLA SEROVAR VARIATION IN RESPONSE TO PERACETIC ACID TREATMENT: A COMPARATIVE STUDY BETWEEN BIOFILMS AND PLANKTONIC CELLS

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Alexandra Calle, Texas Tech University

Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Salmonella* continues to be a leading cause of foodborne diseases in the U.S. This organism can persist in the food processing environment as planktonic cells or attached to surfaces as biofilms. The food industry needs effective antimicrobial treatments to control this organism.

Purpose: Evaluate the response of seven *Salmonella* serovars to peracetic acid (PAA) on planktonic cells and biofilms and establish if the effectiveness is serovar- or attachment conditions-dependent.

Methods: *Salmonella* infantis, Enteritidis, Kentucky, Typhimurium, Newport, Heidelberg, and Dublin were tested individually. Stainless steel coupons placed in a 24-well plate were used to test planktonic cells and biofilms. Each coupon was inoculated with 1.6-ml bacterial culture and incubated at 25°C for 72h (biofilms) or 1h (planktonic cell). Coupons were treated with PAA at 100- and 200-ppm, during 1- and 2-min. After treatments, cells were detached with sonication and enumerated. Four biological repetitions were conducted, and data analyzed using multivariable linear mixed models (R-studio).

Results: initial cell attachment was 4.8 and 6.0 log₁₀CFU/cm² for planktonic and biofilms, respectively. Significant difference was observed between biofilms and planktonic cells for all *Salmonella* serovars (p<0.05). No viable colonies were recovered in the case of planktonic cells after 2 min interventions. Biofilms demonstrated lower reduction, regardless of the intervention (2.12 log₁₀CFU/cm² with 100ppm/1min; 4.74 log₁₀CFU/cm² with 100ppm/1min; 3.81 log₁₀CFU/cm² with 200ppm/1min; and 5.11 log₁₀CFU/cm with 200ppm/2min). Reduction of biofilms with tested treatments appeared to be concentration (p<0.05) and time (p<0.05) dependent. PAA effectiveness varied between *Salmonella* serovars (p<0.05). *S. Kentucky* (0.75–3.19 log₁₀CFU/cm² reduction) and *S. infantis* (0.75–3.19 log₁₀CFU/cm² reduction) exhibited lower reductions than the other serovars.

Significance: *Salmonella* serovars respond differently to PAA treatments when in

biofilm form, highlighting the need for the industry to adjust cleaning and sanitation practices, including using longer contact times or mixed approaches to mitigate *Salmonella*.

P1-11: RAW-MILK HISPANIC HARD CHEESE AGING: A MICROBIAL CHALLENGE STUDY TO ASSESS THE FATE OF FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: *Listeria monocytogenes*, *Staphylococcus aureus*, and *E. coli* O157:H7 are pathogens associated with unpasteurized milk. While manufacturing cheese from raw milk is permitted, pathogen control relies on aging.

Purpose: Evaluate the fate of foodborne pathogens during aging of hard cheese made from raw milk.

Methods: Unpasteurized milk was inoculated with *S. aureus*, *L. monocytogenes*, and *E. coli* O157:H7, reaching approximately 3-4 log₁₀CFU/ml. Hard cheese was prepared following a Hispanic facility protocol, and the aging process was done at 32°C for 70 days. Samples were collected every 10 days for microbial enumeration and chemical analysis (aw, moisture content, and pH). Biomerieux's Tempo® was used to enumerate *S. aureus*, lactic acid bacteria (LAB), aerobic count (AC), Enterobacteriaceae (EB), and *E. coli* (EC), and spread plating on SMAC and MOX agar for *E. coli* O157:H7 and *L. monocytogenes*, respectively. Data was analyzed using Kruskal-Wallis, Dunn's test, and Bonferroni correction with RStudio (version 2023.06.2) ($p < 0.05$).

Results: Enumeration is reported in log₁₀CFU/g. *S. aureus* increased to 5.2 by day 10, declined to 4.3 on day 20, 1.7 on day 30, and <1 log₁₀CFU/g on day 40 ($p > 0.05$). *L. monocytogenes* decreased to 2.2 by day 10 and <1 thereafter ($p > 0.05$). *E. coli* O157:H7 decreased to <1 , remaining at that level ($p > 0.05$). Regarding indicators, LAB increased from 3.7 to 4.4 on day 20, and 4.8 by day 40 ($p > 0.05$). AC increased from 5.7 to 6.6 by day 20, and to 7.8 by day 40 ($p > 0.05$). EB and EC dropped from 3.3 and 3.7 to <1 by day 10 ($p > 0.05$). Chemical properties decreased during aging: pH 6.8-6.5, aw 0.78-0.72, and moisture fluctuated between 40-43%. No significant differences observed due to limited data, replicates and study completion may reveal them.

Significance: Although pathogens decreased over time, high-aging temperature negatively influenced bacterial growth, observed during the first 10 aging days, demonstrating a public health risk.

P1-12: CLEAN-LABEL ANTIMICROBIAL ALTERNATIVES TO CONTROL THE GROWTH OF LISTERIA MONOCYTOGENES IN BUTTERNUT SQUASH SOUP

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* poses a significant concern in ready-to-eat refrigerated soups due to the potential for high post-processing contamination. Additionally, there is a growing demand for clean-label alternatives to conventional antimicrobials such as potassium sorbate.

Purpose: To evaluate the efficacy of clean-label alternatives, such as cultured sugar/dextrose/onion compared to the conventional antimicrobial (potassium sorbate) in controlling the outgrowth of *L. monocytogenes* in butternut squash soup.

Methods: Five treatments of butternut squash soup were prepared including the no antimicrobial (negative control), 0.1% Potassium Sorbate, 0.75% Verdad® Powder N30, 0.75% Verdad® Opti Powder N365, and 0.75% Verdad® Essence ON200. Each treatment was individually packed into 30±2 g before being inoculated with 5-strain of *L. monocytogenes* to a target population of ca. 2.5 log CFU/g. inoculated samples were aerobically packaged, and stored under refrigeration conditions (4.4°C/40°F). on each sampling day, three replicates from each treatment were diluted with buffered peptone and spread-plated onto Modified Oxford agar (35°C for 48 h). Two factors Analysis of Variance with the repeated measured design was used to compare the efficacy between treatments and bacterial growth within treatment over time.

Results: The initial population of *L. monocytogenes* ranged from 2.4 to 2.7 log CFU/g. By day 7, the negative control samples exhibited more than a 1-log increase in *L. monocytogenes* population. in contrast, the addition of antimicrobials inhibited *L. monocytogenes* growth to less than 1 log CFU/g for up to 63 days. No significant differences ($p>0.05$) were observed between the clean-label antimicrobials and the conventional potassium sorbate in controlling *L. monocytogenes* by day 60.

Significance: This study demonstrates that clean-label antimicrobials effectively inhibited *L. monocytogenes* outgrowth to less than 1 log CFU/g for over 60 days, offering a viable alternative to conventional antimicrobials in refrigerated soups.

P1-13: EFFICACY OF CLEAN-LABEL ANTIMICROBIALS ON OUTGROWTH OF LACTIC ACID BACTERIA IN MACARONI AND CHEESE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Ready-to-eat meals have been gaining popularity by providing time-saving, convenience, and accessibility attributes. However, this increasing demand presents challenges such as maintaining product quality and safety.

Purpose: To evaluate the efficacy of three cultured sugar/dextrose products for extending the shelf life of macaroni and cheese.

Methods: Macaroni and cheese were prepared without antimicrobial (negative control), 0.50% Verdad® Powder N30, 0.25% Verdad® Opti Powder N365, and 0.25% Verdad® Opti Powder N370. The prepared samples (30 g) were inoculated with a 4-strain cocktail of lactic acid bacteria (LAB; *Latilactobacillus curvatus*, *Latilactobacillus sakei*, *Leuconostoc carnosum*, *Leuconostoc mesenteroides*) to achieve a population of ca. 1.5 log CFU/g. All samples were aerobically packaged and stored at 4.4°C/40°F until enumeration. Triplicate samples were diluted with buffered peptone, stomached, and spread-plated on de Man, Rogosa, and Sharpe agar (30°C for 72 h). The microbial growth was modeled following Baranyi and Robert's equation to estimate the growth rate and lag time. One-factor ANOVA was used to compare the differences in growth rate and lag time among treatments.

Results: The LAB population in the negative control sample exceeded 6-log on day 12. The samples containing Verdad® Powder N30, Verdad® Opti Powder N365, and Verdad® Opti Powder N370 realized a 6-log LAB population on days 21, 17, and 17, respectively. The tested treatments had a significantly longer lag time ($p < 0.05$) compared to the negative control sample. The samples containing Verdad® Powder N30 and Verdad® Opti Powder N365 had significantly ($p < 0.05$) lower growth rates compared to the other treatments.

Significance: Clean-label antimicrobials effectively extended the shelf life of

macaroni and cheese by extending lag time and decreasing the growth rate of LAB.

P1-14: CONTROL OF YEAST AND MOLD IN HUMMUS USING CLEAN-LABEL PRESERVATION

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Hummus products are vulnerable to spoilage caused by yeast and mold due to a relatively low pH (~5.1) and a lack of thermal processing.

Purpose: To evaluate the potential of clean-label antimicrobials to control yeast and mold growth in hummus.

Methods: Hummus samples with no antimicrobial (negative control), 0.4% and 0.6% Verdad® Powder N30, and 0.8% Verdad® Opti Powder N370 were inoculated with either yeast or mold. Yeast cocktail (2.5 log CFU/g; *Debaryomyces hansenii*, *Pichia membranifaciens*, *Candida zeylanoides*) was enumerated using DG-18 agar with chloramphenicol (25°C, 4 days). Mold cocktail (*Penicillium brevicompactum*, *P. roqueforti*, *P. palitans*) was visually monitored. All samples were stored at 4.4°C. Tukey's test (Minitab 20.2) assessed differences in yeast lag time and growth rate.

Results: Mold appeared in the negative control by day 12, and in treated samples on days 19 (0.4%), 21 (0.6%), and 26 (0.8%). Yeast in the control reached 6-log by day 12, while treated samples reached this level on days 15, 19, and 26, respectively. Treated samples had significantly longer lag times and slower growth ($p < 0.05$).

Significance: Clean-label antimicrobials effectively extended hummus shelf life by delaying yeast and mold growth.

P1-15: INFLUENCE OF POTASSIUM FERRATE CONCENTRATION AND CONTACT TIME ON THE REDUCTION OF LISTERIA MONOCYTOGENES IN WATER

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Nitin Dhowlaghar, Kerry Taste and Nutrition

Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Ferrate (FeVIO_4^{2-}) is a high-oxidizing substance and used as a green oxidant in various applications such as for water purification and wastewater remediation.

Purpose: While ferrate is known to effectively eliminate pollutants, suspended debris, and microbes, its efficacy against foodborne pathogens such as *Listeria monocytogenes* surviving in water is not known.

Methods: This study investigated the antimicrobial effectiveness of potassium ferrate (K_2FeO_4) against *L. monocytogenes* strains Bug600 (serotype 1/2a) and ScottA (serotype 4b) in water. The cell suspension of *L. monocytogenes* was inoculated into water at a concentration of 10⁵ CFU/ml, and then was exposed to potassium ferrate at concentrations ranging from 0 (control) to 16 mg/ml. The survival of *L. monocytogenes* was determined by spread plating of these cell suspensions at 5 and 10 min, and by spot plating at 15, 20, and 30-min intervals on non-selective tryptic soy agar containing esculin.

Results: The results showed that potassium ferrate reduced *L. monocytogenes* Bug600 counts in water by 1 log CFU/ml at 2 mg/ml or by 3 logs CFU/ml at 4 mg/ml within 10 min. At 8 or 16 mg/ml of potassium ferrate, there was a consistent 4 log CFU/ml reduction of *L. monocytogenes* Bug600 within 5- or 10-min in water. Similar findings were observed for ScottA strain.

Significance: Potassium ferrate at high concentrations is effective in killing *L. monocytogenes* water. Further research is needed for refining the disinfection protocols using potassium ferrate to maximize its effectiveness in eliminating persisting *L. monocytogenes* cells in wastewater, particularly on the floors and drains of food processing environments.

P1-16: EFFECTIVENESS OF POTASSIUM FERRATE CONCENTRATION AND CONTACT TIME ON LISTERIA MONOCYTOGENES REMOVAL FROM STAINLESS STEEL SURFACES

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Nitin Dhowlaghar, Kerry Taste and Nutrition

Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* is a foodborne bacterial pathogen that cross-contaminates food products by long-term persisting in the food processing environments even after sanitation cycles.

Purpose: There is a demand for highly effective broad-spectrum disinfectants against foodborne bacterial pathogens. Ferrate (FeVIO_4^{2-}) is a high-oxidizing substance used in synthetic organic transformations, water oxidation catalysis, and wastewater remediation. However, its efficacy against foodborne pathogens such as *L. monocytogenes* is not known.

Methods: This study investigated the antimicrobial efficacy of potassium ferrate (K_2FeO_4) against *L. monocytogenes* on stainless steel surfaces. Stainless steel coupons (SSC) were inoculated with *L. monocytogenes* Bug600 (serotype 1/2a) or ScottA (serotype 4b) at 10^7 CFU/mL. The SSC was exposed to potassium ferrate (PF) at 0 (control) or 1–8 mg/mL for 5 or 10 minutes. After PF treatment, SSC was removed and vortexed with BPW to recover the remaining *L. monocytogenes* cells by spread plating on non-selective tryptic soy agar containing esculin.

Results: The results revealed a PF concentration-dependent reduction in *L. monocytogenes* Bug600 on SSC. At 2 mg/mL of PF, *L. monocytogenes* Bug600 counts were decreased by 3 log CFU/mL within 5–10 minutes. At 4 mg/mL, there was a 4-log reduction, and at the highest concentration of 8 mg/mL, a consistent 5-log CFU/mL reduction of *L. monocytogenes* Bug600 was observed within 5 or 10 minutes. Similar findings were observed for *L. monocytogenes* ScottA.

Significance: Ferrate at high concentrations is effective for removal of *L. monocytogenes* on stainless steel surfaces. Further research is needed to refine the effective disinfection protocol using PF for eliminating the hotspots of *L. monocytogenes* when surviving on certain food-contact and non-food contact

surfaces in food processing environments.

P1-17: UTILIZATION OF SODIUM AND POTASSIUM VINEGAR SYSTEM AS MARINADES TO INCREASE THE SHELF-LIFE OF CHICKEN TENDERS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Natural flavor and weak organic acids based preservation system provide clean label solutions for meat shelf-life and food safety extension.

Purpose: To evaluate the efficacy of Natural Flavor (innovation concept 1; IC 1) individually and in combination with a vinegar (innovation concept 2; IC 2) for control of spoilage microorganisms in ground chicken.

Methods: Ground chicken was treated with 1.5%; IC 1 alone and in combination with 0.75% ; IC 2. Treated samples were packed under aerobic atmosphere and stored at 4 °C for up to 30 days. on each sampling day, duplicate samples were processed to enumerate for lactic acid bacteria (LAB), aerobic plate count (APC), Enterobacteriaceae (EB) and Pseudomonas. Spoilage threshold was considered as 6 log CFU/g. Differences among the treatments were determined using one-way ANOVA using JMP Pro version 16.1.0 (SAS institute inc., NC, US), with significance set at $P \leq 0.05$.

Results: IC1 in combination with IC2 was effective in controlling LAB, APC, EB, and Pseudomonas in ground chicken at 4°C. IC 1 alone provided control of APC, EB, and LAB until days 8, 9, and 10 while untreated control reached 6 log CFU/g by day 7. Treatment of IC1 (1.5%) combined with IC2 (0.75%) exhibited synergistic effect on ground chicken, significantly enhancing the shelf life to 20 days ($p < 0.05$) while IC2 alone provided 14 days of shelf life. All the combination treatments controlled the EB and Pseudomonas population for more than 20 days.

Significance: Combination of Kerry's natural flavor and vinegar based preservation systems offers promising clean label solution for shelf-life extension of ground poultry while meeting consumer expectations for safer food products.

P1-18: IMPACT OF DIFFERENT CONCENTRATIONS OF LIQUID SMOKE AGAINST E. COLI 0157:H7 IN FRESH CARROTS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Fresh produce is susceptible to microbial contamination from field growth to in-plant handling, intervention with clean-label products is necessary to reduce microbial loads, thus ensuring food safety.

Purpose: To evaluate the inactivation of *E. coli* O157:H7 with liquid smoke at concentrations of 1%, 2%, 5%, and 10% applied on baby carrots and the sensory acceptability.

Methods: Baby carrots procured, washed, and UV sterilized were inoculated with 4-5 log CFU/g of *E. coli* O157:H7. inoculated carrots were air dried for 30 minutes before they were dipped in 400 mL of respective liquid smoke (to achieve varying concentrations of 1%, 2%, and 5%) solutions followed by airdrying for 15 minutes. For bacterial enumeration, duplicate samples (50 g) were diluted in 100 mL of neutralizing buffer and plated on SMAC agar followed by incubation at 37 °C for 24 hours. Sensory analysis was conducted to determine the sensorial impact of the liquid smokes at three usage levels.

Results: Overall, smoke solutions showed cidal efficacy against *E. coli* O157:H7 in fresh baby carrots. The maximum log reduction of 1.9 log CFU/g was achieved using 5% smoke treatment solution. Dip treatment of baby carrots in 1% and 2% smoke solution provided 0.2 and 0.7 log CFU/g reduction respectively. The negative control (water wash) did not show any *E. coli* reduction. Sensory evaluation suggested that carrots treated with 1% and 2% were closest to control with no significant impact on organoleptic properties while 5% treatment resulted in mild off taste.

Significance: The findings highlight the potential of smoke as a clean label, sustainable and effective intervention for the fresh produce industry. This approach addresses consumer demand for natural and clean-label solutions.

P1-19: ANTIBIOTIC RESISTANCE IN AEROMONAS SPP. AND VIBRIO SPP. FROM RED TILAPIA

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Fish farming in Cambodia plays a key role in ensuring food security as the population grows. However, misuse and overuse of antibiotics in fish farming may contribute to the increase of antimicrobial resistance.

Purpose: Identify and determine antibiotic resistance in *Aeromonas* spp. and *Vibrio* spp. isolated from red tilapia in five fish farms in Siem Reap Province, Cambodia.

Methods: Temperature water samples were collected from five different farms. Bacteria were isolated using selective media such as *Aeromonas* Selective Agar and TCBS agar. Suspected colonies were subjected to biochemical tests and identified by API 20E/20NE. Antimicrobial sensitivity testing was performed using seven different antibiotics.

Results: Fish raising temperatures ranged from 27 to 30.7°C and pH levels ranged from 6.66 to 9. In a total of 25 farm samples (N=25), 44% presumptive *Aeromonas hydrophilia* (n=11) and 8% presumptive *Vibrio cholerae* (n=2) were identified. Antibiotic resistance in *Aeromonas hydrophilia* (n=11) was: 27.7% to CIP, CL, and OXT; 45.45% to FFC; 54.54% to SXT; 81.81% to AMP; and 100% to E. For *Vibrio cholerae* (n=2), resistance was 50% to OXT and 100% to AMP, CL, E, and FFC. The farms had high percentages of *Aeromonas hydrophilia* and *Vibrio cholerae* with notable resistance to AMP, CL, FFC, OXT, SXT, and E.

Significance: Due to a high propensity for antibiotic resistance, consumers could face food safety issues. Additionally, disease contamination in fish farming production and antimicrobial resistance can contribute to food insecurity.

Award Application: Undergraduate Student Award

P1-20: VALORIZATION OF BIOWASTE MATERIALS FOR THE CONTROL OF FOODBORNE PATHOGENS

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Catherine Baxley, Virginia State University

Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Foodborne illnesses affect millions annually in the United States, with antimicrobial-resistant pathogens further complicating food safety due to the overuse of antibiotics in agriculture. To address these challenges, researchers are exploring natural extracts and innovative membrane separation technology as eco-friendly and efficient methods to extract antimicrobial bioactive compounds like lectins and flavonoids, offering sustainable solutions for the food and pharmaceutical industries.

Purpose: This study aimed to evaluate biowaste materials such as morro seed, moringa seed, strawberry seed, and citrus peel albedo for their antibacterial potential.

Methods: The biowaste materials were subjected to hot water or acidic treatments, followed by extraction using a non-thermal, eco-friendly membrane separation technology, and then tested against four major foodborne pathogens: *Escherichia coli* O157, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus*. Following standard protocols, an agar well diffusion assay and 96-well microtiter plate assay were used for their antibacterial potential.

Results: Citrus peel albedo extract demonstrated strong antibacterial activity, effective at concentrations as low as 32 ppm across all bacterial strains. Moringa seed extract showed selective activity against Gram-positive bacteria with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) at 2,500 mg/L and 5,000 mg/L, respectively. However, extracts from morro and strawberry seeds showed no antibacterial activity even at concentrations as high as 100,000 mg/ml.

Significance: This research underscores the promise of biowaste materials as viable antibacterial agents, contributing to food safety and public health, and promoting environmental sustainability through eco-friendly membrane technology instead of conventional solvent extraction.

P1-21: NATURAL ANTIMICROBIALS FOR CONTROLLING SALMONELLA ON ALFALFA SEEDS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Sprouts are highly susceptible to microbial contamination during germination, necessitating effective interventions to enhance food safety without compromising product quality.

Purpose: This study investigated the efficacy of nanoemulsified benzyl isothiocyanate (NBIT) and nanoemulsified carvacrol (NCR) in reducing *Salmonella* contamination on alfalfa seeds and sprouts.

Methods: Alfalfa seeds inoculated with *Salmonella enterica* serotype Newport at high (6 log CFU/g) and low (2 log CFU/g) inoculum levels were treated with varying concentrations of NBIT (0.5%, 1.0%, 2.0%) and NCR (2.0%). The effects of these treatments on surviving *Salmonella* populations on seeds and sprouts were determined by plating on selective agar media. Further, seed germination and sprout quality were assessed. Each experiment was conducted in triplicate, and the data were statistically analyzed for significant differences ($p < 0.05$) due to treatment.

Results: *Salmonella* were detected at ~ 7 log CFU/g on sprouts grown from contaminated seeds due to potential transfer from seeds during germination. The NBIT and NCR significantly reduced *Salmonella* on both seeds and sprouts. Treatment with 2.0% NBIT reduced up to 2.4 log CFU/g on seeds and 6.4 log CFU/g *Salmonella* on sprouts at the low inoculum level. However, 2.0% NBIT caused notable reductions in germination rates—19% at the high inoculum level and 11% at the low inoculum level—along with slight decreases in sprout size. In contrast, treatments with 0.5% and 1.0% NBIT and 2.0% NCR significantly reduced *Salmonella* on seeds by 1.5 to 2.0 log CFU/g, regardless of inoculum level, and achieved reductions of 5.0 to 6.4 log CFU/g on sprouts at the low inoculum level. Importantly, these lower-concentration treatments preserved seed germination rates and sprout quality comparable to untreated controls.

Significance: These findings highlight the potential of NBIT and NCR as effective antimicrobial agents for improving the microbial safety of alfalfa sprouts while preserving product quality.

P1-22: ANTIMICROBIAL EFFECTS OF ESSENTIAL OILS ON FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Foodborne illnesses due to consumption of food contaminated with pathogens necessitate the development of effective antimicrobial solutions. Consumers' preference for minimal chemicals has led to an interest in natural antimicrobials for pathogen control in food systems.

Purpose: This study evaluated the antimicrobial activity of essential oils: eugenol, carvacrol, neem, and galbanum oils at 2% conc., against three foodborne pathogens.

Methods: Nanoemulsions of eugenol (25.56 nm particle size) and carvacrol (27.39 nm particle size) were prepared while neem and galbanum oils were evaluated in non-nanoemulsified forms. Antimicrobial susceptibility of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*; five isolates of each pathogen were investigated using the disk diffusion assay. Briefly overnight cultures (~6 log CFU/mL) were spread-plated on trypticase soy agar (100 µL) and 6 mm sterile filter paper disks impregnated with antimicrobial (15 µL) were placed on agar. Gentamycin (10 µg) and sterile water served as controls, and inhibition zones (mm) measured after 24 hours at 37°C. Results (inhibition zone) obtained from three independent replicates were analyzed statistically for significant differences.

Results: All essential oils demonstrated antimicrobial activity against the test pathogens, with inhibition zones ranging from 8.13 to 9.93 mm. Carvacrol nanoemulsion exhibited significantly larger inhibition zones against *Salmonella* (9.93 mm) compared to neem, galbanum, and eugenol, which showed comparable activity (8.13–8.33 mm). For *E. coli* O157:H7 and *L. monocytogenes*, zones of inhibition ranged from 8.20 to 9.85 mm, with carvacrol showing significantly greater activity than the neem and galbanum oil. No significant differences in inhibition zones were observed among the bacterial groups for eugenol, carvacrol, neem, or galbanum oils.

Significance: Both nanoemulsified and non-nanoemulsified essential oils displayed antimicrobial activity against foodborne pathogens, suggesting further

investigation on food systems for their potential application

P1-23: AUSTRALIAN NATIVE ESSENTIAL OILS FOR FOOD SAFETY AND PACKAGING APPLICATIONS

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Controlling microbial contamination in food is crucial for sustainability. With growing concerns over synthetic antimicrobials and antimicrobial resistance, alternative solutions are needed.

Purpose: This research investigates the antimicrobial activities of Australian native essential oils, *Backhousia citriodora* (lemon myrtle) and *Melaleuca teretefolia* (honey myrtle), as antibacterial, antifungal, and biofilm-inhibitory agents, with potential applications in active food packaging. **Methods:** The oils were tested against spoilage and pathogenic microorganisms using broth microdilution (liquid phase) and vapor-phase Minimum inhibitory Concentration (MIC) assays. Biofilm inhibition was assessed with a crystal violet assay. Lemon myrtle oil was encapsulated in biodegradable nano-carriers and incorporated into sodium alginate-based packaging. Antimicrobial efficacy was tested in vitro and in a model food using standard plate counts. Release of antimicrobial compounds was analyzed via HS-SPME GC-MS.

Results: Both lemon myrtle and honey myrtle essential oils exhibited significant antibacterial and antifungal activity, especially against Gram-positive bacteria and fungi, in both liquid and vapor phases. MIC values for lemon myrtle oil ranged from 0.09%-0.37% v/v (liquid phase) and 5-20 $\mu\text{L/L}$ of headspace (vapor phase). Lemon myrtle demonstrated 35% biofilm inhibition against *Listeria innocua*. Nanoencapsulated lemon myrtle oil (200 nm particles) incorporated into sodium alginate films resulted in porous, fragile films. However, neat oil-incorporated films effectively inhibited bacterial growth on strawberries for 7 days. Yeast and mold growth was fully inhibited on day 1, with a 1 log reduction from days 2 to 7. HS-SPME GC-MS confirmed the release of the antimicrobial compound 'Citral' over

seven days.

Significance: This study demonstrates the potential of Australian native essential oils as effective antimicrobial agents, particularly in vapor-phase applications, offering a solution to reduce the sensory drawbacks of direct oil incorporation in food products.

P1-24: CROSS-PROTECTION TO FOOD PRESERVATIVES TREATMENTS IN *SALMONELLA* TYPHIMURIUM

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Louise Iara Gomes de Oliveira, Federal University of Paraíba

Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: *Salmonella* Typhimurium is among the leading causes of salmonellosis outbreaks worldwide. To survive adverse conditions, *Salmonella* can develop resistance mechanisms that enhance its survival along the food chain. Studies have demonstrated cross-protection phenomena between resistant variants obtained under selective pressure from antibiotics, natural antimicrobials, and food preservation methods. Further research is needed to understand the mechanisms involved and their implications for food safety.

Purpose: To evaluate the cross-protection of a *Salmonella* Typhimurium variant resistant to meropenem against other antibiotics, heat, and carvacrol, and to identify the genetic changes associated with increased protection.

Methods: A resistant variant (RV) of *Salmonella* was obtained by exposure to an ascending meropenem gradient (1.85× daily), starting from the minimum inhibitory concentration (MIC; 0.0312 µg/mL) and reaching 40× MIC by day 11 (evolutionary assay). Cross-protection of the RV was evaluated against 12 antibiotics, heat (54 °C), and carvacrol (200 µL/L). Genomic DNA was extracted and sequenced using the Illumina HiSeq 4000 platform with 150 bp paired-end reads. Bioinformatic analysis was conducted to identify mutations. Resistance of the RV to antibiotics, heat, and carvacrol was compared to the wild-type strain. Statistical analysis was performed using Sigma Stat 3.5 with significance set at $p \leq 0.05$.

Results: The meropenem-RV doubled its MIC to 0.0625 µg/mL, indicating 100% increased resistance. It also showed a two-fold increase in resistance to cephalothin (MIC: 2 to 4 µg/mL) and imipenem (MIC: 0.25 to 0.5 µg/mL), and demonstrated significantly greater tolerance ($p < 0.05$) to heat and carvacrol.

Genotypic analysis revealed mutations in the *spoT* and *znuA* genes.

Significance: Continuous exposure to antibiotics may result in mutation-related stress responses in *Salmonella*, increasing its tolerance to food preservative treatments.

P1-25: EFFECT OF BACTERIOCINS PRODUCED BY PROBIOTIC BACTERIA ON LISTERIA MONOCYTOGENES INFECTED CORIANDER LEAVES

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James Shelton, Food and Drug Administration

Category: Antimicrobials

Abstract:

Introduction: There is a need to develop strategies to control and mitigate the presence of bacterial pathogens on agricultural products. The use of bacteriocins, antimicrobial peptides produced by microbes, is one such approach. We therefore studied whether bacteriocins produced by a *Lactobacillus plantarum* isolated from a probiotic product would affect *Listeria monocytogenes* survival on coriander.

Purpose: To investigate the presence of and potential use of bacteriocins to mitigate *Listeria* contamination on herbs.

Methods: Coriander plants were grown in growth chambers that mimic agricultural settings. The leaves of coriander plants were spotted as follows: Plant 1 with 5 µl of an overnight *Listeria* culture (7×10^6 CFU) per leaf; Plant 2 with 5 µl of bacteriocin; and Plant 3 with 5 µl of bacteriocin and 5 µl of *Listeria*. These plants were placed in the growth chamber, and at 0, 1, 2, 4, and 24 hours, the treated leaves were placed in tubes containing 5 mL of saline, vortexed, and serial dilutions from these suspensions were plated on Oxford agar plates containing rifampicin and incubated at 37 °C for 48 hours.

Results: Supernatants of overnight cultures of several *Lactobacillus* isolates were tested for bacteriocin activity by their ability to inhibit growth of *L. monocytogenes* on plates. *L. plantarum* DS34_9 was chosen as it was found to inhibit *Listeria* growth the best. Genomic analysis also showed that *L. plantarum* DS34_9 has genes that code for two bacteriocins, Sactipeptides and Pediocin. Experiments with *Listeria* spiked on coriander leaves showed a reduction from 7×10^6 CFU to 9 CFU after 1 hour of incubation, and no colonies appeared at and after 2 hours.

Significance: Preliminary data show that the bacteriocin produced by *L. plantarum* DS34_9 can kill *Listeria* on coriander plants, indicating that bacteriocins could potentially be used in reducing foodborne contamination.

P1-26: ANTIMICROBIAL POTENTIAL OF BENZYL ISOTHIOCYANATE NANOEMULSION AGAINST FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Foodborne pathogens, including *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* pose significant threat to public health and food safety. Novel antimicrobial strategies are essential for effective pathogen control.

Purpose: This study aimed to assess the antimicrobial activity of a 1% benzyl isothiocyanate (BIT) nanoemulsion against multiple strains of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*.

Methods: Five isolates each of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* were evaluated for susceptibility to a 1% benzyl isothiocyanate nanoemulsion using the disk diffusion assay. The particle size (16.78 nm) and polydispersity index (0.34) of nano-emulsified BIT were characterized using a Zetasizer. Overnight bacterial cultures were standardized to ~6 log CFU/mL based on OD600 values, and 100 µL of each culture was spread-plated onto trypticase soy agar (TSA) plates. Sterile filter paper disks (6 mm) were placed on the agar surface and loaded with 15 µL of the BIT nanoemulsion. Gentamycin (10 µg) and sterile distilled water were used as positive and negative controls, respectively. Inhibition zones (mm) were measured following 24 hours of incubation at 37°C. All experiments were performed in triplicate, and the results were statistically analyzed for significant differences.

Results: The 1% BIT nanoemulsion effectively inhibited all tested pathogens. The average inhibition zones were 14.13 mm for *L. monocytogenes*, 12.80 mm for *Salmonella*, and 11.50 mm for *E. coli* isolates. Notably, *L. monocytogenes* strains exhibited the highest susceptibility to the BIT nanoemulsion. Statistical analysis revealed significant differences in inhibition zones between *L. monocytogenes* and *E. coli* strains ($p < 0.05$), underscoring the variable sensitivity of the pathogens.

Significance: These findings underscore the potential of BIT nanoemulsion as a natural antimicrobial agent, demanding further exploration for scalability and integration into sprouts and microgreens.

P1-27: ARTIFICIAL INTELLIGENCE OF THINGS- ENHANCED GLOBAL ANTIMICROBIAL RESISTANCE AUTOMATED SURVEILLANCE SYSTEM USING MULTIPLEX MICROFLUIDIC TECHNIQUE

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: The farm-to-fork continuum represents a fundamental pathway for the spread of antimicrobial resistance (AMR) of foodborne pathogens. To address this crisis, we recently developed a real-time surveillance system to monitor the rise of AMR bacteria in the food chain using microfluidic "lab-on-a-chip" for antibiotic susceptibility testing and image recognition. However, this surveillance system only monitors one antimicrobial each time and requires costly GPU to run a model on desktop computer, which limits its potential applications in agri-foods.

Purpose: This study aims to enhance the efficiency of AMR surveillance of foodborne pathogens by developing a portable, cost-effective AI system.

Methods: As an Artificial intelligence of Things (AIoT) device, Orange Pi 5B was utilized to simultaneously control various components of a portable incubator, including a heater for temperature control, a micro gas pump for managing CO₂ level, and assorted sensors that monitor the bacterial cultivation conditions. Additionally, a camera linked to the Orange Pi could record the colorimetric reaction on multiplex microfluidic chip. An onboard light object detection model was then applied to automatically detect antimicrobial resistance and upload the findings to the cloud. Four typical foodborne pathogens were selected for testing and verification, including *Campylobacter jejuni*, *Campylobacter coli*, *Salmonella enterica* serovar Enteritidis, and *Salmonella enterica* serovar Typhimurium.

Results: The surveillance system uses a lightweight YOLO V5 model for object detection, processing over 30 FPS with 99% classification precision for bacterial growth/inhibition. It cost approximately \$150 excluding the incubator. The collected data could be automatically uploaded to the cloud server, facilitating

instantaneous, large-scale analysis of spatial and temporal AMR trends.

Significance: This affordable AIoT system with multiplexing capabilities holds significant potential for AMR surveillance as it enlarges the scope of surveillance and extends the capacity to concurrently monitor disparate antimicrobials, potentially revolutionizing AMR detection across the global food supply chain.

P1-28: EFFECTS OF PROVONTAGE® 399 ON LISTERIA IN CHICKEN SALAD AT AN ELEVATED PH

Primary Author:

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Ready-to-eat refrigerated deli salads are produced without a heat/kill step and are susceptible to *Listeria* survival/growth unless kept below pH 4.7.

Purpose: This study examined a natural preservative to prevent *Listeria monocytogenes* (Lm) growth in refrigerated chicken salad at pH > 5.2.

Methods: Fresh-prepared chicken salad was challenged with a three-strain, cold-adapted Lm cocktail and tested in triplicate. Treatments included a sterile water positive control (PC), proVONTAGE® 399 (0.75%) natural preservative (NP), and acetic acid negative control (NC). Samples (22 g) were tested on days 0, 1, 3, 7, 14, 28, 42, 56, and 70 for pH and Lm using Brilliant Listeria Agar with supplements to discern Lm from other *Listeria* species.

Results: On day 1, the pH of the PC, NP, and NC salads were 5.4, 5.4, and 4.7. The NP and NC salads maintained pH (± 0.1), whereas the PC salad lowered to 5.0 from days 42–70. The Lm levels averaged $1.28\text{E}+03$ CFU/g on day 0 with no difference between treatments ($p > 0.05$). On day 1, Lm levels dropped to $5.24\text{E}+02$ CFU/g in the PC compared to $1.63\text{E}+02$ in the NC ($p < 0.01$) and <10 in the NP ($p < 0.001$). On day 7, Lm levels rose in all treatments; however, the NP, at $1.44\text{E}+02$ CFU/g, was significantly lower than the PC ($1.30\text{E}+03$, $p < 0.001$) and NC ($2.22\text{E}+02$, $p < 0.05$). On days 14–70, the Lm averaged 30 CFU/g in the NP salads, significantly lower than the PC salads ($5.85\text{E}+02$ CFU/g, $p < 0.001$) and NC salads (70 CFU/g, $p < 0.05$).

Significance: The natural preservative showed a bactericidal effect against *Listeria monocytogenes* in chicken salad at an elevated pH. This provides food manufacturers with an alternative treatment option to ensure the safety of refrigerated deli salads.

P1-29: CARVACROL EFFICACY AGAINST PATHOGENIC ESCHERICHIA COLI PERSISTENT CELLS ON FOOD AND FOOD CONTACT SURFACES

Primary Author:

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Escherichia coli* is a major foodborne pathogen and frequently causes foodborne infections. Natural substances, such as essential oils obtained from plants, are among the most popular and suggested ways for combating bacteria persister cells.

Purpose: in this study, we investigated the antimicrobial activity of carvacrol against pathogenic *E. coli* cocktail persister cells on food (beef) and food contact surfaces (stainless steel, rubber, and pet). We also investigated the visual evaluation of carvacrol efficacy against *E. coli* persister cells under advanced microscopy including field emission scanning electron microscopy (FE-SEM) and confocal laser scanning microscopy (CLSM).

Methods: Minimum inhibitory concentration (MIC), bacterial cell attachment assay on food (beef) and food contact surfaces (stainless steel, rubber, and pet), an assay to determine the reduction of persister cells using carvacrol at different concentrations, FE-SEM, and CLSM were performed to evaluate the prophylactic efficacy of carvacrol.

Results: The results showed that carvacrol affected the bacterial attachment on the tested surfaces at different MIC concentrations ($1/2 \times$ MIC, MIC, and $2 \times$ MIC) with high bactericidal properties. However, higher concentrations of carvacrol $6 \times$ MIC were needed on beef due to the strong growth and attachment of *E. coli*. The persister cells were reduced by more than 2.5 log at $2 \times$ MIC on food contact surfaces and by more than 4 log at $4 \times$ MIC on beef.

Significance: Based on this study, carvacrol can potentially contribute to improving food quality by lowering bacterial contamination on food and food contact surfaces.

Award Application: Developing Scientist Award

P1-30: INHIBITION OF LISTERIA MONOCYTOGENES IN RTE COOKED MEAT PRODUCTS USING A SYNERGISTIC BLEND OF ORGANIC ACIDS AND NISIN (INBAC-ACN/NA)

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* remains a critical zoonotic threat, causing severe illness with some of the highest hospitalization and fatality rates. Cooked meat products are particularly at risk due to their long shelf life and chemical characteristics, which create an ideal environment for *Listeria* growth. Inhibiting its growth throughout the product's shelf life is essential to ensure food safety.

Purpose: Evaluate the effectiveness of preservative INBAC-ACN/NA, against *Listeria monocytogenes* in a cooked meat product at 5°C for up to 113 days.

Methods: Two treatments of cooked meat products were prepared: one containing 0.44% preservative and the other serving as the control with no preservative. Both product types were sliced into portions weighing 13–17 g and inoculated by spraying with a cocktail of five *L. monocytogenes* strains at an initial concentration of 100–200 CFU/g. The samples were vacuum sealed individually and stored at 5°C. The expected shelf life was 90 days, with a 25% extension, resulting in a total study period of 113 days. Three samples were tested at each time point, with the first dilution plated onto TSA agar plates. The detection limit of this method was 1.3 CFU/g. Treatments were compared using a t-test ($p < 0.05$).

Results: The synergistic blend of organic acids and nisin prevented the outgrowth of *L. monocytogenes* for 113 days at 5°C. In the control samples, *L. monocytogenes* increased by more than 3.5 log, while samples containing 0.44% preservative showed a less than 1 log increase. Statistical analysis ($p < 0.05$) confirmed a significant difference between treatments, underscoring the preservative's effectiveness.

Significance: The preservative, applied at a low dosage of 0.44%, effectively inhibits the growth of *L. monocytogenes* in refrigerated cooked meat products, providing a potential solution for reducing listeriosis risks and enhancing food safety.

P1-31: EXPLORING EFFLUX PUMP DEPENDENT AND INDEPENDENT MECHANISMS OF BENZALKONIUM CHLORIDE TOLERANCE EVOLUTION IN *LISTERIA MONOCYTOGENES*

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Quaternary ammonium compounds (QACs), such as benzalkonium chloride (BAC), are widely used sanitizers to control *Listeria monocytogenes* in food processing environments. However, improper use of QAC-based sanitizers can lead to increased QAC tolerance, which poses a significant challenge to food safety and public health.

Purpose: This study aims to investigate the impact of BAC adaptation on cross-tolerance to QACs and commercial QAC-based sanitizers, using strains of *L. monocytogenes* adapted for increased tolerance to BAC.

Methods: Minimum inhibitory concentrations (MICs) for various QACs—Alkyl Dimethyl Benzyl Ammonium Chloride (BAC), Didecyl Dimethyl Ammonium Chloride (DDAC), Dioctyl Dimethyl Ammonium Chloride (DODAC)—and two commercial QAC-based sanitizers (Virex II 256 and 3M Quat) were determined via broth microdilution using twelve *L. monocytogenes* strains (three ancestral: LM10, LM16, LM20; and three evolved lineages for each ancestor). To assess whether efflux pump activity contributes to tolerance, strains were cultured with QACs and the efflux pump inhibitor reserpine (20 µg/ml).

Results: Virex II 256 showed greater efficacy than 3M Quat. Evolved strains exhibited significantly higher MICs ($p < 0.001$) than ancestral strains. MICs increased from 1–3.5 ppm to 4–5 ppm in LM16 and LM20 lineages and to 3 ppm in the LM10 lineage. Reserpine reduced MICs for LM10 and LM20, implicating efflux pumps in tolerance mechanisms, while no effect was observed in LM16, suggesting an efflux-independent mechanism. DDAC had the lowest MIC (≤ 4 µg/ml), followed by BAC (≤ 7 µg/ml), with DODAC and ODDAC showing similar MICs (≤ 10 µg/ml). Some strains showed unchanged MICs even with reserpine.

Significance: Adaptation to BAC stress can lead to cross-tolerance to other QACs, with tolerance mechanisms varying by genetic background.

Award Application: Developing Scientist Award

P1-32: ANTIMICROBIAL EFFECTS OF CAPERS ON MORGANELLA MORGANII IN BROTH AND SOUS-VIDE MACKEREL AS A FUNCTION OF TEMPERATURE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Capers (*Capparis spinosa*) are often used in fish dishes to eliminate unpleasant odors, enhance flavor, and provide antimicrobial activity.

Purpose: The aim of this study was to assess the antimicrobial effects of capers against *Morganella morganii*, a major histidine-decarboxylating organism, in broth and sous-vide mackerel stored at different temperatures.

Methods: Predictive model for *M. morganii* was developed in broth as a function of temperature and model performance was validated with mackerel. Antimicrobial effects of whole and ground dried capers were also compared in broth at the concentrations of 8, 9, 10, and 11%. Sous-vide mackerel was prepared with 9% ground dried capers and inoculated with *M. morganii*. Effect of ground capers against growth of *M. morganii* was investigated at 15 and 25°C.

Results: No growth of *M. morganii* was observed in broth at temperatures below 8°C. Secondary growth models were developed and validated using sous-vide mackerel. The secondary specific growth rate model for broth in this study was suitable to predict growth of *M. morganii* on sous-vide mackerel, whereas the lag time model was not suitable. Ground capers were more effective than whole capers in inhibiting *M. morganii* at both 15 and 25°C in broth, regardless of levels. *M. morganii* was inhibited in broth with 8% ground capers at 15°C, but a concentration of 10% was required at 25°C. Furthermore, the addition of 10% ground capers in sous-vide mackerel at 25°C significantly delayed the growth of *M. morganii*.

Significance: Although capers did not fully inhibit *M. morganii* in sous-vide mackerel at 25°C, their demonstrated antimicrobial activity highlights their potential as a natural preservative for enhancing flavorful taste and extending shelf life of products distributed at room temperature.

Award Application: Undergraduate Student Award

P1-33: SUSTAINABLE CARBON DOTS SYNTHESIS USING ONION PEEL AND THEIR ANTIMICROBIAL AND ANTIOXIDANT CHARACTERIZATION

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Approximately 500,000 tons of onion peels, a byproduct containing up to 100 times more flavonoids and phenolic compounds than onions, are discarded annually, presenting an opportunity to utilize this waste in emerging technologies like carbon dots (CDs) for converting biomass into high-value products.

Purpose: This study aimed to synthesize CDs from onion peel and evaluate their antimicrobial and antioxidant properties, exploring their potential applications in the food industry.

Methods: Carbon dots were synthesized by heating 1.5g of onion peel powder in 50mL of distilled at 200°C for 7h. After cooling, the solution was filtered to remove impurities. The synthesized CDs were characterized using UV-vis spectroscopy, photoluminescence (PL), Fourier-transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), and transmission electron microscopy (TEM). Antimicrobial activity of carbon dots against pathogens was evaluated using minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and time-kill assay. Antioxidant activity was assessed via ABTS radical scavenging.

Results: The successful synthesis of onion peel CDs was confirmed through UV-vis, PL, FT-IR, XPS and TEM analyses. The MIC/MBC values for *Salmonella* Typhimurium, *E. coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* were 2200/2800, 1400/2000, 1200/1800 and 400/600 µg/mL, respectively. The time-kill assay corroborated these findings. CDs from onion peel exhibited stronger antimicrobial activity against Gram-positive bacteria, particularly *S. aureus*. At a concentration of 12.5 µg/mL, CDs demonstrated approximately 54.71% free radical scavenging activity against ABTS radicals, with antioxidant activity increasing proportionally with concentration.

Significance: Carbon dots synthesized from onion peel show potential as

sustainable antimicrobial and antioxidant agents, offering value in recycling this nutrient-rich biomass. Further research is needed to explore their potential in extending the quality and safety of food products.

Award Application: Developing Scientist Award

P1-34: EVALUATION OF A NATURAL ANTIMICROBIAL TREATMENT ON MICROBIAL REDUCTIONS IN GROUND BEEF USING PETRIFILM

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Category: Antimicrobials

Abstract:

Introduction: Ground beef is a highly consumed and perishable product due to its susceptibility to microbial growth, posing significant challenges for food safety and shelf-life extension. Natural antimicrobial treatments can enhance food safety and prolong product shelf life.

Purpose: Evaluate the efficacy of a 5% natural citrus-based proprietary antimicrobial treatment in reducing microbial populations of lactic acid bacteria, mesophilic aerobes, enterobacteria, and coliforms in ground beef stored under refrigerated conditions.

Methods: Ground beef was prepared by grinding freshly processed beef trimmings, followed by the application of the 5% antimicrobial treatment using an immersion process for 30 seconds. Microbial populations were evaluated on days 0, 1, 2, and 3 of refrigerated storage (4°C) using Neogen®'s Petrifilm to quantify lactic acid bacteria, aerobic counts, enterobacteria, and coliforms. Control samples without treatment were also evaluated under identical conditions. Microbial load reductions were analyzed using R® software to compare treated and untreated samples.

Results: on day 0, the antimicrobial treatment reduced mesophilic counts by 0.98 log CFU/g compared to the control, this reduction remained consistent over the evaluation period, ranging from 0.62 to 0.92 log CFU/g through day 3. Lactic acid bacteria were reduced by 0.74 log CFU/g on day 0, with reductions ranging from 0.67 to 0.84 log CFU/g on days 1-3. For enterobacteria, the treatment reduced counts by 0.76 log CFU/g on day 0, with subsequent reductions of 0.75 to 0.61 log CFU/g on days 1-3. Coliform counts were reduced by 0.8 log CFU/g on day 0, with the greatest reduction of 1.1 log CFU/g on day 2, maintaining effectiveness through day 3.

Significance: The results demonstrate that a 5% citrus-based antimicrobial treatment is an effective intervention for extending the shelf life of ground beef.

This treatment addresses consumer demand for natural and clean-label solutions, while further research could explore its scalability and economic feasibility for industrial applications.

P1-35: MICROBIAL QUALITY AND PREVALENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING BACTERIA IN VEGETABLE SALAD FROM LOCAL AND ELITE RESTAURANTS IN IBADAN, NIGERIA

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Category: Antimicrobials

Abstract:

Introduction: Extended-spectrum beta-lactamase (ESBL) producing bacteria are a significant threat to public health due to the harboring of resistance to broad-spectrum antibiotics.

Purpose: In Nigeria, very little information about ESBL-producing bacteria from food samples is available.

Methods: Fifty pure cultures were obtained from (30) ready-to-eat (RTE) vegetable salad purchased from selected local and elite restaurants in Ibadan, Oyo State, Southwest Nigeria. All the isolates were tested against a selected panel of antibiotics, which include third-generation cephalosporin, aminoglycoside, macrolide, glycopeptide, and sulfonamide, by the disc diffusion method and screened for ESBL production through a double-disc synergy test. Detection of ESBL genes with specific primers (CTX-M, TEM, and SHV) using real-time polymerase chain reaction.

Results: Out of fifty strains, seven were confirmed ESBL producers, which were two *Proteus mirabilis*, one each for *Burkholderia cepacia*, *Burkholderia contaminans*, *Bacillus cereus*, *Bacillus velezensis*, and *Serratia rubidaea*. It was observed that 5/7 potential ESBL-producing strains were positive to blaCTX-M, blaTEM, and blaSHV, while no gene was detected in 3/7 strains. Out of the 5 isolates, blaTEM gene was present in two strains: (blaTEM and blaCTX-M) and (blaCTX-M and blaSHV) genes in one strain, respectively, while blaCTX-M gene was present in one strain. The antibiotic susceptibility test indicates that the ESBL-producing isolates were 100% susceptible to Imipenem (IPM). The two *Proteus mirabilis* and *Bacillus cereus* were resistant to nine antibiotics, while *Serratia rubidaea* exhibited resistance to 2 antibiotics. It could be inferred that 96% of the bacteria isolated in this study were multidrug resistant, as they resist three or more classes of antibiotics among the panel of antibiotics used.

Significance: This study highlights the occurrence of ESBL bacteria in RTE vegetable salad from food sources in Ibadan and the need for food surveillance proGrams to control the spread of antibiotic resistance in local and elite restaurants.

P1-36: ENHANCING FOOD PRESERVATION WITH SINGLE ATOM CATALYSTS: A NOVEL APPROACH TO ANTIMICROBIAL PACKAGING

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Traditionally, antimicrobial agents in food packaging face the challenge of high cost, rapid exhaustion, and the inability to inactivate a broad spectrum of microorganisms. Single-atom catalysts (SACs) mimic natural enzymes to catalyze production of reactive oxygen species that inactivate a broad range of foodborne bacteria.

Purpose: To quantify the inactivation of select spoilage and pathogenic bacteria by ROS generated by Co-incorporated SACs in a packaging film.

Method: *E. coli* ATCC 25922, *Listeria innocua* ATCC11288, *Leuconostoc lactis* ATCC 15520, *Listeria monocytogenes*, and *Salmonella enterica* ser. Typhimurium BAA190 were inoculated at 5 log CFU/ml into SACs suspension with concentrations ranging from 0.1 g to 1 g/L (n = 3). Additionally, circular disks of Co-SAC incorporated polylactic acid (PLA) films (10 mm diameter) were incubated in 5 log CFU/ml *E. coli* ATCC 25922 suspensions. Samples were stored at 4°C for 72 h, with sampling intervals every 24 h. Bacteria were enumerated on appropriate selective media, and the surviving colonies were counted.

Result: Higher Co-SACs concentrations and longer incubation enhanced efficacy against *E. coli* and *S. enterica* ($P < 0.05$), but not the other tested bacteria. Significant reductions of *E. coli* occurred: $3.4 \pm 0.34a$ log CFU/ml at 1 g/L Co-SACs, compared to $2.84 \pm 0.06b$ at 0.5 g/L after 72 hours ($P < 0.05$). At 6 h, 1 g/L Co-SACs reduced *E. coli* by $1.22 \pm 0.07c$ log CFU/ml, significantly lower than at 72 h. Similarly, *S. enterica* exhibited a $4.33 \pm 0.03c$ log CFU/ml reduction at 1 g/L Co-SACs ($P < 0.05$). In contrast, *Leu. lactis* showed a minor reduction (0.45 ± 0.06 log CFU/ml), while *Listeria* spp. remained unaffected at all SAC concentrations. Incorporation of Co-SACs (1 g/L) into the film resulted in a small increase in *E. coli* reduction (3.68 ± 0.02 log CFU/ml) after 72 hours.

Significance: Co-SACs demonstrated strong antimicrobial potential towards some selected bacteria, highlighting their promise for enhancing food safety in packaging films.

Award Application: Developing Scientist Award

P1-37: EVALUATION OF THE EFFECTS OF ROOT DECONTAMINATION OF SALMONELLA USING LACTIC ACID BACTERIA IN LIVING CILANTRO (CORIANDER SATIVUM) GROWN USING THE NUTRIENT FILM TECHNIQUE (NFT)

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Cilantro is frequently associated with foodborne illness outbreaks caused by *Salmonella*. Hydroponic systems, such as the Nutrient Film Technique (NFT), can facilitate pathogen survival on plant roots, posing food safety concerns. Post-harvest interventions are crucial to reduce contamination while preserving quality.

Purpose: To evaluate the efficacy of lactic acid bacteria (LAB) treatments in sanitizing cilantro roots contaminated with *Salmonella*.

Methods: A randomized design with repeated measurements was used to assess treatments on days 0, 3, 7, and 14. Cilantro plants grown in NFT systems were inoculated with *Salmonella* (4 log CFU/mL) and treated with a 5-minute dip wash using three LAB-based interventions: Product A (*Lactobacillus acidophilus*), Product B (*Lactobacillus salivarius* L28), and Product C (*Enterococcus faecium* L-20B). Microbial survival was assessed on plant roots and absorbent pads. At each time point, 1 mL of bacterial suspension was extracted for analysis. Bacterial counts were log₁₀-transformed (log₁₀(x+1)) with detection limits set at 1 CFU/mL post-enrichment. Data normality was assessed using the Shapiro-Wilk test, and differences were evaluated with Tukey's HSD.

Results: There was no significant interaction between time and treatment ($p > 0.05$), but both independently influenced microbial reductions ($p < 0.05$). LAB counts remained stable at 9 log CFU/mL on roots, while levels on pads decreased to 6.5 log CFU/mL by day 14. *Salmonella* was undetectable on roots by day 3, but recovery on absorbent pads occurred more frequently. Product C showed detectable *Salmonella* on days 3 and 7, whereas Products A and B achieved non-detectable levels by day 7.

Significance: LAB treatments effectively reduce *Salmonella* on cilantro roots without compromising quality. Higher *Salmonella* recovery on absorbent pads highlights the need to improve packaging decontamination strategies.

Award Application: Developing Scientist Award

P1-38: CONTROL OF GROWTH, SURVIVAL AND BIOFILM FORMATION OF AVIAN PATHOGENIC E.COLI WITH POTENTIAL PROBIOTICS AND THEIR METABOLITES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Avian Pathogenic *Escherichia coli* (APEC) causes a range of clinical manifestations including respiratory, systemic, and reproductive infections in poultry. Notably, its genetic similarities with *E. coli* strains responsible for severe human infections raise concerns about its zoonotic potential. As a sustainable alternative to antibiotics, probiotics can enhance host immunity and prevent colonization of APEC.

Purpose: The aim of this study was to evaluate the efficacy of probiotic species and their metabolites against the growth, survival, and biofilm formation of APEC.

Methods: Antagonistic effects of *Lactobacillus rhamnosus* (LR) and *Lactobacillus plantarum* (LP) against APEC were evaluated using a co-culture media with an initial pathogen load of 10^5 CFU/mL and varying pathogen-to-probiotic ratios (1:1, 1:10, 1:100, 1:1000) to assess dose-dependent responses. Probiotic-induced acidification was monitored via pH measurements. Inhibition of APEC growth and biofilm formation was assessed using cell-free cultural supernatants collected at 24 hours (CFCS1) and 48 hours (CFCS2). Gene expression analysis targeted stress response (*H-NS*, *rpoS*), biofilm formation and virulence (*luxS*, *fimC*, *fyuA*, *iss*), and metabolic and survival pathways (*ftsZ*, *murD*, *fliP*, *ompA*, *ompC*, *radA*, *phoP*). Statistical significance was determined via two-way ANOVA followed by Tukey's post hoc test.

Results: Both LP and LR significantly suppressed APEC growth by 3.73 logs and 3.46 logs ($P < 0.05$) respectively, at pathogen-to-probiotic ratios of 1:1 and 1:10, with complete eradication at higher ratios (1:100, 1:1000) within 24 hours. A 20% (v/v) concentration of CFCS1 reduced bacterial load by approximately 2 logs, while CFCS2 achieved complete elimination within 48 hours ($P < 0.05$). Significant biofilm inhibition (>3 logs) and dispersal (>2 logs) ($P < 0.05$) were observed with 20% and 30% (v/v) of CFCS. Significant alterations in expression of genes with critical roles in survival and biofilm formation were observed.

Significance: These findings highlight the potential of probiotics and their metabolites as viable alternatives for controlling APEC infections.

Award Application: Developing Scientist Award

P1-39: APPLICATION OF AERATED NANOBUBBLE WATER TO CONTROL LISTERIA MONOCYTOGENES AND IMPROVE SPROUT SAFETY

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Sprouts are commonly involved in foodborne outbreaks involving different pathogens including *Listeria monocytogenes* (LM). Besides seed contamination, the sprouting process supports rapid pathogen proliferation. Thus, there is a critical need for effective antimicrobial interventions to improve the microbial safety of sprouts.

Purpose: To determine the antimicrobial efficacy of aerated nanobubble water in controlling LM on alfalfa seeds and sprouts.

Methods: Sanitized alfalfa seeds were inoculated with a five-strain LM cocktail (~4 log CFU/mL) and washed by spraying with tap water (TW) or aerated water (AW; ~30 mg/L O₂). A sub-set of unwashed seeds were included as the control. The seeds were dried, bagged, stored for 3 months and sampled regularly to enumerate LM populations (n=3). In addition, at each sampling, stored seeds from each group were set up for germination and sprouting for 7 days with aerated (AW group) and tap water (for control and TW groups) used as irrigation sources. Sprouts and spent irrigation water were sampled regularly for microbial and sprout quality analysis (n=3). Data were analyzed using GraphPad Prism with significance tested at $p \leq 0.05$.

Results: Seed treatment and sprout irrigation using AW significantly reduced LM populations on stored seeds and germinating sprouts ($p \leq 0.05$). By day 21 of storage, LM populations on seeds were below detection limit (<1 CFU/ml) in the AW samples when compared to ~3 log recovered from control and TW groups. Similarly, irrigation with AW reduced LM populations on sprouts by 4-5 log CFU/ml throughout the study when compared to control and TW (~8 log CFU/ml). Also, use of AW significantly reduced LM survival in spent water to below detection limits. In addition, treatment with AW was seen to significantly increase germination rate, shoot length, and sprout weight.

Significance: Aerated nanobubble water presents a safe and sustainable

intervention to improve sprout safety while supporting sprout growth.

Award Application: Developing Scientist Award

P1-40: EXPLORATION OF ANTIMICROBIAL PRODUCTION CAPABILITIES OF A POTENTIAL STARTER CULTURE, LACTOCOCCUS LACTIS OSY-92, USING CONVENTIONAL AND MOLECULAR TECHNIQUES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Lactococcus lactis* OSY-92 was identified as a potential bioprotective dairy starter culture in a previous study. Understanding the strain's antimicrobial production capability is vital for its future use in the dairy industry.

Purpose: Determine identity, potency, and applicability of the antimicrobial agent(s) produced by *L. lactis* OSY-92.

Methods: The whole genome of *L. lactis* OSY-92, sequenced previously, was mined for biosynthetic gene clusters associated with antimicrobial production. Following genomic analysis, crude extract from *L. lactis* OSY-92 was prepared and tested for antimicrobial activity using the 5µl spot-on-lawn method at various points in the bacterium's growth. Subsequently, reverse transcription quantitative analysis (RT-qPCR) was utilized to analyze the expression of antimicrobial production genes. Gene expression was monitored during lag (0 hrs), exponential (6 hrs), and stationary (24 hrs) growth phases. The 16S rRNA gene was chosen as the housekeeping gene for normalization, and fold change was calculated using the 2- $\Delta\Delta C_t$ method.

Results: The genome of *L. lactis* OSY-92 was found to contain the *lcnBCD* operon, encoding the production, modification, and transport of the antimicrobial peptide lactococcin B. Like other bacteriocins, lactococcin B is effective against closely related strains. Therefore, *L. lactis* ATCC 11454 was chosen as the indicator organism for culture-based assays. Concentrated crude extract of *L. lactis* OSY-92 grown for 24 hours was shown to inhibit the indicator in the spot-on-lawn bioassay. No significant antimicrobial activity was observed in the crude extract harvested from the exponential growth phase culture. RT-qPCR primers were designed to target each gene in the *lcnBCD* operon, and it was shown that the gene encoding the core peptide, *lcnB*, was upregulated in stationary phase by 17.4-fold in comparison to time zero.

Significance: *L. lactis* OSY-92 may have potential as a lactococcin-producing bioprotective dairy starter culture to aid in the production of safe, clean-label dairy

products.

Award Application: Developing Scientist Award

P1-41: EFFICACY OF PERACETIC ACID (PAA) IN COMBINATION WITH A PAA BOOSTER FOR CLEAN-IN-PLACE APPLICATIONS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Peracetic acid (PAA) is a commonly used sanitizer for clean-in-place (CIP) applications in the food industry (e.g., dairy). However, elevated levels may be needed to control persistent microbial targets such as biofilm and bacterial endospores.

Purpose: A formulation containing a mixture of organic acids, chelants, and surfactants was developed and evaluated for its ability to boost PAA performance against biofilm and endospores for CIP applications.

Methods: The CDC biofilm reactor and the Single Tube Method were used to screen concentrations of PAA alone and in combination with the booster to determine efficacy against *Pseudomonas aeruginosa* ATCC 15442 biofilm. To assess sporicidal activity, bacterial spores (*Bacillus subtilis* ATCC 19659 or *Clostridium sporogenes* ATCC 3584) were dried on 304 stainless steel carriers and treated with various concentrations of PAA or PAA plus booster. For both methods, the treatments were applied at 35°C for 10 min followed by neutralization. Viable bacterial counts were obtained by serial dilution plating.

Results: When tested alone, 700 ppm PAA could not achieve the standard 6-log reduction against *P. aeruginosa* biofilm. In contrast, the combination of PAA and booster achieved a greater than 8-log reduction at 450 ppm PAA. The booster had no sporicidal activity but when combined with PAA it reduced the level of PAA needed to achieve a 4-log reduction from 400 ppm to 250 ppm (*B. subtilis*) and from 250 ppm to 100 ppm (*C. sporogenes*). Evidence of a synergistic relationship between PAA and the booster was observed against both organisms with synergy indices ranging from 0.51 to 0.58.

Significance: The results indicate that the booster formulation can increase the

performance of PAA against both biofilm and endospores.

P1-42: INVESTIGATION OF LACTOBACILLUS AND THEIR INDIVIDUAL ANTIMICROBIAL ACTIVITY AGAINST STAPHYLOCOCCUS EPIDERMIDIS

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: *Staphylococcus epidermidis* (SE) is a prevalent commensal microorganism found on the skin of most mammals, including humans. However, it is also an opportunistic pathogen that can cause disease when the skin microbiome becomes dysbiotic, especially if the strain is antibiotic-resistant.

Purpose: This study aimed to investigate the antimicrobial activity of *Lactobacillus plantarum* (LP) and *Lactobacillus rhamnosus* (LR) as alternative approaches to control SE growth.

Methods: A multidrug-resistant SE strain was co-cultured with LP or LR at various ratios in the presence or absence of prebiotics. SE colony counts were measured over time to assess antimicrobial activity. Additionally, cell-free cultural supernatants (CFCS) from LP and LR were collected and applied to SE to investigate inhibitory mechanisms.

Results: SE concentrations decreased significantly over time when co-cultured with LP or LR, with notable reductions at 12 and 72 hours ($p < 0.05$). Cocoa supplementation (1.5–4%) had no significant effect on SE growth in co-culture. CFCS collected from 72-hour LP or LR cultures significantly inhibited SE growth after 4 hours ($p < 0.05$), and no SE was detectable at 72 hours ($p = 0.0003$). CFCS also reduced SE biofilm formation after 24 hours ($p < 0.05$) and bacterial concentration after 48 hours ($p = 0.0143$). Gene expression analysis showed downregulation of metabolic processes (not statistically significant), and fluorescent microscopy revealed reduced cell wall synthesis following CFCS treatment.

Significance: Both LP and LR, along with their metabolites, demonstrated antimicrobial effects against multidrug-resistant SE, suggesting potential as alternative agents for controlling SE colonization.

Award Application: Developing Scientist Award

P1-43: GREEN SYNTHESIS OF ANTIMICROBIAL LIGNOCELLULOSE-BASED NANOCOMPOSITE FILMS WITH NISIN AND OREGANO ESSENTIAL OIL AGAINST FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Ready-to-eat low moisture foods such as packaged nuts are prone to contamination by *Listeria monocytogenes* and *Salmonella* spp., prompting a need for post-process intervention, such as active antimicrobial packaging that offers a promising solution for controlled antimicrobial release and effective pathogen mitigation.

Purpose: This study was aimed to develop an antimicrobial biocomposite by incorporating natural antimicrobials into lignin-containing cellulose nanofibers (LCNF) derived from plant-based waste.

Method: Nanocomposite films consisting of LCNF, chitosan (800 KDa), polyvinyl alcohol, nisin (0.33% w/v), and oregano essential oil (OEO; 0.5%, 1%, and 1.5% v/v) were prepared by solution casting on Teflon plates and punched into 9-mm disks. In vitro antimicrobial activity was tested using a disk diffusion method against four *Salmonella enterica* strains (PT 30, PT 9c, Oranienberg, Anatum), four *Listeria monocytogenes* strains (Scott A, V7, LCDC, 101M), and a control without disks on Mueller-Hinton agar. Film color ($L^*a^*b^*$) was evaluated. Differences in mean values were analyzed using ANOVA with post-hoc Tukey's test at $P < 0.05$ in JMP® Pro 18.0.1.

Result: The antimicrobial film demonstrated broad-spectrum efficacy, with zones of inhibition ranging from 13.4–25.5 mm. It was significantly more effective ($P < 0.05$) against Gram-positive bacteria, as evidenced by greater susceptibility of *L. monocytogenes* (19.11 ± 0.26 mm) compared to *S. enterica* (15.97 ± 0.26 mm). Among the tested strains, the most resistant were *Salmonella* PT 30 and Oranienberg (14.5–16.0 mm), while the most susceptible were *L. monocytogenes* Scott A and V7 (20.57–21.24 mm). For both organisms, the 15% OEO concentration showed the highest efficacy ($P < 0.05$), though films at this

concentration had a slightly darker color, as indicated by a significantly lower L* value (70.27 ± 0.28).

Significance: Plant-based nanocomposite antimicrobial films show potential as a sustainable and effective food safety solution, with their in vivo applications for packaged ready-to-eat low moisture foods warranting further exploration.

Award Application: Developing Scientist Award

P1-44: THE ROLE OF ACCESSORY GENE REGULATOR (AGR)-MEDIATED QUORUM SENSING SYSTEM IN ENHANCING THE BIOSYNTHESIS OF THE NATURAL ANTIMICROBIAL PEPTIDE, PAENIBACILLIN

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: There is growing demand for clean-label foods with natural preservatives. Paenibacillin, an antimicrobial peptide produced by *Paenibacillus polymyxa* OSY-DF, is effective but its low natural production limits the feasibility of implementation. The paenibacillin biosynthetic gene cluster contains genes encoding the Agr quorum sensing (QS) system, which is hypothesized to enhance paenibacillin production via its autoinducing peptides (AIP). Additionally, other Gram-positive bacteria with different AIPs could either enhance or inhibit the Agr QS system in OSY-DF.

Purpose: Investigating the role of the Agr QS system, through its AIPs as genetic regulators, for potentially enhancing paenibacillin biosynthesis.

Methods: *P. polymyxa* OSY-DF was supplemented with 0%, 1%, 3%, 5%, and 10% crude extracts (CE) from 24-hour OSY-DF, 16-hour *S. aureus*, and 24-hour *L. lactis* cultures, each hypothesized to contain their respective AIP signals in their CEs. After 24 hours of growth of OSY-DF with the supplementations, samples were collected for antimicrobial bioassay to semi-quantitatively determine paenibacillin concentration, using *Listeria innocua* as a sensitive indicator.

Results: Supplementing OSY-DF with 1–10% CEs from a 24-hour OSY-DF culture and a 16-hour *S. aureus* culture increased paenibacillin production up to 32 µg/mL, compared to 16 µg/mL in non-supplemented samples. However, OSY-DF supplemented with its own CE exhibited stronger zones of inhibition as compared to CE from *S. aureus*. AIP signals in the CE seem to boost paenibacillin production, with AIPs from OSY-DF and *S. aureus* potentially sharing structural similarities that activate OSY-DF's Agr system. The CE from nisin-producing *L. lactis* however did not impact paenibacillin production. A mutagenesis-based study to knock out the AIP precursor gene (*agrD*) in OSY-DF strain is underway to confirm Agr's role in regulating paenibacillin biosynthesis.

Significance: Understanding the Agr QS-based genetic regulation helps enhance the productivity of the paenibacillin producer, thus improving the feasibility of

using the peptides as effective and natural preservatives for improving food safety.

Award Application: Developing Scientist Award

P1-45: A NOVEL LATERAL FLOW ASSAY BASED ON GRAPHENE QUANTUM DOT-PHAGE PROBE FOR DETECTION OF E. COLI O157:H7

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Lateral flow assays (LFAs) are rapid and cost-effective pathogen detection tools that typically rely on antibodies to capture analytes, but their environmental sensitivity and high production costs highlight the need for alternative biorecognition reagents to improve reliability and efficiency.

Purpose: This study aims to develop a bacteriophage-based lateral flow platform for the detection of *Escherichia coli* O157:H7 in food samples.

Methods: Phage CAM-21 was labeled with highly fluorescent graphene quantum dots (GQDs) using a physisorption method to create a CAM-21-GQD probe. In the LFA platform, phage CAM-21 was used to capture the target pathogen, *E. coli* O157:H7, on the test line, while a suspension of GQDs was used to print the control line. The CAM-21-GQD probe served as the revealing agent to generate a fluorescence signal upon successful pathogen detection. The sensitivity of the LFA was evaluated in both PBS buffer and food samples, including beef and milk. The specificity of the platform was determined by testing *E. coli* O157:H7 and five non-*E. coli* O157:H7 strains. The fluorescence signal was analyzed using an ESEQuant LR3 lateral flow reader.

Results: The LFA completed detection within 30 minutes. Under optimal conditions, the platform achieved a limit of detection of approximately 10 CFU for *E. coli* O157:H7 in PBS buffer and 10⁴ CFU/mL in both milk and beef samples. The specificity tests confirmed that fluorescence was observed only for *E. coli* O157:H7 in the test region, while no fluorescence was detected for non-*E. coli* O157:H7 strains or the blank control (PBS).

Significance: This study demonstrates the potential of a bacteriophage-based lateral flow platform as a rapid, sensitive, and cost-effective method for detecting *E. coli* O157:H7 in food samples, addressing critical needs in food pathogen detection.

Award Application: Developing Scientist Award

P1-46: SYNERGISTIC EFFECTS OF CITROSPET, CITRICIDAL, CAPRYLIC ACID, CARVACROL, LACTIC ACID, AND HIGH-PRESSURE PROCESSING AGAINST STAPHYLOCOCCUS AUREUS AND LISTERIA MONOCYTOGENES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The inactivation of *Staphylococcus aureus* and *Listeria monocytogenes* is a significant challenge limiting the broader adoption of high-pressure processing (HPP) in both domestic and international food commerce.

Purpose: The current study investigates the synergistic impact of HPP, combined with mild heat and five bactericidal compounds to enhance the inactivation of *S. aureus* and *L. monocytogenes* bacteria of concern to the food industry.

Methods: Elevated hydrostatic pressure of 400 MPa combined with mild heat at 25°C was applied to apple cider and pasteurized milk at target inoculations of 10⁷ CFU/ml in a PULSE tube using a Hub880 barocycler unit for 0 min (untreated control) 3, and 5 min with and without 0.01% Citrospet, Citricidal, Caprylic acid, Carvacrol, and Lactic Acid. The results were statistically analyzed using Tukey- and Dunnett 's-adjusted ANOVA.

Results: Under controlled experimental conditions, it was observed that the synergistic application of elevated hydrostatic pressure with mild heat at 25°C could reduce up to >3 logs CFU/mL (i.e., >99.99%) of *S. aureus* and for *L. monocytogenes* could reduce up to >4 logs CFU/mL with Carvacrol and Caprylic acid. The addition of Citrospet, Citricidal, and Lactic Acid did not show any added reduction of *S. aureus* and *L. monocytogenes*.

Significance: This study emphasizes the importance of HPP combined with specific bactericidal compounds in achieving effective inactivation of *S. aureus* and *L. monocytogenes*. This could lead to the development of optimized HPP protocols tailored to specific food products and microbial threats, ensuring enhanced food safety.

P1-47: SYNERGY BETWEEN ACID-PRODUCING BACTERIA STRAINS IMPROVES INHIBITION OF SEVEN DIFFERENT SALMONELLA SEROVARS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Acid-producing bacteria (APB) display antimicrobial properties against foodborne pathogens. They produce organic acids and bacteriocins, lower pH, and create unfavorable conditions for certain bacterial pathogens. This research tested the potential of certain APB as antimicrobial control agents. Combining strains may enhance antimicrobial effects through synergistic interactions, improving pathogen inhibition.

Purpose: To evaluate the synergistic interactions of multi-strain APB combinations to inhibit *Salmonella*.

Methods: Eleven combinations of *Lactiplantibacillus plantarum* (A), *Bifidobacterium bifidum* (B), *Propionibacterium freudenreichii* (C), and *Propionibacterium jensenii* (D) were prepared and tested. The pH of each culture was monitored over 24h. Organic acid production was analyzed using high-performance liquid chromatography from cell-free supernatants. Antimicrobial effects of single APB and combinations against *Salmonella* serovars Enteritidis, Typhimurium, Kentucky, Newport, Dublin, Heidelberg, infantis, were assessed using a kinetic method. Populations of APB and *Salmonella* were enumerated every 12h for 48h using selective media. inhibition percentages were calculated based on reductions in *Salmonella* counts. Data was analyzed using two-way ANOVA with Tukey's post-hoc test using GraphPad Prism10.

Results: When four APB were combined, the highest acid production was observed: 781.67 mM lactic acid, 500.67 mM propionic acid, 30.29 mM acetic acid, 29.4 mM butyric acid, 168.81 mM valeric acid, and a pH of 2.97. Combination A+B had the lowest acid levels and minimal pH changes. After 48h, other APB combinations inhibited *Salmonella* serovars. Combinations B+C, A+C+D, and A+B+C+D completely inhibited *S. Enteritidis*, *S. infantis*, *S. Dublin*, and *S. Typhimurium* ($p < 0.05$). B+C achieved the highest reductions (100%), with log10 reductions up to 8.22. Treatments such as A+B+D and A+B+C+D showed

similarly high inhibition, while others (e.g., B+D and A+C) were less effective, achieving 99.9% to 99.99% inhibition.

Significance: Combining strains of acid-producing bacteria synergistically enhances the antimicrobial activity against *Salmonella*. This APB strains become a promising alternative for food production systems.

P1-48: EVALUATING THE EFFICACY OF CONSISTENT USE OF GRAPESEED 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: Food processing plants house a variety of microorganisms that in combination with foodborne pathogens may attach on food contact surfaces and form biofilms. Biofilms are formed over time and are mostly a result of poor cleaning practices. Biofilm formation could be prevented by regular cleaning with effective sanitizers that can help prevent microbial buildup in food processing plants.

Purpose: The efficacy of consistent use of grapeseed extract on food contact surfaces such as stainless steel 304 (SS304), high density polyethylene (HDPE), polyvinylchloride (PVC), polycarbonate (PC) and buna-N-rubber (BR) inoculated with *Salmonella enterica*/leafy green native microbiota from spinach and cut romaine was evaluated.

Methods: Coupons of various materials were treated by dipping in plant extract solution (2, 3, 5, 7 and 10%) for 2 minutes and dried for 30 minutes. The treated coupons were inoculated with either *S. enterica* or one of the leafy green native microbiota by dipping in tryptic soy broth (TSB), washed and enumerated for 0 h. These coupons were then treated and dipped in TSB for 14–18 h. Washing, enumeration and treatment steps were repeated after 14–18 h, 28–36 h (only washed and treated), and 42–54 h.

Results: Grapeseed extract at 2, 3, and 5% was effective in reducing *S. enterica* to below detection levels (<1 log CFU/coupon) in 42–54 h for all coupon types. Treatments using higher concentrations (7 and 10%) were effective in reducing both leafy green native microbiota to below detection levels except in the case of 7% treatment on SS304, where a 6-log reduction was observed in 42–54 h. In 14–18 h, reduction in population of *S. enterica* and leafy green native microbiota varied based on grapeseed extract concentration and coupon type.

Significance: Results indicate that grapeseed extract at different concentrations can potentially be used to prevent microbial buildup on food contact surfaces and hence, biofilm formation.

Award Application: Developing Scientist Award

P1-49: EXTENDING THE SHELF LIFE OF PLANT-BASED CHEESE SPREAD WITH A CLEAN-LABEL ANTIMICROBIAL

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Plant-based cheese spreads are often susceptible to microbial spoilage and have a limited shelf life due to their high water activity and non-thermal processing of nutrient-rich matrices. The modern food industry prefers natural ingredients that serve multiple functions, and natural clean-label antimicrobials provide a sustainable solution to combat spoilage.

Purpose: To evaluate the effectiveness of a natural, clean-label antimicrobial, DuraShield™ (a combination of cultured dextrose and rosemary extract), in inhibiting the growth of yeast and mold and thereby extending the shelf life of fermented cashew-based cheese spreads.

Methods: Cashew-based cheese spreads were prepared with and without antimicrobials. Four treatments were included in the study: a control (without antimicrobials), Low DuraShield™ (with DuraShield™ at 0.3%), High DuraShield™ (with DuraShield™ at 0.5%), and a synthetic antimicrobial (with sorbic acid at 0.15%). Two sets of experiments were conducted. In Experiment 1, the treatments were stored at 4°C for 36 days, and the natural growth of spoilage microorganisms was monitored. In Experiment 2, the treatments were inoculated with *Penicillium roqueforti* and assessed for mold spoilage over 48 days at 4°C in sterile cups. The experiments were repeated twice, using duplicate samples (n=4).

Results: In Experiment 1, ITS sequencing revealed *Debaryomyces hansenii* as the predominant yeast responsible for spoilage, with the control group reaching 8.7 log CFU/g by day 36. High DuraShield™ and the synthetic antimicrobial effectively inhibited yeast spoilage ($P < 0.5$), keeping growth below 3 log CFU/g. In Experiment 2, *P. roqueforti* reached 6.9 log CFU/g in the control group by day 48. Antimicrobial treatments significantly inhibited its growth ($P < 0.05$), with High DuraShield™ maintaining growth at 4.5 log CFU/g even at day 48. In both experiments, High DuraShield™ had comparable efficacy to synthetic antimicrobial sorbic acid.

Significance: DuraShield™ successfully controlled yeast and mold spoilage in cashew-based cheese spread, and it could be a potential replacement for synthetic antimicrobial sorbic acid.

P1-50: THE EFFICACY OF ANTIMICROBIAL COATINGS IN REDUCING *LISTERIA MONOCYTOGENES* AND *PENICILLIUM EXPANSUM* POPULATIONS ON ORGANIC GALA APPLES

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: There is limited understanding of effective methods to control the growth of fungal and foodborne pathogens on organic apples during long-term storage, highlighting the need for further evaluation in this area.

Purpose: Evaluate the survival of *Listeria monocytogenes* (LM) and *Penicillium expansum* (PE) individually inoculated on organic apples over 60 days of refrigerated storage.

Methods: Organic Gala apples were spot inoculated and coated with sodium alginate incorporated with 2% essential oils (EO) (v/v) [cinnamon bark oil (CBO), oregano oil (OO), coriander oil (CO), clove bud oil (CIBO), or an equal proportion (% v/v) mixture of all four (MO)], as well as controls consisting of coating without essential oils (C), or no coating (NC) for each inoculum type [LM (~8.5 log CFU/apple) or PE (~6 log CFU/apple)]. Apples were stored at 3 °C. LM and PE populations were assessed at 1, 30, and 60 days of storage by spiral-plating on selective and differential agar plates (n=9 per treatment combination).

Results: A significant decrease in LM population was observed for CBO, CO, MO, and C ($p < 0.001$), with each treatment achieving at least a 2.5-log reduction after 60 days of storage. Treatments OO, CIBO, and NC showed a 1-log reduction. Comparatively, PE populations remained stable over the 60-day period, with no

significant differences detected between treatments ($p > 0.05$).

Significance: LM populations declined in all treatments, with no benefits of EO-incorporated coatings beyond that of a coating-only control during the first 60 days of storage. An examination of population dynamics beyond the first 60 days of storage will be beneficial to better assess the efficacy of EO coatings for the control of PE and LM in organic Gala apples.

P1-51: LISTERIA MONOCYTOGENES CONTROL IN READY-TO-EAT PRODUCTS WITH A CLEAN LABEL ANTIMICROBIAL

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The ability of *Listeria monocytogenes* (LM) to survive in extreme environmental conditions, including high salt concentrations and wide temperature ranges, makes it a significant challenge for food safety, specifically in ready-to-eat (RTE) products. This establishes the need for an effective antimicrobial to reduce LM growth.

Purpose: To evaluate the ability of a natural, clean-label antimicrobial, DuraShield™ NPV (NPV: rosemary extract + cultured dextrose + buffered vinegar), to inhibit the growth of LM in cooked chicken meat, cured hotdog, and plant-based meat.

Methods: For the cooked chicken, boneless skinless chicken breasts were vacuum tumbled in brine with phosphates, salt, sugar, and 1% NPV. Cured hot dogs were made by emulsifying ground pork with various ingredients, and 1% NPV was added to the emulsion before cooking in collagen casings. Additionally, a synthetic antimicrobial treatment (2% sodium L-lactate and sodium diacetate) was included for comparison. Plant-based meat crumbles were produced using textured vegetable protein, water, soy and pea proteins, and 1% NPV. After cooking at 74°C and chilling, all samples were inoculated with a cocktail of LM and stored at 4°C in vacuum-sealed packaging. Growth was monitored against a control group without antimicrobials (N=4 samples/experiment).

Results: In cooked chicken, LM grew to 8 log CFU/g in control samples within 24 days, while NPV maintained growth below 3 log CFU/g ($P<0.05$). In cured pork hot dogs, LM grew from 3.5 log to 6.5 log CFU/g by day 80 in control samples, whereas NPV inhibited LM growth, reducing it from 3.8 to 2.8 log CFU/g ($P<0.05$), closely matching the synthetic antimicrobial treatment. For plant-based meat crumbles, LM reached 6.7 log CFU/g after 105 days at 4°C, while NPV controlled it below 1

log CFU/g from an initial count of 3.2 log CFU/g ($P < 0.05$).

Significance: A natural clean-label antimicrobial, DuraShield™ NPV, can be used to control *L. monocytogenes* growth in RTE products.

P1-52: APPLICATION OF A MICROBIAL PEPTIDE AGAINST AN ENVIRONMENTAL ISOLATE OF LISTERIA MONOCYTOGENES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* (Lm) is a persistent foodborne pathogen in food processing environments linked to multiple outbreaks. Antimicrobial peptides produced by microbial sources are promising alternatives to conventional antibiotics, offering a natural and effective approach to controlling pathogens in the food processing environment.

Purpose: This study aims to evaluate the antimicrobial activity of a lab-isolated *Bacillus velezensis*-derived antimicrobial peptide against Lm isolated from a dairy processing environment.

Methods: *Bacillus velezensis*, previously isolated from the biofilm of a whey reverse osmosis membrane, was cultured in tryptic soy broth (37°C/24h) to produce the cell-free extract (CFE) by centrifugation and microfiltration. The CFE was concentrated using SnakeSkin™ dialysis tubing to produce dialyzed CFE (DCFE). The DCFE was partially purified using a C-type gravity column packed with Sephadex G-75 resin by size exclusion chromatography. The resulting fractions with higher protein content were freeze-dried to yield freeze-dried protein fractions (FDPF). The pH, protein concentration, and antimicrobial activity of the CFE, DCFE, and FDPF were evaluated using well diffusion assays against the Lm isolate. SDS-PAGE was performed to assess the molecular weight and purity of the DCFE and FDPF.

Results: The pH of CFE, DCFE, and freeze-dried protein fractions (FDPF) ranged from 6.8–7.0. Protein concentrations were 2.8 mg/mL (CFE), 90 mg/mL (DCFE), and 2.2–50 mg/mL (FDPF). The DCFE exhibited antimicrobial activity against Lm, with inhibition zones of 7 ± 0.1 mm. SDS-PAGE analysis of DCFE revealed multiple protein bands in the 2–250 kDa range. Among the FDPF, four fractions exhibited inhibition zones of 6 ± 0.2 mm against Lm and produced distinct bands within the 5–10 kDa range on SDS-PAGE, confirming the effective purification of the antimicrobial peptide.

Significance: The antimicrobial peptide derived from *Bacillus velezensis* showed

promise as a natural intervention for controlling *L. monocytogenes* in food and dairy processing environments.

Award Application: Developing Scientist Award

P1-53: MULTI-DRUG RESISTANCE PROFILING AND GENETIC INSIGHTS OF NON-*E. COLI* ENTEROBACTERIACEAE ISOLATED FROM WASTEWATER IN SOUTHEAST WYOMING

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Antimicrobial resistance (AMR) is a global health crisis fueled by selective pressures from antibiotic use in clinical and environmental systems. Wastewater treatment plants (WWTPs) are hotspots for the persistence and dissemination of AMR genes. While much research focuses on *Escherichia coli*, other Enterobacteriaceae genera remain underexplored despite their critical role in AMR dissemination.

Purpose: This study aims to identify and characterize AMR genes and mobile genetic elements (MGEs) in Enterobacteriaceae isolates from influent samples at the Laramie WWTP in Wyoming, focusing on non-*E. coli* genera.

Methods: Wastewater influent samples were collected twice weekly (March-April 2024), representing 24-hour composite sampling. A total of Enterobacteriaceae isolates (N=100) were obtained through serial dilution and plating on antibiotic-supplemented agar. Among these, multidrug-resistant (MDR) non-*E. coli* isolates (n=27) were identified using MALDI-TOF. Antibiotic susceptibility was determined via broth microdilution. Genomic analysis included quality trimming with Trimmomatic, de novo assembly using SPAdes, annotation with Prokka and identification of resistance genes via Abricate.

Results: Resistance was highest for Ampicillin (80.8%), Cefoxitin (57.7%), Streptomycin (50%), and Ceftiofur (42.3%). Key AMR genes included blaCTX-M-15, blaTEM-1, blaSPR-1, blaCTX-M-55, blaSHV-187, qnrB1, blaCMY-2, and tetA, along with aminoglycoside-modifying enzymes aph(3'')-Ib and aph(6)-Id. MGEs such as incFII_1_pKP91, incFIB(K)_1_Kpn3, and ColRNAI_1 were widespread, facilitating horizontal gene transfer. Virulence genes, including fliM, fliA, and ompA, were detected across genera. MDR was widespread, notably against beta-lactams, aminoglycosides, and fluoroquinolones.

Significance: This study highlights the role of WWTPs in driving AMR among non-*E. coli* Enterobacteriaceae, an often-overlooked group. Antimicrobial resistance

genes (ARGs) from these MDR isolates persist in wastewater and can spread through treated effluent into surface water. in regions like Wyoming, where treated wastewater is reused for irrigation, these ARGs risk contaminating agriculture and the food supply chain. Strict monitoring and improved treatment are essential to mitigate these environmental and public health risks.

P1-54: NATURAL PRESERVATIVES VS. PATHOGENS: A MIC STUDY OF *Listeria monocytogenes* AND *Salmonella enterica* STRAINS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Ready-to-eat (RTE) dips, including hummus, are prone to contamination by *Listeria monocytogenes* and *Salmonella enterica*. Natural preservatives may offer a solution by inhibiting pathogen growth while maintaining product safety and quality.

Purpose: This study aimed to assess the antimicrobial effectiveness of three natural preservatives against *L. monocytogenes* and *Salmonella* to improve food safety in RTE dips.

Methods: *L. monocytogenes* and *Salmonella* strains were grown in tryptic soy broth with yeast extract and 80 µg/ml rifampicin for 48 hours at 37°C, then diluted in 1× phosphate-buffered saline to achieve 7 log CFU/ml. Provantage 399 (7 mg/ml), Galimax Flavor F-100 (20 mg/ml), and Galimax Flavor VNPF-100 (10 mg/ml) were dissolved in water and acidified with 4% vinegar (pH 4.6). MIC testing was conducted in 96-well plates, with preservative solutions in row 1, serial dilutions across rows 2–8, and tryptic soy broth as the diluent. Each well received 10 µl of resazurin dye (0.5 mg/ml) and 10 µl bacterial inoculum post-dilution (~5 log CFU/ml per well). Positive and negative controls were included. MIC results were confirmed by streaking 10 µl onto tryptic soy agar.

Results: Provantage 399 showed strong activity against *Listeria*, with MIC values of 1.75 mg/ml for *L. monocytogenes* G1091 under unacidified and acidified conditions. For the other *Listeria* strains, MICs ranged from 3.5–7 mg/ml under acidification and 7 mg/ml at unacidified pH. Provantage 399 exhibited no activity against *Salmonella*. When acidified, Galimax Flavor F-100 inhibited *L. monocytogenes* and *Salmonella* with MICs of 5 mg/ml (*S. Newport*) and 10 mg/ml (*S. Montevideo* and *S. Typhimurium*). Similarly, acidified Galimax VNPF-100 inhibited all *L. monocytogenes* strains at 5–10 mg/ml and all *Salmonella* strains at 10 mg/ml.

Significance: The study highlights the potential of natural preservatives to inhibit *L. monocytogenes* and *Salmonella* under acidified conditions, offering a clean-

label solution to enhance food safety in RTE products.

Award Application: Developing Scientist Award

P1-55: NEXT-GENERATION PROBIOTICS AS NOVEL STRATEGY TO CONTROL FOOD-BORNE CAMPYLOBACTER JEJUNI INFECTIONS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Campylobacter jejuni* is a foodborne pathogen responsible for self-limiting diarrhea in humans, primarily caused by the consumption of contaminated poultry and poultry products. While infections are typically treated with antibiotics, the increase in antimicrobial resistance has highlighted the urgent need for alternative therapeutic approaches.

Purpose: In this study we aim to evaluate the effect of Next-generation probiotics (NGP) on inhibiting the growth of *C. jejuni* in vitro.

Methods: Thirty-eight probiotic strains were screened for their ability to inhibit *C. jejuni* growth using an agar-well diffusion assay. The best probiotics were selected for their effect in inhibiting growth of *C. jejuni* when co-cultured together in liquid media. Probiotic supernatants were assessed for their ability to inhibit biofilm formation and pre-formed biofilms. We also evaluated their effect on *C. jejuni* adhesion, invasion, and survival in human intestinal cell lines. Additionally, RT-qPCR was used to analyze changes in the expression of virulence-associated genes of *C. jejuni*. All experiments were conducted in duplicates, and data were analyzed using two-way ANOVA with Tukey's multiple comparisons test.

Results: All the probiotics demonstrated inhibition of growth of *C. jejuni*. However, eight probiotics demonstrating the highest inhibition of growth were selected for further development. These probiotics effectively inhibited *C. jejuni* growth in liquid media. Four probiotics demonstrated up to 100% inhibition of biofilm formation and pre-formed biofilms of *C. jejuni*. The probiotics significantly ($p < 0.05$) reduced *C. jejuni* adhesion, invasion, and survival in human intestinal cells and downregulated genes associated with virulence factors, biofilm, and motility. Future studies will focus on evaluating the top two probiotics for their efficacy in reducing *C. jejuni* colonization in chickens.

Significance: These results will facilitate the development of NGPs as promising alternatives to antibiotics to treat *C. jejuni* infections.

Award Application: Developing Scientist Award

P1-56: A CLEAN-LABEL CASEIN FERMENTATE FOR MOLD PREVENTION IN YOGURT

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Mold growth is a common challenge in yogurt production. While synthetic preservatives are widely used, increasing consumer demand for natural, clean-label solutions has created a need for alternative preservatives. This study evaluates the efficacy of the clean label Natural Protectant-casein (NPc) as a natural antifungal preservative for yogurt.

Purpose: This study aims to validate NPc as a safe and natural solution for preventing mold growth in yogurt without compromising its probiotic value.

Methods: NPc was produced by fermenting a 20 g/L casein digest solution with *Aspergillus oryzae* (10^5 spores/mL) at 30°C and 220 rpm for six days. The culture was filtered, sterilized, and freeze-dried into powders. NPc was incorporated into yogurt at ~1.1% w/w, and samples were spiked with 1,000 spores of *Penicillium commune*, *Penicillium chrysogenum*, *Penicillium roqueforti*, and *Mucor circinelloides*. Both control and NPc-treated yogurts were stored at 4°C and monitored weekly for mold growth through visual inspection and plate-count experiments. Lactobacilli counts were also tracked to evaluate the probiotic value of NPc-treated yogurt.

Results: Mold growth became evident in control yogurt by week 2, with colonies spreading and sporulating over time. In contrast, NPc-treated yogurt exhibited no visible mold growth across all tested molds for five weeks, with no changes of mold colony numbers in NPc-treated yogurt ($p > 0.05$), indicating complete inhibition of mold germination and growth. Additionally, lactobacilli count in NPc-treated yogurt stayed above 8 log CFU/mL, showing that NPc addition did not compromise the probiotic quality of the yogurt.

Significance: NPc is a fermented casein using the GRAS fungus *A. oryzae*, making it a safe and effective clean-label mold preservative. NPc's ability to inhibit mold growth without affecting probiotic content highlights its potential as a valuable

additive for yogurt and other dairy products, aligning with consumer demand for natural and safe food solutions.

Award Application: Developing Scientist Award

P1-57: BACILLUS METABOLITES' ANTILISTERIAL POTENTIAL AGAINST LISTERIA MONOCYTOGENES WRLP42 WHEN CULTURED IN SWEET WHEY

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* contamination and growth in foods, particularly dairy products, remains a challenge for the industry. *Bacillus* spp. are a promising resource for secondary metabolites with antimicrobial activity that could be useful for food safety and preservation; however, they remain largely unexplored.

Purpose: Evaluate the antilisterial potential of *Bacillus* strains cultured in laboratory media and sweet whey.

Methods: *Bacillus* strains (n=6) were cultured in sweet whey and tryptic soy broth supplemented with yeast extract (TSBYE) at 37C for 36 h. At 12, 24, and 36 h, cultures were filter-sterilized (0.25 µm) to produce cell-free supernatant (CFS). The CFS was diluted in TSBYE and inoculated with *L. monocytogenes* WRLP42 (6 log CFU/mL) in a 96-well plate and incubated at 37C for 24 h with optical density (OD595) monitoring (FilterMax F5) at 5-min intervals. Lag phase, maximum OD, and growth rates were calculated using JMP Pro18 (logistic 4P).

Results: All TSBYE-CFSs of *B. subtilis* HD13 and *B. subtilis* Y487 prevented the growth of *L. monocytogenes* WRLP42. Sweet whey-CFSs of these same strains were less effective and did not result in a significant delay of growth of *L. monocytogenes* WRLP42 (Tukey-Kramer p >0.05). *B. spizizenii* ATCC 6633-CFS cultured in TSBYE produced a significantly reduced growth rate and increased lag phase of *L. monocytogenes* WRLP42 (Tukey-Kramer p <0.05).

Significance: *B. subtilis* and *B. spizizenii* antilisterial metabolite production is dependent on growth media. Optimization of their production in ingredient streams is needed to advance their application in food formulation.

P1-58: MATERIAL TYPE AND ACTIVE INGREDIENT COMBINATIONS IMPACT STAPHYLOCOCCUS AUREUS CROSS-CONTAMINATION RISK

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Optimizing wiping materials and cleaning products is crucial for limiting pathogen spread on food contact surfaces. Data on cross-contamination among different wipe-chemistry combinations remains limited.

Purpose: The purpose of this study was to evaluate the risk of cross-contamination from various wiping materials paired with different chemistries on surfaces that were not previously contaminated with *Staphylococcus aureus* (e.g., boards, gloves, and wipes).

Methods: Three wipe materials impregnated with water, quaternary ammonium, hydrogen peroxide, and ethylated alcohol were tested against *S. aureus* (ATCC 6538) on a Formica surface to determine bactericidal efficacy of disinfectants under conditions mimicking real-world use. The Formica boards were inoculated with approximately 7 log₁₀ CFU. Boards were dried for one h after inoculation then wiped for one meter before swabbing a 10x10 cm sampling zone (World Bioproducts LLC) after defined label contact time. Following the wiping procedure, the glove was imprinted on tryptic soy agar (TSA; Fisher Scientific) for five s. The plates were incubated for 24 h at 37°C to evaluate cross-contamination risks using different wiping materials and chemistries.

Results: All QAC and EA chemistries resulted in cross-contamination of the wipe, board, and glove. Nonwoven towelettes exhibited significantly higher bactericidal efficacy and transferred less *S. aureus* than other materials ($p < 0.05$), with no significant difference between QAC and EA chemistries in the inoculation zone ($p > 0.05$). Material type significantly impacted cross-contamination ($p = 0.0001$), with nonwoven towelettes transferring lower bacterial loads to the sampling zone ($p < 0.05$). EA and hydrogen peroxide products also reduced bacterial transfer to uncontaminated areas compared to QAC and water ($p < 0.05$).

Significance: The combination of products and wiping materials should inform sanitation standard operating procedures as improper use may elevate cross-contamination risks to the environment and user.

P1-59: GLUCOSE/LACTOSE-ANTIBODY CONJUGATES AS GROWTH INHIBITORS AGAINST *L. MONOCYTOGENES*

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The application of sugar oxidases as bacterial growth inhibitors through the release of hydrogen peroxide subsequent to sugar oxidation has limitations, including non-specificity and changes in sensory attributes. The objective of this study was to synthesize antibody-oxidase conjugates and assess their inhibition potential against target pathogens compared to unconjugated sugar oxidases.

Purpose: Lactose oxidase (LOx) and glucose oxidase (GOx) were conjugated to an antibody specific for *Listeria monocytogenes* (Ig) using biotin-streptavidin interaction and a conjugation kit, respectively.

Methods: The conjugates (GOx-IgG, LOx-IgG) were added to inocula of 2 log CFU/ml in 2.5% glucose/lactose-enriched PBS. Assays were performed in 96-well culture plates to test different treatment concentrations in serial dilutions from 60 μ L to 10^{-4} μ L. Solutions of unconjugated enzyme (LOx, GOx), unconjugated antibody (Ig), unconjugated enzyme and antibody (LOx+IgG, GOx+IgG), and PBS were applied as experimental controls. For the samples with LOx conjugates, streptavidin-conjugated LOx (S-LOx) was included as an additional control. All treatments were incubated at 37°C with agitation for 24 hours, and optical density readings were taken every 15 minutes to monitor microbial growth. Differences between control and conjugate treatments were analyzed using a mixed-effect model, and significant differences between pairs of treatments were assessed by a post hoc Tukey test ($p < 0.05$). All experiments were performed in biological triplicates.

Results: Treatments with both enzyme and antibodies showed greater inhibition potential than all other treatments. However, for LOx, LOx+IgG performed better than LOx-IgG conjugates at inhibiting 2-log CFU/ml *L. monocytogenes* at a concentration of $\leq 9.15 \times 10^{-4}$ μ L LOx. In contrast, for GOx, a dose of 7.5×10^{-3} μ L GOx-IgG was twice as effective as the unconjugated enzyme.

Significance: Conjugates of antibody-sugar oxidases represent a promising food safety tool that can potentially target specific food pathogens with reduced

secondary effects.

Award Application: Developing Scientist Award

P1-60: THE EFFECT OF NATURAL PRESERVATIVES AGAINST HEAT RESISTANT ORGANISMS IN APPLE JUICE AND ORANGE JUICE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Heat resistant organisms pose a spoilage risk that cannot be controlled by typical pasteurization processes. Artificial preservatives are an option; however, consumers have shown increased interest in natural products.

Purpose: To determine the efficacy of natural preservatives against *Alicyclobacillus* spp. (ACB) and heat resistant molds (HRM) in juice.

Methods: 100% Apple Juice and 100% orange juice were preserved with natural flavor (plant polyphenols and bioflavonoids at 0.5%, 1.0%, and 1.25%), X-TRA GUARD™ S (rowan berry extract at 1500 ppm and 2500 ppm), or Nagardo® (mushroom glycolipids at 5 ppm, 15 ppm, and 50 ppm). Each sample was inoculated with 102 CFU/ml heat shocked ACB spores and incubated at 45°C. The samples were plated on acidified PDA at day 0 and then weekly for 14 days. Additional preserved juice samples were inoculated with 102 CFU/ml heat shocked HRM ascospore cocktail (*Eurotium herbariorum*, *Byssosclamyces*, and *Neosartorya*) and incubated at 28°C. The samples were plated on acidified PDA at day 0 and then monitored for mold growth. All samples were tested in triplicate (natural flavor was only tested in orange juice).

Results: All natural preservatives were effective against ACB in apple juice at tested concentrations, demonstrating bactericidal or bacteriostatic effects compared to non-preserved control juice. The natural flavor and rowan berry extract were effective against ACB in orange juice at all tested concentrations and the mushroom glycolipids were effective at 15 ppm and 50 ppm. The natural flavor and rowan berry extract were effective against HRM in both apple and orange juice at all tested concentrations and the mushroom glycolipids were effective at 50 ppm when compared to the control, which showed visible signs of mold spoilage.

Significance: Preservatives derived from plant and microorganism sources may replace artificial preservatives to ensure product quality and safety while maintaining consumer-friendly labels. The effectiveness of these preservatives is dependent on the juice matrix and the organism(s) of concern.

P1-61: MULTIDRUG-RESISTANT ESCHERICHIA COLI ISOLATED FROM PASTURED BROILER FARMS IN THE SOUTHEASTERN UNITED STATES

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Interest in pasture-raised chickens has been increasing over the years. However, the pasture environment can be susceptible to contamination with antibiotic-resistant (AR) bacteria and antibiotic resistance genes (ARGs) from different sources.

Purpose: Here, we aimed to evaluate the spread of AR and ARGs in pasture broiler farms in the Southeastern USA.

Methods: Samples (feces, soil, ceca, and whole carcass rinses) were collected from 40 pastured broiler flocks across nine farms in the Southeastern USA. *Escherichia coli* isolates underwent phenotypic AR profiling against 30 antibiotics belonging to 12 classes using the Kirby-Bauer disk diffusion assay. In-depth whole-genome sequencing (WGS) was conducted on multidrug-resistant (MDR) *E. coli* (n=28) to evaluate the resistome, virulome, plasmidome, sequence types (STs), and pathogenicity. Bioinformatics analyses were conducted using tools available at the Center for Genomic Epidemiology. Conjugation assays were performed on select isolates to investigate the mobilization of plasmid-borne ARGs.

Results: Only 28 (5%) of the *E. coli* isolates from the farms were classified as MDR. These isolates exhibited resistance to penicillins, β -lactam and β -lactamase inhibitor combinations, cephalosporins, aminoglycosides, tetracyclines, folate pathway inhibitors, and nitrofurans. Multilocus sequence typing revealed 19 different STs. WGS identified 2–7 ARGs per isolate, including *tet*, *cmiA1*, *bla*_{TEM}, *aadA*, *sul*, *floR*, *aph(6)-Id*, *aph(3')-Ia*, *aph(3'')-Ib*, *dfrA*, and *aac(3)-Via*. Isolates harbored virulence genes and plasmid types (IncI2 α , IncFII, IncFIB) carrying ARGs such as *aph(6)-Id*, *aph(3'')-Ib*, *sul2*, *tet(A)*, and *bla*_{TEM}. Conjugation assays confirmed the mobilization of *bla*_{TEM}-carrying IncI2 α and IncX2 plasmids into naïve rifampicin-resistant *E. coli* and *Salmonella enterica* serovar Enteritidis.

Significance: The detection of transmissible ARGs in MDR *E. coli* highlights the

need to monitor and identify environmental drivers of antimicrobial resistance in pasture-raised poultry systems in the USA.

Award Application: Developing Scientist Award

P1-62: INHIBITION OF SPOILAGE BACTERIA IN DELI SALAD CULTURED CELERY JUICE (VEGSTABLE® SECURE)

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Ready-To-Eat (RTE) deli salads pose a risk for spoilage microorganisms due to their complex composition and high water activity. Traditionally, this has been controlled by the addition of organic acid salts into the formulation. Consumer interest in natural ingredients has made synthetic antimicrobials unattractive for labelling. Culturing vegetable juice to be high in antimicrobial acids presents a clean label alternative to synthetic ingredients.

Purpose: Determine the antimicrobial efficacy of cultured celery juice against spoilage organisms in a potato salad model at 10°C for up to 28 days.

Methods: Potato salad was formulated with 1-5% Cultured Celery Juice A and 2.5-3.8% Cultured Celery Juice B. The wild spoilage organisms were monitored. Salads were inoculated with a cocktail of lactic acid bacteria (LAB). The salads were stored at 10°C and the total aerobic plate count (APC), LAB, and fungal plate count was monitored for the expected shelf-life using individual packages in duplicate. The cell count was compared to a negative control containing no antimicrobial.

Results: Cultured Celery Juice A used at 1-5% showed a reduction in LAB growth rate, delaying the outgrowth by 7-14 days at 10°C. In an uninoculated study, potato salad formulated with 2.5 and 3.8% Cultured Celery Juice B prevented the outgrowth of spoilage bacteria, below log 7 for 28 days. Fungal growth above log 5, was prevented by Cultured Celery Juice B at 3.8% for the study duration of 28 days, and 2.5% usage delayed the growth for an additional 8 days compared to a negative control.

Significance: Cultured celery juice rich in organic acids as a novel antimicrobial ingredient used in RTE deli salads such as potato salad to control aerobic bacteria, lactic acid bacteria, and fungi.

P1-63: GENOTYPIC CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE OF ESCHERICHIA COLI FROM WILDLIFE FEEDLOTS AND RETAIL MEAT IN LARAMIE, WY

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Antimicrobial resistance (AMR) is a global health threat driven by environmental contamination with antibiotics, biocides, and their residues. Biocides, commonly used in agriculture and healthcare, exert selective pressure, fostering resistant bacteria. Wildlife exposed to these environments may act as reservoirs, introducing resistant bacteria into the food supply. Despite these concerns, the scale of this interconnected problem remains unclear. Therefore, we hypothesize that biocide-resistant isolates from environmental and food sources display similar resistance profiles due to cross-contamination between wildlife and food.

Purpose: This study aimed to investigate biocide-resistant *Escherichia coli* (*E. coli*) from wildlife feedlots and retail meat, as biocides contribute to resistance selection and may promote cross-contamination, driving AMR transmission. The goal was to identify shared and distinct resistance mechanisms, assess bacterial persistence, and evaluate the potential risks to foodborne AMR.

Methods: *E. coli* isolates (N=46 from retail meat and N=77 from wildlife feedlots) were obtained using the National Antimicrobial Resistance Monitoring System (NARMS) protocols. Biocide-resistant isolates (n=71) underwent whole-genome sequencing (WGS) for resistance profiling, including quality assessment with Trimmomatic, de novo assembly with SPAdes, and genome annotation via Prokka. Abricate was used to find resistant genes using databases such as NCBI, Virulence Finder, Plasmid Finder, and Resistance Finder databases.

Results: WGS identified a range of resistance genes (*tet(A)*, *tet(B)*, *sul1*, *sul2*, *blaCMY-2*) and plasmids (*Col156_1*, *incFIB*, *pENTAS02_1*) across both sources, suggesting overlapping selective pressures. Virulence factors such as *espR1*, *fim*, and *csg* were abundant, indicating pathogenic potential. Wildlife-specific genes (*cmlA1*, *aadA5*) highlighted unique adaptations, while retail meat isolates predominantly carried *blaCTX-M-55*.

Significance: This study emphasizes wildlife as a reservoir for diverse AMR genes and highlights the role of biocide-resistant bacteria in the transmission of AMR. Findings underscore the need for integrated monitoring systems to track AMR across environmental, wildlife, and food systems to mitigate AMR risks.

Award Application: Developing Scientist Award

P1-64: CHARACTERIZATION AND OPTIMIZATION OF HOMEMADE ELECTROLYZED WATER FOR SAFEGUARDING PRODUCE SAFETY AND QUALITY

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Electrolytically modified water, including electrolyzed water, is a practical, green alternative for improving food safety and quality by reducing microbial pathogens and spoilage contaminants. However, the effectiveness of homemade electrolyzed water (HEW) is experimentally under-explored.

Purpose: This study evaluated the antibacterial and preservative characteristics of HEW generated using commercial HEW devices and food-grade mixtures.

Methods: HEW was prepared using NaCl, vinegar, and tap water across four commercial generators. Concentration and pH were adjusted using iodized NaCl and vinegar. Antibacterial activity was assessed through agar diffusion, spraying, and immersion, followed by bacterial inoculation and colony/MPN enumeration. HEW preservability was evaluated via solution turbidity in tomato and plant health. Tomato quality was assessed using automated metrics: color (ΔE^*), hardness, pH, vitamin C, lycopene, β -carotene, flavonoids, and phenolics.

Results: HEW demonstrated limited antibacterial activity on bacterial lawn agar, but immersion treatment (>5 -log CFU/mL reduction) showed significantly better bacterial reduction than spraying (3-log CFU/mL) ($P < 0.05$). Slightly acidic HEW (pH 5.3) prepared with 23% NaCl and applied via immersion or spraying significantly enhanced antibacterial activity. Immersed tomatoes and sick plants showed preservability improvements. No significant differences ($P > 0.05$) were observed in tomato organoleptic or nutritional values post-treatment.

Significance: This study highlights the preservative potential and produce-friendliness of HEW while identifying key parameters—NaCl concentration, pH, generator type, shelf-life, and immersion method—that influence antibacterial effectiveness.

Award Application: Developing Scientist Award

P1-65: REPLACEMENT OF POTASSIUM SORBATE WITH NATURAL PRESERVATIVES IN BANANA FILLING: STABILITY ASSESSMENT DURING STORAGE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Synthetic chemical preservatives, such as potassium sorbate, are widely used in the food industry to prevent microbial spoilage, but are under increasing pressure due to consumer demand for "natural" and "chemical-free" products and environmental concerns. **Aim:** The aim of this study was to evaluate the stability of an industrially produced banana filling for the pastry industry where potassium sorbate was replaced by 'clean label' commercial preservatives during storage.

Methods: The banana filling was stored at 22 °C and 62% relative humidity, and five treatments were tested: (a) positive control with potassium sorbate, (b) negative control without preservatives, (c) addition of formulation A (1.13%), (d) addition of formulation B (1%), and (e) a combination of formulations A (0.38%) and B (1%). Formulation A contained oregano, rosemary, plum and linseed extracts, while formulation B contained red fruit extracts. Microbiological (total microorganisms, moulds, yeasts, aerobic and anaerobic spore forming bacilli), physical (aw, °Brix, colour, viscosity, shear force) and physicochemical (pH) analyses were performed throughout the storage period.

Results: No growth of total microorganisms, moulds, yeasts or spore germination was observed during storage. pH and aw values remained stable, while °Brix increased slightly. The banana fillings became more reddish and yellowish over time, indicating possible chemical changes affecting quality. The rheological evaluation showed a pseudoplastic behaviour with small variations.

Significance: The tested clean label preservatives are a promising alternative to potassium sorbate, but further optimisation is needed to address texture and colour issues and to ensure sensory attributes are maintained during storage. These findings contribute to the advancement of natural preservative strategies in the food industry, while meeting consumer and environmental demands.

P1-66: EMERGENCE OF AZOLE-RESISTANT YEASTS IN WINE GRAPE PRODUCTION

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The widespread use of fungicides such as azole-based compounds in fruit production may play a role in selecting for multi-drug resistance (MDR) of emergent human pathogens. Fluconazole-resistant *Candida* (notably *Candida auris*) has gained global attention due to risks for nosocomial transmission, though presence in food and agriculture systems remains largely unexplored

Purpose: Isolate and characterize azole-resistant *Candida* that may be present in vineyards with a history of intensive fungicide use

Methods: Grape clusters from Chardonnay, Cabernet Franc, and Chambourcin cultivars were collected across Maryland (n=4 vineyards) in the 2024 growing season. Grapes were juiced and fermented with musts shaking at 28°C for 3–5 days. Following CDC protocols for *C. auris* isolation from environmental samples, aliquots of fermented juice were incubated in Sabouraud Dextrose broth supplemented with fluconazole for 5–7 days, then plated on CHROMagar *Candida* and incubated for 2 days, both at 37°C. Isolates with morphologies resembling putative *Candida* species were processed for colony PCR with primers for a *C. auris* gene and subsequent DNA extraction and ITS amplicon sequencing

Results: All isolates (n=18) were negative for the *C. auris* PCR assay, though positive for the ITS gene, indicating alternative fluconazole-resistant fungi. ITS sequencing identified *Candida tropicalis*, relatives of *Candida* such as *Meyerozyma guilliermondii* (*Candida guilliermondii* anamorph) and *Pichia kudriavzevii* (formerly *Candida krusei*), and other yeast strains including members of *Hanseniaspora* and *Saccharomyces*

Significance: Agriculture operations are increasingly dependent on fungicide use, which has the potential to select for drug-resistant pathogens. We demonstrate that wine grapes are a unique model system to study emergent MDR *Candida* and related taxa as novel food safety risks

P1-67: EFFECT OF PH ON BEVERAGE PRESERVATION USING NATURAL ANTIFUNGAL SOLUTIONS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Beverages with high nutrient content, water activity (a_w), and sugar concentration are highly susceptible to yeast and mold contamination. The need for clean-label preservatives with antifungal properties is critical to extend the shelf life of these beverages.

Purpose: This study aims to evaluate the antifungal efficacy of natural flavor (Flavoset® 5400L) in beverage formulations.

Methods: Varying concentrations of Flavoset® 5400L (0–2.00%) were prepared in potato dextrose broth (PDB) and adjusted to pH 3.40 and 5.00 using hydrochloric acid. Samples were aliquoted into 100-well honeycomb plates and inoculated with yeasts (*Candida tropicalis*, *Zygosaccharomyces bailii*, *Dekkera bruxellensis*, *Brettanomyces naardensis*, and *Saccharomyces cerevisiae*) or molds previously isolated from various beverages (*Penicillium spinulosum*, *P. fellutanum*, *P. commune*, *Aspergillus niger*, *Byssoschlamys fulva*). Growth curves were generated using Bioscreen-C Pro at 30°C. Lag time (h) and maximum growth rate (μ_{max} ; 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 one-way ANOVA ($p \leq 0.05$).

Results: Flavoset® 5400L exhibited significant antifungal activity ($p \leq 0.05$) against all tested fungal strains at pH 3.40 compared to the no antimicrobial control. Specifically, the MIC for yeasts ranged from 0.25% (*S. cerevisiae*) to 0.75% (*C. tropicalis*). For molds at pH 3.40, the MIC ranged from 0.25% (*B. fulva*, *P. commune*) to 1.00% (*P. spinulosum*). At pH 5.00, significantly higher MICs were observed for all the yeast and mold strains (1.00% for *Z. bailii*, *S. cerevisiae*, *A. niger* and 2.00% for *C. tropicalis*, *P. commune*, and *P. spinulosum*).

Significance: The utilization of Flavoset® 5400L as an antifungal agent presents an effective natural preservative option for beverage systems. Besides, the higher

MIC required for beverages at pH 5.00 underscores the importance of tailored preservation strategies for different pH levels.

P1-68: EFFICACY OF NATURAL PLANT EXTRACT SYSTEM AGAINST SPOILAGE MICROORGANISM IN REFRIGERATED DOUGH

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Natural plant extracts qualify for clean label solutions and provide benefits such as flavor, shelf-life extension, and food safety impact.

Purpose: To assess the minimum inhibitory concentrations (MIC) against Aerobic Counts, lactic acid bacteria, and yeast for a clean-label solution such as the natural flavor Flavoset 5400L.

Methods: Samples of Flavoset 5400L were prepared at two concentrations (0.3% and 0.4%) in Brain Heart Infusion broth (BHI) at pH 7. Samples were aliquoted into 100-well plates then inoculated with a cocktail of seven different strains of APC, two strains of LAB, and three strains of yeast. Optical density (OD) was measured at 600 nm using Bioscreen C and incubated at 30°C. Growth curves were fitted using a modified Gompertz equation to determine the maximum growth rate (μ_{max} ; h^{-1}). The MIC was defined as the lowest concentration at which no growth occurred. Data were analyzed using one-way ANOVA ($P < 0.05$).

Results: Flavoset 5400L at a dosage of 0.3% exhibited complete inhibition against APC and LAB. For yeast, the 0.4% level was significantly ($p \leq 0.05$) effective in reducing the growth rate and increasing the lag time for all strains ($1.046 h^{-1}$, $1.142 d$) compared to the control ($1.54 h^{-1}$; $0.41 d$).

Significance: The study highlights the antimicrobial efficacy of a clean-label plant extract system against spoilage microorganisms and provides the industry with a natural ingredient.

P1-69: EXPLORING ANTIMICROBIAL RESISTANCE IN SMALL-SCALE FOOD ANIMAL FARMS ACROSS TENNESSEE, ALABAMA, AND GEORGIA

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Antimicrobial resistance (AMR) is a global health crisis driven by agricultural practices. While AMR research has primarily focused on large farms, small-scale farms have received little attention. Understanding AMR dynamics in various farming systems is key to reducing resistance and protecting public health.

Purpose: This study examined AMR spread in small-scale cattle, poultry, sheep, and goat farms across Tennessee, Alabama, and Georgia. It targeted urgent-threat bacteria such as *Escherichia coli*, *Klebsiella*, and *Enterococci*, and assessed on-farm antibiotic usage and management practices.

Methods: Researchers visited farms and collected samples of soil (n=216), water (n=111), manure (n=216), feces (n=137), and milk (n=81). Microbial analyses included selective media and biochemical testing (Kirby-Bauer method). A survey captured antibiotic use practices. GIS mapping visualized AMR patterns.

Results: Isolates from cattle and poultry farms exhibited multidrug resistance, especially to erythromycin (85%), nalidixic acid (78%), and ampicillin (37.3%). On goat and sheep farms, *S. aureus* (69%), *S. saprophyticus* (38%), and *E. coli* (36%) were common ($p < 0.05$), showing resistance to penicillin (81.1%), ampicillin (79.6%), and cephalothin (49.9%). Pathogenic bacteria—including *E. coli* O157:H7, *Shigella*, and *Salmonella*—were found in milk samples, with resistance to novobiocin, tetracycline, and erythromycin. Multidrug resistance indices ranged from 0.25–0.92, with a common pattern of ERY>AMP>NAL across the region. AMR levels were higher during warm seasons. Over 80% of respondents reported using antibiotics, primarily oxytetracycline and penicillin.

Significance: Small-scale farms are significant AMR reservoirs. Targeted antimicrobial stewardship education for small producers is vital to mitigate AMR spread in agricultural and environmental settings.

Award Application: Developing Scientist Award

P1-70: IN VITRO ACTIVITY OF CINNAMON BARK ESSENTIAL OIL AND LACTIC ACID COMBINATION AGAINST SALMONELLA

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: An array of essential oils have been shown to have antimicrobial activity against *Salmonella*, but they can impact organoleptic properties. The combination of acidic pH along with essential oils (EOs) could help improve antimicrobial efficacy with less impact on sensory properties.

Purpose: To evaluate the minimum inhibitory concentration (MIC) of *Salmonella* using cinnamon bark EO in tryptic soy broth (TSB) media at neutral pH 7.0 or acidified with lactic acid at pH 6.0 and 5.0.

Methods: *Salmonella* cocktail at stationary phase was prepared by combining equal portions of five different strains implicated in produce-related outbreaks. TSB was adjusted with lactic acid to pH 6.0 or 5.0 when mixed with EO or water. EO was dissolved in ethanol as a stock solution. The MIC was determined by microtiter plate assay. Each well contained 200 μ L total and consisted of 100 μ L double strength TSB, 20 μ L of log 6.0 CFU/mL inoculum, and the balance made of distilled deionized water and EO stock solution to achieve the desired EO concentration. Water and aqueous ethanol were used as positive controls. After incubation at 37 °C for 24 hours, the lowest antimicrobial concentration with optical density change at 630 nm below 0.05 was recorded as the MIC. Each experiment was carried out with two biological replicates and three technical replicates per biological replicate (n=6).

Results: The MIC obtained from the cinnamon bark EO was 0.04% (v/v) for pH 7.0, 0.01% (v/v) for pH 6.0, and 0.00188% (v/v) for pH 5.0, suggesting a significant impact on the antimicrobial efficacy of cinnamon bark EO and demonstrating the combination of multiple intrinsic factors (pH and antimicrobial) to achieve desired inactivation with less concentration of EOs.

Significance: Improvement in the in vitro antimicrobial efficacy by combining acidic pH and cinnamon bark EO demonstrates the potential for further exploring synergistic effects in food systems.

Award Application: Developing Scientist Award

P1-71: IDENTIFYING AND QUANTIFYING ANTIMICROBIAL RESISTANCE GENES (ARGS) IN WASTEWATER SOURCES FOR PUBLIC HEALTH

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The presence of Antimicrobial Resistance Genes (ARGs) is a constant public health concern, especially those found in wastewater as the environment provides ideal conditions for vertical and horizontal gene transfer in microbes. These genes and microbes can be a cause for concern in wastewater reuse and source water contamination.

Purpose: To identify and quantify the presence of ARGs at diverse wastewater sites.

Methods: from September-October 2024, wastewater samples were collected from a community site (CW; n=2), a hospital site (HW; n=2), and influent at two wastewater treatment plants (WW1; n=2, WW2; n=3) were chosen because of their diverse populations. 50 ml of wastewater was concentrated using centrifugation. 250 ul of concentrate was extracted using the DNeasy PowerSoil Pro Kit (Qiagen) to a final volume of 50 ul. Extracts were then diluted to a concentration of 1 ng/ul and screened for ARGs using quantitative PCR (qPCR), with a 96-well Microbial DNA qPCR Array containing pre-aliquoted primer-probe mix, corresponding to 90 different ARG targets and 6 controls. Select ARGs were then chosen from positive screening samples, encompassing a broad range of drug classes, to quantify using droplet digital PCR (ddPCR).

Results: 71 of the 90 ARG targets were positively detected in at least one of the sites tested. ARGs belonging to Aminoglycoside, β -lactamase, Erythromycin, Fluoroquinolone, Macrolide Lincosamide StreptoGramin B, Multidrug efflux pump, Tetracycline efflux pump, Vancomycin, and β -lactam classifications were found. Quantitative ddPCR

Results were obtained from the following genes (sample averages, in copies/100 ml): blaCTX-M-A/B (HW=4.15*10³, WW2=2.08*10⁴, CW=4.66*10⁵) IMP (WW2=7.323, CW=8.775), blaSHV (HW=3.33*10⁶, WW1=5.35*10², WW2=4.85*10³, CW=6.24*10⁵), KPC (HW=5.07*10³, WW1=7.89*10³,

WW2=6.45*10⁴, CW=2.19*10⁵), VIM (WW1=3.89*10⁶, WW2=6.34*10⁴, CW=5.31*10⁷), and OXA-48 (HW=5.76*10⁸, WW2=4.26*10⁴).

Significance: The results describe potential public health risks from ARGs in wastewater and provide a model for monitoring wastewater to screen for a wide variety of ARGs for further quantification.

P1-72: GENOMIC INSIGHTS INTO LISTERIA MONOCYTOGENES FROM FOOD PRODUCTS IN KOSOVO: A WHOLE-GENOME SEQUENCING STUDY

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Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* is a persistent foodborne pathogen that can contaminate food processing environments, posing serious risks to public health. Whole-genome sequencing (WGS) offers a high-resolution approach to investigate the pathogen's genetic variability, virulence factors, and resistance traits, thereby providing critical insights for targeted surveillance and control strategies.

Purpose: The primary objective of this study was to use WGS to examine the population structure, resistance determinants, and virulence potential of *L. monocytogenes* isolates derived from multiple food sources in Kosovo.

Methods: Between March 2016 and September 2022, a total of 117 isolates were obtained from food products, including hard cheese, sausages, and smoked meats. Genomic DNA was extracted from each isolate and sequenced on an Illumina platform. Sequencing read quality was assessed using FastQC (Version 0.12.0), trimmed with Trimmomatic (Version 0.36), and de novo assembled with SPAdes (Version 3.15.5). Genes encoding stress resistance, virulence factors, and antibiotic resistance were identified with BLASTN ($\geq 80\%$ coverage, $\geq 80\%$ identity) in the BIGSdb-Lm database. Multilocus sequence typing (MLST) was performed to determine sequence types. Genes associated with disinfectant/metal resistance (e.g., Tn6188_qac, cadA) and virulence factors (inlA, inlB, inlC, inlE) were also evaluated.

Results: Isolates displayed considerable genetic diversity, clustering into a range of sequence types and clonal complexes. Approximately 70% carried Tn6188_qac, indicating resistance to quaternary ammonium compounds, and around 20% contained cadA, associated with cadmium resistance. Over 90% of isolates possessed intact inl genes, suggesting a sustained pathogenic capacity.

Significance: This comprehensive WGS-based investigation underscores the diverse genetic landscape and resilience of *L. monocytogenes* in Kosovan food products. The findings highlight the importance of ongoing genomic surveillance

and the implementation of robust hygiene practices to minimize foodborne listeriosis threats and enhance overall food safety.

P1-73: ANTIMICROBIAL POTENTIAL OF BACILLUS MEGATERIUM CELL-FREE SUPERNATANT AGAINST LISTERIA AND SALMONELLA BIOFILMS IN HYDROPONIC SYSTEMS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The attachment and biofilm formation of *Listeria monocytogenes* in hydroponic systems resist standard cleaning methods, posing significant risks. Antimicrobial peptides from biofilm origin show promise as broad-spectrum agents against various bacterial pathogens, due to their ability to disrupt microbial membranes.

Purpose: This study investigated the broad-spectrum antimicrobial potential of *Bacillus megaterium* Cell-free supernatant (CFS) by targeting against Gram-negative (*Salmonella enterica* ATCC 14028, *Salmonella* Tennessee, *E. coli* O157:H7 H1730) and Gram-positive (*Listeria monocytogenes*: Scott A, V7, M101, LCDC) bacteria and examined its effect on *Listeria* and *Salmonella* biofilm on various hydroponic contact surfaces.

Methods: Broad spectrum efficacy of *B. megaterium* CFS previously isolated from biofilm microflora was assessed through an agar well assay. For biofilm testing, 1 cm² coupons of PVC and vinyl were inoculated with *L. monocytogenes* (5.87 log CFU/cm²) and *S. enterica* (5.32 log CFU/cm²). After 5 days of incubation, coupons were treated with CFS (223 mg/mL) for 5 min.

Results: Both Gram-positive and Gram-negative strains of bacteria showed antimicrobial susceptibility to *B. megaterium* CFS with a strong inhibition. *Salmonella enterica* exhibited greater inhibition (16±0.04 mm) as compared to *Salmonella* Tennessee (12±0.10 mm) and *E. coli* O157:H7 (11.5±0.08 mm). *Listeria monocytogenes* Scott A showed higher inhibition (17±0.09 mm) compared to other outbreak strains. For the biofilm study, a significant (p<0.05) reduction in *Listeria monocytogenes* and *Salmonella enterica* was observed on PVC and vinyl surfaces, with reductions of 2.15±0.08 and 1.99±0.16 log CFU/cm² respectively. Also, *B. megaterium* CFS treatment significantly (p<0.05) reduced *S. Enterica* biofilms by 2.75±0.11 and 2.82±0.09 log CFU/cm² on PVC and vinyl surfaces, respectively.

Significance: These findings highlight the efficacy of *B. megaterium* CFS against a

broad spectrum of environmental pathogens, suggesting its potential as a disinfection technique for mitigating biofilm formation and microbial risks in hydroponic systems.

Award Application: Developing Scientist Award

P1-74: SYNERGISTIC EFFECT OF CLOVE & CINNAMON AGAINST ASPERGILLUS FLAVUS IN GEORGIA PEANUTS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Aspergillus flavus* poses significant economic and health risks in peanuts due to contamination by aflatoxin B1 (AFB1).

Purpose: This study examines the synergistic effects of Clove and Cinnamon Essential Oils (EOs) as biocontrol agents against *A. flavus* and the production of AFB1 in Georgia peanuts.

Methods: *A. flavus* isolated from peanuts was cultured on Potato Dextrose Agar and incubated at 25°C for 6 days. The synergistic activity of the two EOs was tested at 250, 500, 1000, and 2000 ppm (Pinto et al. 2009). Ten µL of 1mL *A. flavus* suspension was inoculated at center of 30 Rose Bengal agar plates. After 5 days of incubation, various concentrations of Clove EO were micro-pipetted at center of 10 of the plates. The experiment was repeated with Cinnamon EO in another set of 10 plates. A third set of 10 plates was inoculated with combined EOs. Tween 20 (0.05%) served as -ve and fungicide as +ve controls. Experiments were replicated thrice and incubated similarly. After incubation, the Minimal Fungicidal Concentration (MIC) and Maximum Fungicidal Concentration (MFC) of the EOs were determined by measuring fungal colony diameters. AFB1 quantification was done by Ion Monitoring (SIM) based Liquid Chromatography and Mass Spectrometry (LCMS) using Orbitrap Exploris™ 240. AFB1 standard was used. A t-test was performed to assess statistical significance.

Results: MIC indicated reduced fungal growth with increasing concentrations of Clove and Cinnamon oils. The reduction was significantly greater when they were combined, especially at 2500 ppm. Similarly, quantification indicated that AFB1 reduction was significantly higher at 2500 ppm when both EOs were combined.

Significance: Our findings prove that Cinnamon and Clove EOs have a synergistic antifungal effect against *A. flavus* and have potential when combined as a biocontrol agent against *A. flavus* in Georgia peanuts. Future studies will focus on the synergistic effect of these EOs against contaminated peanuts.

P1-75: SURVEILLANCE OF SALMONELLA ENTERICA IN RETAIL MEAT REVEALS A MEGAPLASMID IN SALMONELLA PANAMA WITH A NOVEL MULTIDRUG RESISTANCE PROFILE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Megaplasms drive the global expansion of multidrug-resistant (MDR) *Salmonella*, as seen in pESI-like megaplasms in *S. infantis* and *S. Muenchen* and pESM in *S. Minnesota*. Their high genomic plasticity is demonstrated by the acquisition of *bla*CTX-M-65 (American pESI-like strains) and *bla*CTX-M-1 (European pESI-like strains). Identifying and characterizing these mobile elements is crucial for understanding the emergence of uncommon *Salmonella* serovars.

Purpose: To investigate the presence of megaplasms in *Salmonella* spp. isolated from retail meat in Chile and analyze their genomic features and phylogenetic relationships.

Methods: We conducted a six-month surveillance study across 100 retailers in Santiago, Chile. Chicken, turkey, and pork samples were collected, and *Salmonella* was isolated using standard methods. Presumptive colonies were confirmed via *invA*-PCR, and serogroups were determined by multiplex PCR. Whole-genome sequencing was performed using the Illumina NextSeq 500 platform. Plasmids were reconstructed with *mob-suite* and compared with the PLSDDB database to assess relationships with known mobile genetic elements.

Results: Approximately 200 *Salmonella* isolates were recovered, primarily from chicken. Notably, one *S. enterica* serovar Panama (ST48) isolate from a pork sample carried *SPI-1-5,9,13-14* and *C63PI* and harbored *cdtB* (cytolethal distending toxin). It also contained a 279.9 kb *incHI2* conjugative plasmid carrying *sul1*, *floR*, *bla*TEM-1, *dfrA12*, *aadA2*, *aph(3'')-Ib*, *aph(6)-Id*, *tet(B)*, *qacEΔ1*. Similar

plasmids were identified in database strains of serovars Johannesburg, Saintpaul, Worthington, Muenster, Anatum, and Panama. However, the Chilean megaplasmid displayed a unique resistance profile, suggesting a local adaptation event.

Significance: This study reveals a novel megaplasmid spanning multiple *Salmonella* serovars, indicating a broader host range than pESI- or pESM-like megaplasmids. The distinct resistance gene profile of the Chilean isolate underscores the genomic plasticity of these elements and highlights the need for continued surveillance to track their evolution.

Award Application: Developing Scientist Award

P1-76: EFFECT OF ENVIRONMENTAL STRESSES ON *SALMONELLA ENTERICA*'S RESPONSE TO PLANT-BASED ANTIMICROBIAL COMPOUNDS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Salmonella* is known for its ability to withstand a broad range of environmental stresses, potentially contributing to its resistance to antimicrobial treatments. These adaptive responses, especially under stress conditions, pose challenges to ensuring food safety.

Purpose: This study investigates the effects of carvacrol, thymol, and curcumin on the growth and survival kinetics of *Salmonella enterica* under various environmental stresses.

Methods: Pure cultures of *S. enterica* were pre-exposed to environmental stress conditions: (i) high salt (2.5% and 5%), (ii) low pH (5.5 and 6), (iii) low temperature (4°C), and (iv) nutrient-rich, normal growth (37°C) conditions. The pre-conditioned cells, adjusted to an initial inoculum concentration of 10^{5-6} CFU/mL, were treated with carvacrol (0.1 to 25 mM), thymol (0.1 to 25 mM), and curcumin (0.25 to 5 mM) at a 1:5 (v/v) ratio in a 96-well plate. Growth, death, or survival kinetics were monitored by measuring optical density at 590 nm over a 24 h period at 1 h intervals using a plate reader. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for each condition. Mechanistic studies, including cell permeability and reactive oxygen species (ROS) assays, were also performed. All experiments were conducted in triplicate, and the data were analyzed using ANOVA in SPSS™.

Results: MIC values of carvacrol, thymol, and curcumin were determined to be 5, 2.5, and 5 mM, respectively. Survival kinetics varied significantly between stressed and non-stressed cells depending on antimicrobial treatment. For example, salt-stressed cells of *S. enterica* exhibited greater resistance to curcumin and carvacrol treatments compared to cells grown under low pH, normal growth, and low temperature conditions. In contrast, thymol treatment showed the opposite trend, with the least resistance observed in salt-stressed cells.

Significance: The findings provide valuable insights into the use of environmentally stressed organisms in challenge studies and the development of

effective antimicrobial strategies.

P1-77: ANTIMICROBIAL EFFICACY OF ELECTROSTATICALLY APPLIED PROPIONIC ACID ALONE OR IN COMBINATION WITH LACTIC ACID AGAINST *E. COLI* O157:H7 ON FRESH BELL PEPPERS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Contamination of fresh produce with enterohemorrhagic *E. coli* O157:H7 presents a persistent challenge in food safety. Generally Recognized as Safe (GRAS) organic acids like propionic acid (PA) and lactic acid (LA) offer promising natural antimicrobial solutions, combining pathogen control effectiveness with minimal processing compatibility.

Purpose: This study aimed to evaluate the effectiveness of PA alone or in combination with LA against *E. coli* O157:H7 on bell peppers under two scenarios: bacteria under different attachment periods, and sustained antimicrobial activity over extended periods.

Methods: Green bell pepper samples (5 g) were surface inoculated with 100µl of three-strain cocktail of GFP-tagged ampicillin-resistant *E. coli* O157:H7 (F4546, ATCC4395, and H1730) to ~8 log CFU/g. Samples were treated using an electrostatic sprayer gun for 30 s with PA (0.5, 1, 2, and 5% v/v) alone or combined with LA, followed by a 5-min drying period. Two intervention approaches were investigated: treatment after different bacterial attachment periods (1h, 24h, 48h), and sustained antimicrobial effect over extended periods (1h, 24h, and 48h). Data were statistically analyzed with one-way ANOVA and post-hoc Tukey test.

Results: in the first approach, statistically significant differences ($p < 0.05$) were observed among different concentrations at all time periods. The 5% (PA:LA) combination significantly reduced bacterial load to 3.42 ± 0.35 , 4.36 ± 0.66 , and 3.32 ± 0.41 log CFU/g at 1h, 24h, and 48h, respectively. in the second approach, no statistically significant differences ($p > 0.05$) were observed for extended antimicrobial treatment, but the higher log reduction was observed for 5% PA: LA at 4.74 ± 0.08 , 5.30 ± 0.02 and 5.77 ± 0.06 log CFU/g at 1h, 24h, and 48 h respectively.

Significance: This study offers insightful information for improving antimicrobial treatments emphasizing early intervention in pathogens for microbial safety of fresh produce.

P1-78: EFFICACY OF SILVER NANOPARTICLES AS A WASHING SOLUTION TO REDUCE *E. COLI* O157:H7 ON THE SURFACE OF SWEET POTATOES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Silver nanoparticles (AgNPs) have emerged as a promising alternative to traditional sanitizing agents. Sweet potatoes, being highly susceptible to microbial contamination and spoilage, particularly benefit from AgNPs' advanced antimicrobial properties

Purpose: This study evaluated the efficacy of silver nanoparticles as a washing solution to reduce the presence of *E. coli* O157:H7 on sweet potatoes

Methods: Sweet potatoes, with and without soil, were spot inoculated with 200 μ L of GFP-tagged ampicillin-resistant *E. coli* O157:H7 (8 log CFU/mL) and dried inside a biosafety cabinet for 30 minutes. Sweet potatoes (both contaminated and non-contaminated) were washed with AgNP solution at concentrations (5, 10, 15 ppm) for 5 and 10 minutes. *E. coli* O157:H7 counts were enumerated using TSA plates with ampicillin (50 μ g/mL). One-way and two-way ANOVA tests with Tukey's HSD were used for statistical analysis

Results: The efficacy of silver nanoparticles against *E. coli* O157:H7 was significantly affected by the presence of soil on the sweet potato. At all concentrations, clean sweet potatoes showed >1.2 log CFU/cm² reduction compared to 0.6–1.2 log CFU/cm² for soil-covered samples. Concentrations of 10 and 15 ppm yielded significantly higher reductions (>1.73 log CFU/cm²) on clean samples. Increasing treatment time did not significantly ($P>0.05$) reduce *E. coli* O157:H7 levels, with reductions of 1.19 log CFU/cm² and 1.23 log CFU/cm² at 5 and 10 minutes, respectively. Cross-contaminated samples showed significantly lower counts, often below the detection limit (<0.04 log CFU/cm²)

Significance: Silver nanoparticles were effective in reducing *E. coli* O157:H7 from sweet potatoes. Concentration and organic matter presence significantly influenced AgNP antimicrobial effectiveness

Award Application: Developing Scientist Award

P1-79: EVALUATING POLYPHENOLS AS ANTIMICROBIAL AGENTS TO REDUCE MULTI-DRUG RESISTANT SALMONELLA ON CHICKEN MEAT SURFACES

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: The rise of antimicrobial-resistant foodborne pathogens underscores the urgent need for innovative mitigation strategies in food systems. Poultry remains a significant vector for *Salmonella* outbreaks, necessitating effective pre- and post-harvest interventions.

Purpose: This study explores the effects of polyphenols—4-hydroxycinnamic acid and itaconic acid—in reducing *Salmonella enterica* serovar Typhimurium DT104 (DT104) contamination on chicken meat surfaces under both pre- and post-inoculation conditions.

Methods: DT104, a multidrug-resistant strain resistant to five antibiotics, was selected as the model organism. Minimum inhibitory concentrations (MICs) of the polyphenols were initially determined using a standard 96-well plate microdilution protocol. UV-sterilized chicken tenders (1 g each) were inoculated with DT104 (10^5 CFU/mL) and treated with polyphenols either individually or in combination. Pre-inoculation treatments applied polyphenols before bacterial exposure, while post-inoculation treatments targeted bacteria attached to the meat. Samples were incubated at 10°C, 30°C, and 37°C, and bacterial counts were recorded at 2-hour intervals over 14 hours using XLD agar.

Results: Results indicated a significant reduction in DT104 growth in polyphenol-treated samples compared to controls. Importantly, although individual polyphenols showed no anti-DT104 effect in MIC analysis, significant antimicrobial effects were observed in chicken samples. This could be due to the hurdle principle, where meat creates stress environments for DT104 in the presence of polyphenols. On the chicken samples, individually, 4-hydroxycinnamic acid and

itaconic acid resulted in 5- to 75-fold reductions. Combined treatments achieved synergistic effects, with reductions up to 3,000-fold at 37°C and 1,000-fold at 10°C.

Significance: Polyphenols demonstrate substantial potential as antimicrobial agents to reduce *Salmonella* contamination on chicken meat. Their application, alone or synergistically with existing interventions like peroxyacetic acid, could offer a promising strategy to mitigate antimicrobial-resistant *Salmonella* in poultry processing systems.

Award Application: Developing Scientist Award

P1-80: ANTIMICROBIAL SUSCEPTIBILITY OF MICROBIOTA ASSOCIATED WITH SPINACIA OLERACEA VAR. CAPITATA AND BRASSICA OLERACEA L. FROM FARMS AND RETAILS.

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Leafy green vegetables are a highly variable group of perishable food that broadly can be defined as vegetables grown for their edible leaves. The emergence of various antibiotic-resistant bacteria particularly in the food chain has become a major area of concern.

Purpose: This study aimed to evaluate the antimicrobial susceptibility of foodborne pathogens isolated from leafy greens at the farms and retails.

Methods: Sixteen isolates were analysed for antimicrobial susceptibility test against various antibiotic agent. Samples were streaked onto Mueller hinton agar plates and incubated at 37°C for 24h. Target organisms were *E. coli*, coagulase-positive *Staphylococci*, *Listeria* and *Bacillus*. Using Kirby Bauer disc diffusion technique the isolates were subjected to a panel of nine antimicrobial agents: penicillin (P) (10_μg), ampicillin (AMP) (10_μg), gentamicin (CN) (10_μg), ceftazidime (CAZ) (30_μg), chloramphenicol (C) (30_μg), tetracycline (TE) (30_μg), vancomycin (VA) (30_μg), erythromycin (E) (15_μg) and ciprofloxacin (CIP) (5_μg).

Results: Generally, thirteen isolates (13/16; 81.25%) were susceptible to vancomycin and only three isolates (3/16; 19%) were resistant. Twelve isolates (12/16; 75%) were susceptible to gentamycin and four (4/16; 25%) were resistant. Ten isolates (10/16; 63%) were susceptible to chloramphenicol and tetracycline, four isolates were resistant (4/16; 25%) and two isolates were intermediate (2/16; 13%).

Significance: Leafy greens at the farm and retail level in South Africa may assume an important role in the maintenance and dissemination of antimicrobial resistant pathogens.

P1-81: ANTIMICROBIAL RESISTANCE PROFILES, VIRULENCE FACTORS, AND RESISTANCE GENES OF ESCHERICHIA COLI IN FRESH VEGETABLES FROM SELECTED TRADITIONAL MARKETS IN SOUTH WEST NIGERIA

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Traditional markets in Africa and low-income countries are major vegetable suppliers, yet they can harbor antimicrobial-resistant pathogens, raising food safety concerns.

Purpose: This study investigated the resistance profile, virulence and AMR genes of *Escherichia coli* in fresh vegetables from selected traditional markets in South West Nigeria.

Methods: A total of 509 vegetable samples—tomatoes, carrots, and cucumbers were collected in a sterile sampling container during three visits (March–October 2023), covering dry and rainy seasons, from nine markets across Ogun State's senatorial districts. The samples were transported to the laboratory under low temperatures using an iced bag. *Escherichia coli* was isolated, and its resistance profile and associated genes were assessed.

Results: Of the samples, 73 (14.4%) tested positive for *E. coli*: 34 (14.4%) from tomatoes, 27 (24.1%) from carrots and 12 (7.5%) from cucumbers. Carrots in Ogun Central showed the highest contamination (9.8%), while cucumbers in Ogun East had the lowest (1.3%). Among 34 *Escherichia coli* isolates, 12 originated from Ogun Central, 9 from Ogun East, and 13 from Ogun West. Isolates showed resistance to all antibiotics except gentamicin and levofloxacin. Virulence genes *stx1* and *stx2* were detected in 3 and 2 isolates, respectively. Dry season contamination was observed compared to the rainy season. Also, 47% of isolates carried resistance genes and 15% carried virulence genes, indicating low pathogenic potential.

P1-82: CHARACTERISTICS OF HISPANIC-STYLE CHEESE THAT INFLUENCE GROWTH OF LISTERIA MONOCYTOGENES IN QUESO FRESCO AND QUESO COTIJA

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Hispanic-style cheeses (HSCs) are a diverse category of cheese, including fresh (Queso Fresco; QF) and ripened (Queso Cotija; QC) varieties. Recent recalls of HSCs associated with *Listeria monocytogenes* contamination raise concerns regarding the microbial safety of this cheese category.

Purpose: Compare the behavior of *L. monocytogenes* in commercial QF and QC during storage to identify product characteristics that may prevent growth.

Method: HSCs (n=40; 20 QF and 20 QC), from five commercial manufacturers per cheese type were purchased at retail. Cheeses (20 g portions) were inoculated in duplicate (two cheeses/production lot) with a 5-strain *L. monocytogenes* cocktail (QF 3 log CFU/g; QC 5 log CFU/g), vacuum sealed, and stored at 7°C for 28 days. *L. monocytogenes* was enumerated every 4 days (Harlequin Listeria agar; 37°C, 48 h). Each experiment was completed twice to represent biological independence. Microbial (standard plate count [SPC; TSAYE]; lactic acid bacteria [LAB; MRS]; *E. coli*/coliforms and yeast and mold [3M Petrifilm]) and compositional (pH, water activity [aw], moisture content, NaCl) analyses were performed on uninoculated samples.

Results: QFs varied in pH (6.06-6.46), aw (0.95-0.98), and NaCl (1.02-3.01%). Most QFs (80%; 16/20) supported *L. monocytogenes* growth (≥ 1 log CFU/g increase), except QFs from one manufacturer (-0.43–0.84 Δ log CFU/g). None of the QCs supported *L. monocytogenes* growth at observed pH (4.91-5.63), aw (0.90-0.94), and NaCl (1.72-5.39%). one QC showed *L. monocytogenes* die-off (-1.30 Δ log CFU/g). Differences in *L. monocytogenes* behavior between the two cheese types were explained by pH and aw. in QF, NaCl levels were inversely correlated with *L. monocytogenes* growth ($p < 0.05$; multiple linear regression).

Significance: For QF, *L. monocytogenes* growth was inconsistent, likely due to

varying salt concentrations, whereas QC did not support *L. monocytogenes* growth. These findings inform strategies to improve the safety of HSCs.

Award Application: Developing Scientist Award

P1-83: ORGANIC ACID INHIBITION OF LISTERIA MONOCYTOGENES GROWTH IN MODEL HISPANIC-STYLE CHEESES

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Recent *Listeria monocytogenes* outbreaks in Hispanic-style cheese (HSC) highlight the need for intervention methods, with previous research showing lactic (LA; 40 mM) and propionic acid (PA; 25 mM) as potential inhibitors based on their minimal inhibitory concentrations in broth (BHI; 37°C, 48 h).

Objective: Evaluate the effectiveness of acidification supplemented with organic acids in a model HSC to prevent *L. monocytogenes* growth.

Methods: A model HSC, based on queso fresco, was prepared by adding whole milk (3.8 L), vinegar (60 mL; 5% acidity), and cheese salt (8 g) to a slow cooker. For organic acid treatments, LA (40 mM) or PA (25 mM) was added. The mixture was heated to 37°C, held for 5 min, then coagulated with vegetarian rennet for 35 min. Curds were formed (4.4 × 5 cm) and pressed (60 g) for 3 h. Resulting mini cheeses (20 g; n=4) were surface-inoculated with a five-strain *L. monocytogenes* cocktail (5 log CFU/g), vacuum sealed, and stored at 7°C for up to 20 days. Every four days, cheeses were enumerated for *L. monocytogenes* on Harlequin Listeria selective agar (37°C, up to 48 h). Experiments were repeated twice.

Results: The pH of the cheeses varied significantly by acid addition compared to the control ($p < 0.05$; one-way ANOVA with Tukey's HSD), with ranges of 5.33–5.65 for LA, 5.64–5.75 for PA, and 6.03–6.43 for the control. Control cheeses supported the growth of *L. monocytogenes* (~1.81 log CFU/g increase over 20 days), while both treatments significantly inhibited growth ($p < 0.05$; two-sided Welch's t-test) over 20 days (LA ave. 0.19 log CFU/g; PA ave. 0.22 log CFU/g decrease).

Significance: This proof-of-concept study demonstrated the effectiveness of organic acid supplementation to reduce *L. monocytogenes* growth in a model HSC. Future efforts will determine efficacy at larger scale in a queso fresco cheese and evaluate changes in textural and/or sensory properties.

P1-84: RAPID DETECTION OF AFLATOXIN M1 IN GOAT MILK PRODUCTS BASED ON ELISA METHOD

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Goat milk has high nutritional content and low allergenicity. In China, the market share of goat milk products is relatively small, but their market potential is much higher than that of cow milk. It should be noted that goat dairy products are susceptible to aflatoxin M1 contamination. Enzyme-linked immunosorbent assay (ELISA) is a fast and reliable method for mycotoxin detection. Thus, it's necessary to investigate the feasibility of ELISA for the detection of aflatoxin M1 in goat milk products.

Purpose: To verify the applicability of ELISA method in goat milk products for rapid determination of aflatoxin M1.

Methods: Two types of goat dairy samples, liquid milk and milk powder, were used for analysis. The aflatoxin M1 negative samples were spiked with three different concentration levels. All samples were pre-treated and tested according to the manual of Veratox® for Aflatoxin M1 Kit. The recovery rate and coefficient of variation were calculated to evaluate the suitability of the kit for goat milk products. Control samples were also tested by HPLC method and the relative deviations between them were compared.

Results: The recovery rates of aflatoxin M1 in all tested goat milk samples were within the range of 60%–120%, which were consistent with the requirements of GB Standard 5009.24-2016 that should be within the range of 50% to 120%. The coefficient of variation within each batch of sample was less than 20%, which was also consistent with the requirements of GB/T Standard 33411-2016. The relative deviation between the control samples and HPLC results were within 20%, which met the internal control standards of the laboratory.

Significance: The Veratox for Aflatoxin M1 ELISA Kit meets the rapid detection requirements for goat milk products, which is helpful for quality control in dairy industries in China.

P1-85: PERFORMANCE EVALUATION OF GLIADIN R5 ELISA KIT IN PROCESSED SNACKS

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: Gliadin, an alcohol-soluble protein that belongs to gluten, has been identified as a major causative factor in several disorders, including wheat allergy and gluten intolerance (celiac disease). Sandwich ELISA based on R5 monoclonal antibody is a CODEX-approved assay for the detection of gliadin in wheat, rye, and barley. Therefore, the feasibility of ELISA kits in processed foods also needs to be validated.

Purpose: Performance evaluation of Sandwich ELISA kits on gliadin in processed snacks containing gluten.

Methods: Three different flavors of gluten-free baked rice crackers and three processed snack control samples with different gliadin contents were used for analysis. Gluten-free samples were spiked with three levels using gliadin standard solution. The gliadin in all samples was first incubated with the renaturing cocktail solutions, then extracted with 80% ethanol, and the supernatant was collected after centrifugation. All samples were tested according to the manual of the Veratox® Gliadin R5 ELISA kit. The actual detection values of the snack control samples were used to assess the accuracy of the ELISA kit. The recovery rates and coefficient of variation of gluten-free samples were calculated to evaluate the suitability of the kit for processed foods.

Results: For control A, B, and C with three different gliadin contents, the testing results were all within the target ranges shown in CoA documents. The recovery rates of gliadin in rice crackers were in the range of 90%–110%, which was consistent with the requirements of GB/T Standard 27404-2008. The coefficient of variation within each batch of all samples was less than 20%, which was also consistent with the requirements of GB/T Standard 33411-2016.

Significance: The Gliadin R5 ELISA kits meet the rapid detection requirements for gluten components in processed products, providing a valuable tool for allergen control during food manufacturing.

P1-86: IMPACT OF CLEAN LABEL SOLUTIONS IN CONTROLLING LISTERIA MONOCYTOGENES AND SPOILAGE MICROORGANISMS IN CHEESE DIP

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Recalls and outbreaks linked to *Listeria monocytogenes* in soft cheeses have been recognized. These outbreaks and recalls highlight the need to reduce the risks associated during soft cheese making process and the implementation of improved food safety measures.

Purpose: Reducing food safety risks due to outgrowth of *Listeria monocytogenes* in cheese dip by incorporating a clean-label preservative.

Methods: Cheese dip was manufactured and treated with 0.25% IsoAge DV100 (dry vinegar), 0.55% DF5001 (cultured whey), 1.10% DF5015 (cultured skim milk), and no preservative (control). Samples were inoculated with a five-strain cocktail of *L. monocytogenes* (serotype 1/2a, 1/2b, 4a, and 4b) at approximately 3 log CFU/g, modified atmosphere packaged, and stored for 120 days at 7°C. Samples (duplicates) were removed from storage, homogenized, and plated onto Modified Oxford for *Listeria*, Tryptic Soy Agar (TSA) for APC, de Man Rogosa and Sharpe Agar (MRS) for LAB, and Potato Dextrose Agar (PDA) for yeast/mold. Differences among treatments were determined using one-way ANOVA at $p < 0.05$.

Results: No outgrowth of *Listeria monocytogenes* was observed in the treatments (0.25% IsoAge DV100, 0.55% DF5001, 1.10% DF5015) and control; however, better control of *Listeria* was observed in treatments than in control. Addition of clean-label solutions increased the lactic acid bacteria in the samples over time. Yeast count in control samples reached the spoilage limit (>6 log CFU/g) at day 30; however, yeast counts in samples with 0.25% IsoAge DV100, 0.55% DF5001, and 1.10% DF5015 did not show spoilage for more than 120 days.

Significance: The results of this study demonstrate the possibility to use clean-label preservatives to increase shelf life and reduce food safety risks of cheese

dip.

P1-87: CONTROL OF SPOILAGE MICROORGANISMS IN COTTAGE CHEESE USING FERMENTATE SYSTEM

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Cottage cheese is a nutrient-rich substrate with a pH of around 5.2. Therefore, it is susceptible to microbial spoilage. Fungi and yeast are the common causes of spoilage in cottage cheese. Increasing consumer demand for clean-label products necessitates the use of natural antimicrobials in cheese.

Purpose: This study aimed to investigate the growth inhibitory effect of clean-label antimicrobials against yeast and mold in cottage cheese over a period of 45 days.

Methods: Cottage cheese was prepared by incorporating 0.10% cultured skim milk (DuraFresh 5015) and 0.15% commercial market sample. Samples were refrigerated at 4°C and analyzed in duplicate up to 45 days for each variable. The cottage cheese was evaluated for aerobic plate count (APC), lactic acid bacteria (LAB), and yeast and mold at days 0, 22, and 45. Dilutions were plated onto Tryptic Soy Agar (TSA) for APC, deMan Rogosa and Sharpe Agar (MRS) for LAB, and Potato Dextrose Agar (PDA) for yeast/mold. Data were analyzed using one-way ANOVA ($P < 0.05$).

Results: No visual mold was observed in either the commercial sample or the sample formulated with 0.10% cultured skim milk during the 45-day shelf life at 4°C. Yeast remained below the detection limit ($<1 \log \text{CFU/g}$) in samples formulated with 0.10% cultured skim milk, and lactic acid bacteria were not affected during the shelf-life study. However, yeast growth in samples containing the commercial market sample exceeded $2 \log \text{CFU/g}$ by day 22 and continued to increase through day 45.

Significance: The cultured skim milk treatment is substantially more effective in controlling yeast compared to the commercial market sample over a period of 45 days.

P1-88: LISTERIA CONTROL AND CLEAN LABEL INTERVENTIONS IN HISPANIC CHEESE

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Hispanic-style cheeses account for a significant portion of listeriosis outbreaks. Hispanic cheeses, such as Queso Fresco present a unique environment for microbial growth due to their moderate salt levels, moisture, and relatively neutral pH. These factors provide an ideal platform for the growth of pathogenic organisms like *Listeria*, posing significant food safety risks.

Purpose: Evaluate the efficacy of clean-label interventions in controlling *Listeria monocytogenes* and enhancing the safety and shelf-life of Queso Fresco.

Methods: Queso fresco was manufactured and treated with 0.8% IsoAge DV100, 1% IsoAge DV100, and no preservative (control). Samples were inoculated with five-strain cocktail of *L. monocytogenes* (serotype 1/2a, 1/2b, 4a and 4b) at ca. 3 log CFU/g, vacuum-packaged, and stored for 60 days at 4°C. Sampling (duplicates) were removed from storage, homogenized, and plated onto Modified Oxford medium for enumeration. Differences among treatments were determined using one-way ANOVA at $p < 0.05$.

Results: For *L. monocytogenes*, the control treatment exhibited fastest outgrowth > 2 log CFU/g by 10 days of storage at 4°C. Treatments with IsoAge DV100 at 0.8% and 1% significantly ($p < 0.05$) controlled *L. monocytogenes* outgrowth (< 2 log CFU/g) for 60 days of storage. Uninoculated samples stored at 4°C did not spoil during the shelf life. No visual difference was observed in the treatments overtime.

Significance: Integrating these clean-label solutions into cheese formulations offers viable approach for achieving both safety in controlling *Listeria* and shelf-life extension.

P1-89: CLEAN-LABEL INTERVENTIONS FOR SPOILAGE PREVENTION IN GOAT CHEESE

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Goat cheese is particularly susceptible to spoilage during storage due to its nutrient-rich composition. Spoilage of cheese is often caused by early or late blowing. Additionally, spoilage can occur due to yeast growth, resulting gas production, unpleasant odors, and visible defects. To prevent spoilage during cheese processing storage, clean-label alternatives have emerged as effective intervention strategies.

Purpose: Increasing shelf life by incorporating clean label alternatives and controlling spoilage microorganisms in goat cheese.

Methods: Goat cheese was manufactured and treated with 0.5% Flavoset 5400L and no preservative (control). Samples were inoculated with *Pichia* Fermentants at ca. 2 log CFU/g, vacuum packaged, and stored for 60 days at 7°C. Samples (duplicates) were removed from storage, homogenized, and plated onto Potato dextrose agar (PDA) for yeast. Differences among treatments were determined using one-way ANOVA at $P < 0.05$.

Results: Yeast count in goat cheese samples without antimicrobial (control) were bloated and reached the spoilage limit > 6 log CFU/g at day 15, however the yeast counts in goat cheese samples with 0.5% Flavoset 5400L were below the spoilage limit (6 log CFU/g) at day 60. The addition of Flavoset 5400L is effective in extending the shelf life of goat cheese samples.

Significance: The results of this study demonstrate the possibility to use clean label alternatives in goat cheese to increase shelf life. Clean label solutions are very effective in controlling spoilage microorganisms.

P1-90: IMPROVING FOOD SAFETY PRACTICES IN QFT CHEESE MANUFACTURING: INSIGHTS FROM INSPECTIONS AND SAMPLING

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Queso fresco-type cheeses (QFT cheeses) are soft, fresh, ready-to-eat cheeses commonly consumed in Hispanic communities. QFT cheeses are highly susceptible to post-processing microbiological contamination and are frequently linked to foodborne outbreaks.

Purpose: The purpose of this study was to evaluate U.S. FDA's inspection observations, surveillance sampling, and compliance sampling activities related to QFT cheeses, with the aim of identifying patterns of noncompliance with federal regulations and highlighting opportunities to improve food safety practices within the QFT cheese industry.

Methods: FDA inspection data were extracted from the Human Foods Data Platform using R Studio via SQL queries and summarization.

Results: Between 2010 and 2022, FDA performed 57 inspections of domestic QFT cheese firms. of these inspections, 78.9% (45/57) were categorized as Voluntary Action indicated (VAI), 12.3% (7/57) as official Action indicated (OAI), and 8.8% (5/57) as No Action indicated (NAI). The top three cited inspection deficiencies were related to Cleaning and Sanitizing Operations, Manufacturing Conditions, and Lack of Effective Pest Exclusion. 14.8% (13/88) of the FDA's environmental surveillance samples and 30.4% (7/23) of its environmental compliance samples tested positive for *Listeria* species.

Significance: These findings highlight key areas where QFT cheese manufacturers can focus on improving their food safety practices to reduce incidents of microbiological contamination and mitigate the risk of causing future foodborne outbreaks.

P1-91: MULTIDRUG-RESISTANT INCFIB PLASMIDS ENCODING SIDEROPHORES ENHANCE ESCHERICHIA COLI GROWTH UNDER IRON-LIMITED BOVINE CECAL CONDITIONS

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: incFIB plasmids, carried by members of the Enterobacteriaceae, frequently harbor both antimicrobial resistance (AMR) genes and accessory siderophores (sitABCD and/or iucABC-iutA). We hypothesize that the iron-poor environment of the preweaned dairy calf gut selects for bacterial strains carrying these accessory siderophore genes and this coincidentally enriches antimicrobial-resistant strains in these animals.

Purpose: This study evaluated the selection advantage conferred by siderophore-encoded incFIB plasmids carried by *E. coli* under iron-deficient conditions in calf cecal contents.

Methods: AMR *E. coli* strains harboring incFIB plasmids were identified via automated microdilution antimicrobial sensitivity tests and whole-genome sequencing. Plasmid influence on growth was assessed by curing strains with ethidium bromide. Plasmid recombination was performed via two-step conjugation using *Klebsiella michiganensis* as an intermediate and electroporation. Growth kinetics were tracked for 60 hours in standard vs. iron-deficient media (iron depleted with 2,2'-dipyridyl) using isothermal microcalorimetry. Expression of *sitA* and *iucA* was quantified by qPCR in wild-type, cured, and recombined strains.

Results: We obtained four wild-type incFIB+ (siderophore and AMR) strains, four incFIB plasmid-free susceptible strains, four cured strains, and six recombined strains. Under iron-replete conditions, no significant growth differences were observed between the strains. However, in iron-depleted cecal media, plasmid-bearing strains produced up to 3.5 times more total heat than plasmid-free strains, indicating a clear growth advantage. In ten incFIB+ wild-type and recombined strains, qPCR showed up to a 3-log increase in *sitA* and *iucA* expression under iron-limited conditions, highlighting the importance of siderophore genes.

Significance: These findings suggest that incFIB plasmids co-encoding AMR and siderophore genes confer a competitive edge to *E. coli* in the iron-deficient dairy calf cecum. Understanding how iron availability affects AMR persistence will aid in developing strategies to control resistant bacteria and ensure dairy product safety.

P1-92: HEAT STRESS IN DAIRY COWS: CAN HEAT STRESS IN DAIRY COWS AFFECT MILK MICROBIOLOGICAL AND PHYSICOCHEMICAL PROFILES (QUALITY)

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Heat stress can cause significant losses in the dairy industry primarily due to reduced milk yield and higher somatic cell counts. Therefore, its impact on the chemical and microbial composition of milk is an area of interest that is not yet well explored.

Purpose: This study was to determine the effect of a heat-stress environment in dairy cows on the microbial and chemical composition of raw milk.

Methods: Twenty-four lactating Holstein cows were divided into Control (CT) group (n=12) and Heat Stress (HS) group (n=12), respectively, which had entire or limited access to fan and sprinkler cooling systems during a 14-day period in June. The cow respiration rates were measured daily to monitor the heat stress condition. Raw milk samples were collected hygienically at day 0 and day 14 from both groups (samples from 4 cows per group served as one replication). Three milk replicates per group were used for microbiological and chemical analyses.

Results: The respiration rate (12pm: $89.45 \pm 12.36/\text{min}$ vs $69.15 \pm 9.27/\text{min}$) was higher ($P < 0.001$) in the HS group compared to the CT group during the trial, indicating different heat stress conditions between the two groups. Raw milk obtained from the HS cows showed a reduction ($P \leq 0.05$) in homofermentative LAB and mold counts from day 0 (LAB: 3.4 log CFU/g; mold: 2.0 log CFU/g) to day 14 (LAB: 2.4 log CFU/g; mold: 1.1 log CFU/g). Additionally, milk from HS cows had higher ($P \leq 0.05$) moisture and lower ($P \leq 0.05$) fat and total solids content after 14 days. These changes were not observed ($P > 0.05$) in raw milk obtained from the CT group.

Significance: Understanding the impact of heat stress in cows on milk quality composition is essential for industry to prevent negative impacts on dairy products. HS cows were lower in fat, which can reduce frozen products and cheese yield.

P1-93: THE EFFECT OF A PROBIOTIC YOGHURT ON GUT MICROBIOME, LOW-GRADE INFLAMMATION AND WEIGHT STATUS OF OBESE SOUTH AFRICAN WOMEN

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: The prevalence of obesity and its associated comorbidities, such as low-grade chronic inflammation and altered gut microbiome profiles, is a growing public health challenge in South Africa.

Purpose: This study investigates the impact of a probiotic-enriched yogurt on the gut microbiome, systemic inflammation, and the effects of these enriched-gut microbiome on the weight status of obese South African women.

Methods: Probiotic-enriched yogurt was prepared using LGG (*Lactobacillus rhamnosus* GG) and BB-12 (*Bifidobacterium animalis* subsp. *lactis*). The probiotic yogurt sample was analyzed at day 0 and 7 to ensure the concentrations are beyond the recommended levels. Using a randomized controlled trial design, participants consumed either probiotic yogurt or a placebo (plain yogurt) for 12 weeks, with assessments conducted at baseline and post-intervention. Gut microbiome composition was analyzed using next-generation sequencing, while markers of inflammation (e.g., C-reactive protein, interleukins) and weight-related parameters (BMI, waist circumference) were measured using standard protocols.

Results: Preliminary results suggest that probiotic yogurt consumption leads to significant modulation of the gut microbiota, marked by increased abundance of beneficial bacteria, reduced inflammatory markers, and modest weight improvements.

Significance: This study highlights the potential of functional foods as an adjunctive strategy for managing obesity and its metabolic consequences in vulnerable populations.

P1-94: A RAPID METHOD TO DETECT SLOW-GROWING MOLDS IN YOGHURT

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Traditional enumeration of molds in China follows GB 4789.15-2016, which requires 5 days of incubation on Rose Bengal agar plates. In a previous study, three extremely slow-growing fungi were found in local yoghurt plants: *Aureobasidium pullulans*, *Talaromyces wortmannii* and *Cladosporium cladosporioides*. They can only form tiny colonies with limited hyphae on Rose Bengal agar plate over 5 days, which can be easily neglected. Soleris® offers a rapid alternative method to detect molds by adding 2 mL of 1:10 diluted yoghurt to the vial DYM-109C supplemented with antibiotics, which inhibits the growth of lactobacilli and other bacteria. CO₂ produced by molds diffuses through a membrane to the agar plug containing a dye indicator. The color change in the dye indicates the presence of molds.

Purpose: This study aimed to compare the performance of the alternative Soleris method to GB 4789.15-2016 for slow-growing mold detection in Yili's yoghurt.

Methods: Two types of yoghurt from Yili were inoculated individually with *A. pullulans*, *T. wortmannii* and *C. cladosporioides* at high (45 CFU/g, 1 sample for each type of yoghurt and each fungus), medium (15 CFU/g, 4 samples) and low (5 CFU/g, 4 samples) levels. Samples were analyzed parallelly by GB method and Soleris. Detection rate of the two methods was compared. The eLOD₅₀ of Soleris was also calculated.

Results: Soleris has an overall higher detection rate than GB method. The eLOD₅₀ of Soleris was less than 4×LOD₅₀, meeting verification requirements.

Significance: Soleris offers a sensitive and rapid method to detect slow-growing molds in yoghurt in 48 hours and was comparable to GB method. The reduction in incubation time can save food factories 3 days of additional incubation and help

free up storage space.

P1-95: ANALYSIS ON MICROBIAL CONTAMINATION IN RAW MILK AND YOGURT PRODUCTION PROCESS

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: The increasingly hot and humid summer weather in Korea, exacerbated by climate change, creates ideal conditions for the growth and proliferation of spoilage fungi. These environmental changes can significantly affect food production processes by increasing the risk of microbial contamination. In this context, analyzing the microbiota of raw milk and detecting microbial contamination are crucial for identifying microorganisms responsible for potential contamination and ensuring the safety and quality of food products.

Purpose: This study aimed to analyze microbial contamination, including spoilage fungi and foodborne pathogens (*Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes*), throughout the yogurt production process, from raw milk to finished products.

Methods: A total of 36 samples, including raw milk, pasteurized milk, surface samples (10 cm²), bottles, and final yogurt products, were collected. The microbiota of raw and pasteurized milk was analyzed through metagenomic sequencing. Total bacterial counts, as well as the presence of lactic acid bacteria, fungi, *L. monocytogenes*, *E. coli*, and *Salmonella* spp., were tested per Korea Food Code. Fungi were isolated on potato dextrose agar (PDA) and incubated at 28°C for 7 days. Pure isolates were obtained by sub-culturing and identified using ITS sequencing and BLAST analysis.

Results: Metagenomic analysis revealed Proteobacteria as the dominant phylum (78.1%) and *Pseudomonas* spp. (73%) as the dominant genus in raw milk. Total bacteria, lactic acid bacteria, and fungi were detected in raw milk, but *L. monocytogenes*, *E. coli* and *Salmonella* spp. were not. No bacteria were found after heat treatment. Among 35 samples, 17 fungal species, including *Cladosporium* and *Fusarium*, were isolated. Four spoilage fungi—*C. oxysporum*, *C. pini-ponderosae*, *C. colombiae*, and *F. annulatum*—exhibited high query cover (97–100%) and identity (97.6–99.8%).

Significance: The identified fungal species, including spoilage fungi pose risks to product quality and safety. Further research is required to identify the sources and

routes of fungal contamination and to develop effective control strategies for maintaining product quality.

Award Application: Developing Scientist Award

P1-96: DUAL SPECIES BIOFILMS OF LISTERIA MONOCYTOGENES AND RALSTONIA INSIDIOSA DISPLAY ALTERED EXTRACELLULAR POLYMERIC SUBSTANCE PRODUCTION IN A STRAIN-DEPENDENT MANNER

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: *Ralstonia insidiosa* (Ri), a ubiquitous water bacterium, enhances the biofilm formation of *Listeria monocytogenes* (Lm), which may facilitate persistence in food processing environments. However, the mechanism is unclear.

Purpose: To investigate whether Ri increases the production of Lm's carbohydrate-based extracellular polymeric substances (CB-EPS) under cheese processing conditions over 10 days.

Methods: *L. monocytogenes* strains Lm009, Lm010, and Lm019 from artisan cheese production environments, and Ri891 and Ri602 were grown in 1× (Lm) or 1/20× (Ri) tryptic soy broth (TSB) overnight. Overnight cultures were diluted 1:40 with fresh 1/20× TSB and inoculated into 96-well plates as single-species and dual-species combinations. Plates were incubated at room temperature or 15°C. On days 1, 5, or 10, plates were rinsed with phosphate-buffered saline and incubated with 0.1% Ruthenium Red (RRD) dye for 60 minutes at 22°C. RRD was removed to a fresh plate, and OD₄₅₀ was measured. CB-EPS production was measured as: EPS = OD(blank) – OD(sample). Repeated measures ANOVA was used to determine the effects of time, temperature, and biofilm type (single/dual species) on CB-EPS production.

Results: Overall, CB-EPS production was highest on day 1 at 22°C and day 5 at 11°C ($p < 0.05$). However, at 11°C, Lm19 had the highest CB-EPS on day 10. CB-EPS production was lower for dual-species biofilms for Lm009 and Lm010. For Lm019 at 11°C, co-cultivation with Ri891 marginally increased CB-EPS ($p > 0.05$). For Lm010, co-cultivation with Ri602 significantly reduced CB-EPS, but less than co-cultivation with Ri891 ($p < 0.05$). For Lm009, both Ri891 and Ri602 reduced CB-EPS by similar levels. At 22°C, Lm010 had the highest CB-EPS ($p < 0.05$), but CB-EPS was lower in dual-species biofilms than in single-species biofilms of either Ri or Lm (all strains).

Significance: Co-cultivation with Ri causes temperature- and strain-specific changes in CB-EPS production for *Listeria monocytogenes*, potentially altering EPS composition.

Award Application: Undergraduate Student Award

P1-97: GENERALIZED LINEAR MIXED MODELS FOR RETAIL FOOD INSPECTION DATA: A CASE STUDY EVALUATING OHIO'S CERTIFIED FOOD PROTECTION MANAGER CERTIFICATION

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Presentation type/Format: Technical

Category: Data Management and Analytics

Abstract:

Introduction: Data generated from retail food safety inspections can be statistically analyzed to provide valuable information about retail foodborne illness risk factors and inspection programs, but advanced statistical approaches are needed to sufficiently account for correlations between establishments, inspectors, and locations.

Purpose: To develop a statistical model evaluating how a 2019 Ohio policy change, which required establishments to employ a Certified Food Protection Manager (CFPM), affected overall inspection performance of Risk Level III and IV food establishments in Franklin County.

Methods: A generalized linear mixed modeling (GLMM) approach was used to evaluate how compliance with Ohio's CFPM requirement was associated with the odds of a satisfactory standard inspection earned by Risk Level III and IV establishments in Franklin County from March 1, 2019 to February 28, 2020. Satisfactory inspections were defined as inspections with fewer than three critical or seven total violations. Random variables were included in the model to account for correlation between repeat inspections at the same establishment, within the same zip code, and by the same inspector.

Results: Risk Level III and IV establishments that complied with Ohio's CFPM requirement were 2.0 (95% CI: [0.9, 4.6], $p=0.092$) and 2.2 (95% CI: [1.7, 2.7], $p<0.001$) times as likely to earn a satisfactory inspection compared to those who did not comply with the CFPM requirement. Additionally, inspectors were identified as a larger contributor to inspection variance (29% and 38%) than establishments (26% and 30%) among Risk Level III and IV inspections.

Significance: This analysis provides an example of how GLMMs were used to

evaluate the effectiveness of Ohio's CFPM requirement while accounting for variation that arises due to repeated inspections at establishments, by inspectors, and within communities. It is critical that appropriate analytic methods, such as GLMMs, are used when conducting analyses of inspection data to inform foodborne illness prevention activities and policies.

Award Application: Developing Scientist Award

P1-98: EVALUATION OF CLEANING, DISINFECTION AND FOOD HANDLING PRACTICES IN DOMESTIC KITCHENS

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Presentation type/Format: Poster

Category: Data Management and Analytics

Abstract:

Introduction: Proper cleaning and disinfection of surfaces in domestic kitchens are essential for preventing cross-contamination and the persistence of pathogens. The aim of this study was to identify hygiene and handling practices in domestic kitchens through surveys.

Purpose: To assess cleaning, disinfection, and surface-use practices in domestic kitchens and identify potential risk factors for cross-contamination.

Methods: An online survey was distributed using non-probability sampling, primarily through social media, over a 10-month period (January to October 2024). The survey consisted of two sections: demographics and questions related to surface use, cleaning, and disinfection practices. Contingency tables and Pearson's chi-square tests were used to evaluate associations among variables.

Results: A total of 322 individuals participated. The majority were aged 18–25 years (56.5%), women (71.4%), held a bachelor's degree (46.6%), and were from middle socioeconomic backgrounds (41.3%). Sixteen percent had only one cutting board (CB), with most made of plastic (71.4%) and wood (62.1%). Nearly half (40.4%) used the same CB interchangeably for different foods, and 45.3% used the same CB for raw and cooked foods. Most participants (56.5%) estimated washing their CB took 30 seconds to 1 minute. Refrigerators were cleaned (35.7%) and/or disinfected (32.9%) once a month. Sponges were washed (46.9%) and disinfected (19.6%) daily, while 8.3% and 24.8% reported never cleaning or disinfecting them. Dishcloths were washed (39.1%) and disinfected (21.7%) daily. Liquid soap was the most common cleaning agent (67.5%–56.5%), and hypochlorite was the most used disinfectant (66.3%–69.8%). An association was found between sex and the number of cutting boards, with women reporting more ($p < 0.05$).

Significance: Certain cleaning, disinfection, and food handling practices in

Querétaro may contribute to cross-contamination and the persistence of pathogenic microorganisms on kitchen surfaces and utensils. Interventions targeting household hygiene could help reduce these risks.

Award Application: Undergraduate Student Award

P1-99: OVERVIEW OF FOOD CONTAMINATION BY FOODBORNE BACTERIA IN JAPAN USING PUBLISHED DATA

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Presentation type/Format: Poster

Category: Data Management and Analytics

Abstract:

Introduction: A quantitative microbial risk assessment requires a comprehensive understanding of the variability in foodborne bacterial contamination, which differs significantly depending on food items, bacterial species, and cultural food practices.

Purpose: This study examines food contamination in Japan using previous survey data from public institutions to assess the extent of bacterial contamination.

Methods: Over the past 20 years, surveys on food contamination by major food poisoning bacteria (*Campylobacter coli/jejuni*, *Listeria monocytogenes*, enterohemorrhagic *Escherichia coli*, and *Salmonella* spp.) in Japan have been conducted. Published data were collected and categorized into two types: "Contamination ratio" and "Contamination concentration." The "Contamination ratio" refers to prevalence data that indicates the ratio of positive samples. In contrast, "Contamination concentration" measures the concentration of bacterial counts. Using the collected data, we visualized the sample size, the contaminated ratio, and distribution of the mean values of bacterial concentration (log CFU/g) for each type of food and bacteria. By comparing the contamination ratio for each type of food or bacteria, we investigated which foods were more contaminated in Japan. We visualized the distributions and calculated the mean and variance of contamination concentration values to estimate the extent of bacterial contamination. All the analyses were conducted using Python (version 3.12.7).

Results: A total of 25 prevalence survey datasets and 6 concentration survey datasets were collected, encompassing 95,615 samples in the prevalence surveys. The contamination ratio was less than 10%, except for *Campylobacter coli/jejuni* on chicken meat, which exhibited 15 to 75%. Conversely, the contamination ratio for vegetables was consistently below 1% for each bacterium. The contamination values for meat products displayed a bimodal distribution: the higher peak ranged from 6 to 7 log CFU/g, while the lower ranged from 1 to 2 log CFU/g.

Significance: An overview of food contamination data in Japan reveals key

characteristics. Understanding the current state of food contamination in Japan provides insights into the risk of food poisoning.

Award Application: Developing Scientist Award

P1-100: STATISTICAL TOOLS FOR ENVIRONMENTAL MONITORING PROGRAMS IN FOOD PROCESSING FACILITIES: A SYSTEMATIC REVIEW

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Presentation type/Format: Poster

Category: Data Management and Analytics

Abstract:

Introduction: Environmental Monitoring programs (EMPs) utilize statistical tools to analyze microbial sampling data, identify risk zones, and verify control measures in food processing facilities. These tools enhance the effectiveness of EMPs by providing data-driven insights to mitigate microbial contamination risks.

Purpose: A systematic review was conducted to synthesize literature relevant to the research question, "What statistical analysis methods are currently used to analyze environmental sampling data in food processing facilities?"

Methods: Literature published between 2000-2024 and 2024 was identified through a comprehensive search of keywords using SCOPUS and PubMed databases. This review followed PRISMA guidelines that included screening, full-text review, extraction, and synthesis. Records were screened for relevance based on eligibility criteria, and titles and abstracts were reviewed by a single reviewer using Covidence. Studies were considered eligible for inclusion if they (i) were peer-reviewed articles published in English, (ii) focused on microbial sampling of food processing environment, and (iii) utilized statistical methods to analyze microbial sampling data.

Results: of the total 2,561 records, 45 were selected for full-text review, and 34 of these met the inclusion criteria and were included for detailed qualitative analysis. Descriptive statistics and visual analysis ($n = 30$), including mean microbial counts ($\log \text{CFU/cm}^2$), standard deviation, percentages of positive samples, and trend analyses over time, were used to pinpoint contamination hotspots in different zones. Several studies tested data normality ($n = 4$) before applying parametric and non-parametric tests ($n = 14$). Subsequent post hoc analyses ($n=5$) were conducted to pinpoint specific group comparisons that contributed to the overall findings. Additionally, various studies utilized advanced statistical methods such as regression analysis and multivariate approaches ($n = 11$) to explore microbial contamination dynamics in food processing environments.

Significance: While descriptive statistics are commonly used to analyze

environmental monitoring data, there is a need for employing advanced statistical methods to predict contamination risks in food processing environments.

Award Application: Developing Scientist Award

P1-101: GRAPH ANALYTICS FOR SAMPLE-EFFICIENT ENVIRONMENTAL MONITORING IN FOOD PROCESSING ENVIRONMENTS

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Presentation type/Format: Poster

Category: Data Management and Analytics

Abstract:

Introduction: As environmental monitoring and testing for the presence of pathogens such as *Listeria monocytogenes* in food processing environments is expensive and time-consuming, there is a need for sampling strategies that judiciously select testing areas to detect potential contamination with fewer tested samples.

Purpose: Our research aimed to develop both active learning sampling algorithms and graph-centrality based open-loop sampling algorithms that exploit the probabilistic model underlying the potential pathogen spread within food processing facilities to identify the most relevant testing areas during environmental monitoring.

Methods: The algorithmic development was based on a graph model that captures cross-contamination relations among agents in a food processing environment extracted from an existing agent-based model of *Listeria* spread. We considered two approaches to the sampling strategy: an active learning approach based on closed-loop sequential decision-making and a graph-centrality based open-loop approach. Several algorithms were developed under each approach based on various cost metrics and graph centrality measures. The algorithms' performance was compared with respect to the 'cost' metric, describing the total contamination state detection errors (false negative and false positive) over the simulation length in scenarios with the varying numbers of daily samples tested, the ratios of critical (food contact surfaces) to non-critical agents (non-food-contact surfaces), numbers of critical agents, and the decision threshold, a parameter that balances error trade-offs with facility priorities in risk management.

Results: Preliminary simulation findings highlight the effectiveness of both the active learning closed-loop and open-loop algorithms in reducing the sampling cost and improving the detection accuracy across scenarios. Furthermore, when compared, our closed-loop algorithms outperform open-loop algorithms.

Significance: The developed novel active learning algorithms have the potential to optimize environmental monitoring by reducing the expenses and time associated with sampling and testing, using fewer samples while maintaining reliability.

Award Application: Developing Scientist Award

P1-102: HARNESSING MACHINE LEARNING FOR ENHANCED FOOD SAFETY AND PREDICTIVE ANALYTICS

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Presentation type/Format: Poster

Category: Data Management and Analytics

Abstract:

Introduction: Ensuring food safety involves navigating complex datasets encompassing microbial, chemical, and environmental factors. Traditional methods, while reliable, often fail to scale with the growing data demands. This study explores ML's potential in revolutionizing data-driven food safety protocols.

Purpose: The objective was to evaluate the efficacy of machine learning models in predicting foodborne contamination and assessing microbial risks across diverse food matrices.

Methods: The study utilized a dataset comprising microbial profiles, environmental conditions, and contamination events from over 1,000 food samples across varied categories. Preprocessing techniques, including feature scaling and dimensionality reduction, were applied. Models such as Random Forest, Gradient Boosting, and Neural Networks were trained and validated using an 80-20 split. Metrics like accuracy, F1-score, and ROC-AUC were employed to compare model performance. Post-training, SHAP (SHapley Additive exPlanations) values were used to identify key risk factors influencing predictions.

Results: The Random Forest model outperformed others, achieving an accuracy of greater than 90% and an F1-score of greater than 0.80. Key risk factors identified included ambient temperature fluctuations and pathogen load, which correlated strongly with contamination events. Results indicated a significant potential to automate and enhance risk assessment processes, reducing reliance on manual interventions.

Significance: Integrating ML into food safety management systems can elevate the predictive accuracy of contamination risks and streamline decision-making. This research underscores the value of ML in addressing contemporary challenges in food protection, offering actionable insights to safeguard public health.

Award Application: Developing Scientist Award

P1-103: DEVELOPMENT OF DDPCR ASSAY FOR THE DETECTION OF BOVINE MILK IN FOODS

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Anne Eischeid, US FDA

Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: The Federal Food, Drug, and Cosmetic Act lists milk as a major food allergen in the United States. Current immuno-detection methods are standard but can be limited by food processing and cross-reactivity. DNA-based detection methods have proven to be species-specific, sensitive, and robust in complex food matrices.

Purpose: This study aims to transfer a previously validated real-time PCR assay to droplet digital PCR format and evaluate its sensitivity, specificity, robustness, and precision.

Method: Species-specific primers and probe were previously designed for a real-time PCR assay targeting the COI mitochondrial gene for *Bos taurus*. PCR parameters were optimized for droplet digital PCR. Four complex food matrices (dark chocolate, vegan cookies, dairy-free muffins, and orange juice) were spiked with known concentrations of bovine milk. Assay performance was evaluated in terms of linearity, dynamic range, sensitivity, and precision. Cross-reactivity was tested with pork and goat milk.

Results: The assay showed a linear detection range from 0.001–10 ng/μL milk DNA, with an R^2 of 0.9997 and a limit of quantification (LOQ) of 0.1 copies/μL (0.001 ng/μL DNA), corresponding to 1000 ppm milk in food. The droplet digital PCR showed comparable sensitivity to real-time PCR, better performance in the presence of PCR inhibitors (e.g., dark chocolate), no cross-reactivity with non-target species, and improved precision (RSD values <10% for all but the lowest concentration).

Significance: The high prevalence of milk allergy in the United States underscores the need for sensitive, species-specific detection methods. Despite low DNA concentrations in milk, this droplet digital PCR assay successfully detects bovine milk in complex food matrices with high specificity, precision, and robustness.

P1-104: METABARCODING FOR DETECTION OF FOOD ALLERGENS: TARGET AND PRIMER SELECTION

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: FDA needs analytical methods for detection of major food allergens to help enforce labeling requirements of the Food, Drug, and Cosmetic Act. DNA-based methods such as PCR are robust for this purpose but are generally targeted to individual allergenic foods and limited in their multiplexing capability.

Purpose: This work is focused on development of a next generation sequencing (NGS)-based approach known as metabarcoding, which includes PCR amplification followed by massively parallel sequencing of specific DNA target regions which are commonly called “DNA barcodes.”

Methods: This work was focused on the critically important initial stage of allergen metabarcoding method development: selection and testing of target regions and PCR primer pairs. A total of 26 candidate primer pairs, targeting 8 different regions of plant and animal genomes, were selected from the scientific literature. Each pair was tested for PCR amplification on a panel of DNA extracted from all major allergenic foods, and results were assessed via agarose gels.

Results: Primer pairs were evaluated based on their ability to amplify DNA of the expected product size from a wide variety of allergenic foods with minimal nonspecific amplification. From the initial 26 primer pairs, 10 were selected for a second round, and 6 were selected from a third round of testing as optimal for use in further development of allergen metabarcoding. Selected primer pairs target the 16S rRNA and COI genes of animals and the mitochondrial P6 loop and rbcL gene in plants.

Significance: As a nontargeted and highly multiplexed approach, metabarcoding can enhance enforcement through broad-based detection and identification of both known and potential allergens in food products.

P1-105: SEAFOOD ALLERGEN CROSS-CONTACT RISK ASSOCIATED WITH REUSE OF SHARED BREADING MIXTURES

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: Breading systems are widely used in the retail and foodservice industries, and can be reused up to 7 days under certain conditions. However, the risks of allergen cross-contact from reusing breading mixtures remain underexplored.

Purpose: The objective is to determine whether seafood allergens, particularly from shrimp and fish, can be transferred to other foods when breading mixtures are reused.

Methods: Twenty batches of shrimp and cod (25-30 g/batch) were prepared separately in a mixing bowl containing 200 g of breading mixture. After the 1st, 5th, 10th, 15th, and 20th batches, the breading mixture was manually stirred, and 0.5 g samples were collected from three distinct locations in the bowl. Residual shrimp protein concentration was quantified using a Crustacean Residue ELISA kit (ELISA Systems), while cod protein concentration was determined using Fish Protein ELISA Kit (Neogen). All quantified

Results were expressed as mean $\pm$ standard error of the mean.

Results: Shrimp proteins were not detected in the breading mixture after a single batch of shrimp was breaded. However, shrimp protein concentrations progressively increased with repeated use, reaching approximately 1.0 ± 0 ppm, 5.5 ± 0.4 ppm, and 10.6 ± 0.2 ppm after 10, 15, and 20 batches, respectively. For cod, a similar trend of increasing fish protein concentrations with repeated reuse of the breading mixture was observed. After 20 batches, cod protein

concentrations reached 587 ± 16.0 ppm in the breading mixture.

Significance: This project directly addresses food safety and allergen management in the retail/foodservice setting. It helps for better understanding allergen cross-contact risks associated with reusing breading systems.

P1-106: MASS SPECTROMETRY ANALYSIS OF MILK PROTEINS IN HEATED OIL SYSTEMS

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Joseph Baumert, University of Nebraska Lincoln

Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: Understanding allergen cross-contact in systems with shared cooking media, including shared frying oil, is needed to develop and implement food safety controls. To evaluate these systems, the performance of analytical methods quantifying allergens must be well understood.

Purpose: The objective of this work was to evaluate the targeted mass spectrometry quantification of milk protein from milk-derived ingredients in oil under conditions representing shared frying systems.

Methods: Milk-derived ingredients (non-fat dry milk [NFDM], sodium caseinate, or whey protein concentrates) were spiked into oil and heated for 2–20 minutes at 150°C and 170°C. Subsequently, total milk protein was quantified using a targeted, bottom-up LC-MS/MS method, which included protein extraction, reduction, alkylation, and tryptic digestion. Resulting peptides were desalted, lyophilized, and resuspended for LC-MS/MS. Independent samples were heated in duplicate and LC-MS/MS was conducted in duplicate.

Results: The relative recovery across heat-treated samples was compared to the unheated controls for all 11 target peptides. NFDM samples showed minimal loss in concentration (80–100% recovery) when heated for 2–5 minutes and a significant loss (<10% recovery) at 10–20 minutes. This was peptide-specific; some peptides slightly increased in concentration at 2–5 minutes (>100% recovery). Sodium caseinate and whey protein concentrates showed similar patterns, though sodium caseinate had the most stable peptides across time and temperature. A significant difference ($p < 0.05$) existed in fold change percentages among the ingredients. Thermal processing, ingredient type, and matrix components such as lactose resulted in protein aggregation and chemical modifications that affected detection.

Significance: This research will help inform allergen risk assessment, precautionary labeling decisions, and the development and implementation of allergen control strategies.

Award Application: Developing Scientist Award

P1-107: COMPARISON OF MANUAL AND FULLY AUTOMATED ELISA TEST PROCEDURES USING HYGIENA'S GLUTENTOX® ELISA RAPID G12 ASSAY

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: Automation has increasingly become an essential requirement for laboratories due to time and cost reasons and plays an important role in automation of ELISA assays.

Purpose: The purpose of this study was to compare the performance of the ELISA assay using a manual protocol and a fully automated platform.

Methods: A high-level concentration of gluten was prepared from wheat flour to artificially inoculate five matrices: soy flour, cornbread, rolled oats, seasoning mix and evaporated milk. After the gluten was added, matrices were thoroughly mixed until fully incorporated. Serial dilutions were created to obtain final contamination levels of 0, 5, 10 and 20 ppm. From each level, 6 x 0.5 g test portions were extracted, with the extraction solution, UGES, at room temperature (soy flour, rolled oats, seasoning mix and evaporated milk) or at 50 °C in a water bath (cornbread), for 40 minutes, and tested following the ELISA assay instructions using both manual and automation protocols.

Results: The concentration of gluten in each replicate sample was obtained from the OD450 measurements compared to the standards. All calculated results from the samples were at the targeted inoculation levels (0, 5, 10 and 20 ppm) with high reproducibility and within the AOAC performance requirements for recovery, ranging from 50-150%. Results between manual and automated test procedures were assessed using the relative standard deviation (RSD). All percentages for repeatability for the same samples were within acceptable limits in all cases (<20%), most of them within the range of 0.07 to 4.7% (at different concentrations of gluten), with the highest variation being 12% (rolled oats and

corn bread, at 5 ppm gluten).

Significance: The AOAC RI PTM #042301 certified GlutenTox ELISA Rapid G12 assay showed that it can be consistently used in a fully automated ELISA analyzer with reproducible quality. This ELISA method is recommended for laboratories demanding automated ELISA solutions for the detection and quantification of gluten in food samples.

P1-108: IDENTIFICATION OF ANTIGENICITY CHANGES IN TENEBRIO MOLITOR LARVA (TML) AND THEIR DETECTION IN PROCESSED FOODS USING INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: *Tenebrio molitor* larva (TML), commonly recognized as an edible insect and alternative protein source, contains proteins such as arginine kinase and tropomyosin, which are known to trigger food allergies.

Purpose: Since processed foods often undergo multiple heat treatments that can alter antigenicity, this study aims to develop a method to detect TML adulteration, monitor antigenicity changes, and protect allergic consumers.

Methods: The changes in the antigenicity of TML, a known allergenic source, were investigated under various heat treatments, including autoclaving, microwaving, and baking, for different durations. Microwave-dried TML (MTML) was used to prepare cookies, which served as samples for analysis. An indirect enzyme-linked immunosorbent assay (iELISA) based on a monoclonal antibody (MAb) specific to thermal-stable soluble proteins (TSSPs) of TML was employed to detect antigenicity changes and the presence of TML in processed foods.

Results: The antigenicity of TML was measured with an O.D. of 2.0 in the absence of heat treatment. Antigenicity was maintained under various heat treatments, with O.D. values of 1.5 after autoclaving for 15 minutes, 2.4 after microwaving for 90 seconds, and 2.3 after baking for 12 minutes. However, prolonged heating led to a decrease in antigenicity, with O.D. values reduced to 0.6, 0.8, and 0.7 after autoclaving for 60 minutes, microwaving for 120 seconds, and baking for 20 minutes, respectively. Using iELISA, all 15 TML-containing samples tested positive and all 9 TML-free samples tested negative, with the assay demonstrating a detection limit of 300 ng/mL and no cross-reactivity with other food allergens. These results indicate that TSSPs in TML retain partial antigenicity after heat treatment, supporting their use as reliable marker proteins for detecting TML adulteration in processed foods.

Significance: The iELISA developed in this study is a valuable tool for rapidly detecting the presence of TML in processed foods, even after undergoing typical food processing methods and durations used in manufacturing.

P1-109: ALLERGENICITY CHANGES OF THERMAL STABLE-SOLUBLE PROTEINS IN SHRIMP (LITOPENAEUS VANNAMEI) AND ANALYSIS OF SHRIMP PRESENCE IN PROCESSED FOODS USING INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

:
.: (TSSP) </p>
(iELISA), TSSP , . :
(100°C, 60), (121°C, 60), (170°C, 0~20) TSSP
. (100%, 75%, 50%, 30%, 10%, 5%, 3%, 1%, 0.5%, 0.3%, 0.1%, 0%)
, , . 231 (11 × 3 × 7) 3 .
iELISA . : TSSP 60 . 7.5 .
TSSP 5 . iELISA , 0.3%, 0.1%, 0.5%
. : TSSP MAb iELISA
.

P1-110: COMPARISON OF SIX COMMERCIAL SESAME ELISA KITS ON THEIR RELIABILITY OF DETECTING AND QUANTIFYING SESAME RESIDUE FROM BUFFER & CRACKER MATRICES

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: With sesame being recently added to the allergens requiring labeling by the FDA, there is little information on sesame detection which producers can use to create food safety plans.

Purpose: Sesame ELISAs were evaluated to determine allergen recovery from sesame flour spiked into buffer at known concentrations and sesame flour, extract, and seeds spiked into crackers.

Methods: 10, 50, and 100 ppm sesame flour spikes were prepared in buffer and were evaluated using six sesame ELISA kits, namely A, B, C, D, E, and F. 10, 50 and 100 ppm sesame flour spikes were spiked into crackers. Additionally, 10, 50, and 100 ppm sesame extract spikes were prepared prior to spiking into crackers. The sesame seed spikes were prepared by adding a sesame seed to a 15 g cracker sample (corresponding to 20 ppm sesame protein) before or after homogenization. The samples were homogenized using either a benchtop blender or a freezer mill. ELISA kits B and C were used to evaluate the sesame flour, extract, and seed spikes in crackers.

Results: Recovery varied among the six kits and buffer spike concentrations. Kits B and C were the most consistent in recovering sesame from the buffer spikes with both having a recovery of $90\pm 9\%$. For the sesame extract and flour samples, kit B had an average recovery of $102\pm 10\%$ and $72\pm 8\%$, respectively, while kit C had an average recovery of $81\pm 10\%$ and $146\pm 18\%$ respectively. Spiking a sesame seed into homogenized crackers resulted in more consistent recovery than spiking into cracker pieces.

Significance: Sesame ELISA kits differed in recovery and detection across different spiking materials and types of sesame residue used for spiking. These results are useful for understanding the limits of ELISA testing methods. Further evaluation of allergen recovery from different matrices incurred with sesame is warranted.

Award Application: Undergraduate Student Award

P1-111: IDENTIFICATION OF ABUNDANT AND PERSISTENT GLUTEN PEPTIDES POST-FERMENTATION

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: Current analytical

Methods, including ELISA, are inadequate for quantitative detection of gluten in fermented and hydrolyzed foods, creating a deficit that implicates numerous stakeholders, including those with Celiac disease (CD), manufacturers, and the government.

Purpose: The objective was to identify abundant and persistent gluten target peptides in a commercial fermentation system for development of a mass spectrometry (MS)-based fermented gluten method.

Methods: Protein extracts from wheat flour and samples throughout production of a fermentation-derived ingredient were digested separately by three proteases (trypsin, chymotrypsin, ProAlanase) and prepared for untargeted MS (Thermo Scientific™ Orbitrap Exploris™ 240). Triticum aestivum peptides were identified using Proteome Discoverer™ 3.0 and peptides from gluten proteins were quantified. Abundant peptides from intact gluten sources were designated candidate target peptides for the calibrant and any fully or semi-cleaved gluten peptides detected in post-fermentation samples were transferred to a targeted MS method. Targeted MS confirmed the presence of gluten peptides in these samples and quantitative assessment allowed for selection of the best-performing peptides.

Results: Untargeted MS of the wheat flour calibrant yielded identification (statistically validated with a false discovery rate (FDR) < 1%) of 164 (trypsin), 108 (chymotrypsin), and 117 (ProAlanase) peptides from gluten proteins, with the 50 most abundant selected as candidate peptides for intact gluten. For the fermentation-derived ingredient, 240, 100, and 127 gluten peptides were identified (FDR < 1%) in samples digested with trypsin, chymotrypsin, and ProAlanase, respectively. A qualitative targeted MS method was generated to confirm target presence using product ion scans. Abundance and quality criteria determined more than 16 high-performing gluten target peptides for this fermentation.

Significance: Quality target peptides for use in a novel fermented gluten MS method were identified. In addition to a method development approach for filling the current analytical gap, subsequent methods inform appropriate labeling of fermented and hydrolyzed foods to protect individuals with CD.

Award Application: Developing Scientist Award

P1-112: DETERMINATION OF N-NITROSAMINES AND N-NITROSATABLE SUBSTANCES MIGRATING FROM FOOD-CONTACT RUBBER MATERIALS USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract:

Introduction: N-nitrosamines in foods are a growing concern due to their potential health risks.

Purpose: In this study, a gas chromatography-mass spectrometry (GC-MS) method was developed and validated for the determination of N-nitrosamines and N-nitrosatable substances migrating from 91 food-contact rubber materials.

Methods: Fifteen N-nitrosamines were extracted by dichloromethane from different food simulants, including water, 4% acetic acid, and alcohol solutions at concentrations of 10%, 20%, 50%, and 95%. The developed method demonstrated excellent linearity ($R^2 > 0.9966$) with a concentration range of 0.05–0.5 mg/L for N-nitrosamine standards, with detection limits as low as 0.01 µg/kg and quantification limits as low as 0.03 µg/kg. This method also showed high accuracy and precision, with average recoveries of 81.2–119% and relative standard deviations (RSDs) below 10% at different spiking levels in natural rubber material, synthetic rubber films, and rubber pacifier samples. Inter-laboratory validation across three research institutions confirmed its robust repeatability, accuracy, and precision ($R^2 = 0.9968$ – 0.9998 , average recovery = 87.1–102.3%, and RSD < 9%).

Results: Among the 91 analyzed food-contact rubber samples, four were found to contain N-nitrosamines and N-nitrosatable substances.

Significance: These findings indicate that the GC-MS method serves as a reliable tool to monitor N-nitrosamines and N-nitrosatable substances migrating from food-

contact rubber materials into food.

P1-113: DEVELOPMENT OF GAS CHROMATOGRAPHY-THERMAL ENERGY ANALYSIS FOR DETERMINING N-NITROSAMINES AND N-NITROSATABLE SUBSTANCES MIGRATING FROM FOOD-CONTACT RUBBER MATERIALS

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract:

Introduction: Migration of N-nitrosamines, known carcinogenic compounds, from food-contact materials into food poses significant health concerns. Monitoring their presence is critical to ensure consumer safety.

Purpose: To develop and validate a sensitive and selective gas chromatography method coupled with thermal energy analysis (GC-TEA) for detecting N-nitrosamines and N-nitrosatable substances migrating from food-contact rubber materials.

Methods: The optimized GC-TEA method was used to detect 15 N-nitrosamines extracted from various food simulants, including water, 4% acetic acid, and alcohol solutions at 10%, 20%, 50%, and 95%, using dichloromethane as the extraction solvent. The method showed good linearity ($R^2 > 0.9984$) across a concentration range of 0.025–0.5 mg/L. Limits of detection and quantification ranged from 0.026 to 0.188 $\mu\text{g/kg}$ and 0.087 to 0.625 $\mu\text{g/kg}$, respectively. Migration tests on three food-contact rubber materials (natural rubber, synthetic rubber film, and rubber pacifier) demonstrated average recoveries of 85.7% to 106.0% and relative standard deviations (RSDs) below 10% across different spiking levels. Inter-laboratory validation was performed across three independent labs, confirming excellent repeatability, accuracy, and precision (R^2 values > 0.998 , recoveries of 82.7%–106.2%, and RSDs $< 9\%$).

Results: The validated GC-TEA method was applied to 91 food-contact rubber material samples, including rubber seals, natural latex sheets, vulcanized rubber test pieces, thermoplastic rubber spoons, rubber door seals, milk tubes,

disposable rubber gloves, and O-rings. Four samples tested positive for N-nitrosamines and N-nitrosatable substances.

Significance: These results highlight the effectiveness of the GC-TEA method for monitoring the migration of N-nitrosamines and N-nitrosatable substances from food-contact rubber materials into food.

P1-114: ANALYSIS OF COMMONLY CONSUMED FOODS FOR PERCHLORATE

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract:

Introduction: Perchlorate is commonly found in food and is a known endocrine disruptor that can interfere with thyroid function and affect the regulation of metabolism in adults as well as disrupt normal growth and development of the central nervous system in fetuses and infants.

Purpose: in this study, we tested perchlorate levels in various fresh and packaged foods previously reported to contain perchlorate and compared the estimated intakes to health-based exposure limits.

Methods: We purchased and tested 2-3 samples each of 73 products (n=196), including 63 supermarket foods and 10 fast food items, for perchlorate by liquid chromatography tandem mass spectrometry (LC-MS/MS). The samples included baby food, baked products and grains, beverages, dairy, fast food/mixtures, fruits and vegetables, meat and meat products, seafood products, others, with various packaging types. We estimated perchlorate means for each food, food category, and package type. We estimated perchlorate intake in a serving of the foods for young children and adults using the means and label serving sizes, and compared the intakes to both the U.S. Environmental Protection Agency (EPA) reference dose and the European Food Safety Agency (EFSA) tolerable daily intake (TDI) for perchlorate.

Results: Perchlorate was measurable in 131 (56 products) or approximately 67% out of the 196 samples, at 2.1 - 84 ppb. The estimated perchlorate intakes for adults were well below the TDI, but intakes of children under 6 years old from one serving of some baby foods, fast foods, and fruits and vegetable averaged 0.07-0.25 ug/kg bw/day or 20-80 percent of the TDI, leaving little room for tolerable exposure from other likely sources.

Significance: Some commonly consumed foods can expose young children to concerning levels of perchlorate.

P1-115: MICROBIAL CONTAMINATION IN FINISHED COMPOST FROM COMPOSTING FACILITIES ACROSS CALIFORNIA

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Composting is an environmentally friendly option for recycling organic waste into organic fertilizers, but inadequate pathogen reduction can lead to the survival of foodborne pathogens such as *Salmonella* spp. in the finished compost. This poses a risk of foodborne illness when compost is applied to agricultural soils as the crops are eaten raw or with limited processing.

Purpose: Assess the microbial contamination in finished compost from compost facilities across California.

Methods: A cross-sectional study was conducted in California's composting facilities (CF), including commercial facilities (CM), urban sites (UB), and vermicomposting sites (VM). The presence of foodborne pathogens (*Salmonella* spp., Shiga Toxin-producing *E. coli* (STEC), and *Listeria monocytogenes*,) and fecal coliform concentration [$\log_{10}(\text{MPN/Gram dry weight (gdw)})$] were characterized by targeted selective isolation and PCRs.

Results: Overall, 172 samples of finished compost [KN1] were collected among 32

CFs with total fecal coliforms averaging 3.10 and ranging from 0.11 to 6.69 log₁₀(MPN/gdw). Among those, 108 samples were from 21 CMs with a mean fecal coliform concentration of 2.13 [0.11, 6.69], 48 samples were from 7 UBs with a mean fecal coliform concentration of 4.68 [0.25, 6.66], and 16 samples were collected from 3 VMs with a mean fecal coliform concentration of 4.90 [0.25, 6.03]. Compost unit type was significantly associated with fecal coliform concentration ($p < 0.05$). *Salmonella* was detected in 1 sample from CM. *Listeria monocytogenes* was detected in 3 samples (1 CM and 2 UB samples). No pathogenic *E. coli* was detected in any collected samples.

Significance: Results document the presence of foodborne pathogens was rare in finished compost while the fecal coliform concentration was variable across different composting facilities in California. Data from this study will provide the producers and end-users of compost with potential risks associated with different composting practices.

Award Application: Developing Scientist Award

P1-116: MICROFLUIDIC OPTICAL APTASENSOR FOR MYCOTOXIN DETECTION IN AGRI-FOODS

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract:

Introduction: Mycotoxins are a category of chemical hazards in agri-food systems that pose threats to both public health and food security. Current standard analytical methods (predominantly LC-MS) are costly, labour-intensive, and complex.

Purpose: This study aims to develop a novel microfluidic optical aptasensor for rapid detection of small molecules based on analyte-tuned growth of gold nanoseeds combined with machine-learning-enhanced spectrum analysis.

Methods: This growth pattern of aptamer-coated gold nanoseeds in the absence of target molecules was determined by varying influencing factors, including Apt:AuNS ratio (0-40 pmol per 1 μ L AuNS), aptamer sequences (8Z31 vs. Z143), addition of HAuCl₄ (3 conc., in 2 protocols). In the presence of the mycotoxin (zearalenone), the reaction conditions were investigated referring to the LSPR wavelength, such as HAuCl₄ amount (7 steps) and MgCl₂ concentration (3 levels). Five machine-learning models were developed to process the raw absorption spectra (400-900 nm) for enhanced prediction of zearalenone concentration.

Results: We discovered and optimized a previously unreported growth pattern of aptamer-coated nanoparticles in the presence of different concentrations of analyte. The entire analysis was miniaturized on a customized microfluidic platform, allowing for automated spectrum acquisition with precise liquid manipulation. A machine learning model, based on random forest with feature engineering, was developed and evaluated for spectrum analysis, significantly enhancing the prediction of mycotoxin concentrations. This approach extended the detection limit determined by the conventional method ($\sim$ 72 ppb with high variation) to a wider range of 10 ppb to 100 ppm ($n=4$ in each of the 9 conc.) with high accuracy (overall mean absolute percentage error of 5.7%). The developed analytical tool provides a promising solution for detecting small molecules and monitoring chemical hazards in agri-food systems and environment.

Significance: The developed analytical tool provides a promising solution for detecting small molecules and monitoring chemical hazards in agri-food systems and environments.

P1-117: RISK ASSESSMENT OF SELECTED VETERINARY DRUG RESIDUES IN CATTLE IN THE USA

Primary Author:

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Joseph Eifert, Virginia Tech

Presentation type/Format: Technical

Category: Food Chemical Hazards

Abstract:

Introduction: Veterinary drug residues occur when veterinary drugs or their metabolites are detected in meat, organs, or products such as milk from food-producing animals, posing potential risks to human health.

Purpose: The primary goal of this study is to evaluate the national US cattle drug residues sampling reports and highlight the most detected drug residues in cattle and the possible risks associated with them to enhance food safety.

Methods: Veterinary drug residues sampling reports and datasets for the years 2021, 2022, and 2023 were evaluated to rank the most frequently detected drug residues in cattle. These samples were collected mainly by the National Residue program (NRP) administered by the USDA Food Safety and Inspection Service (FSIS). R Studio statistical platform was used to analyze the datasets and highlight significant trends.

Results: Upon analysis, 3,391 residues were detected across all species, with 3,107 (92%) from cattle. Across the 3,107 cattle samples, veterinary drug residues were detected in 12 different types of cattle, including dairy cows, beef cows, bulls, calves, heifers, and others. The top 10 violative residues, which exceed legal safety limits, were as follows: Desfuroylceftiofur (351), Penicillin (161), Flunixin (122), Sulfadimethoxine (94), Sulfamethazine (79), Tilmicosin (74), Neomycin (70), Florfenicol (62), Meloxicam (46), and Ampicillin (35), respectively. The top 10 violative residues account for about 35.5% of the total detected from cattle samples. Contrarily, the highest 5 detected residues at non-violative concentrations include Tulathromycin (687), Oxytetracycline (233), Eprinomectin (98), Penicillin (86), and Desfuroylceftiofur (82), respectively. The violative residues were associated with adverse health effects on humans such as allergenicity, toxicity, carcinogenicity, organ damage, and antimicrobial resistance.

Significance: Proper risk assessment approaches and robust residue monitoring measures are essential to ensure that cattle are free from drug residues to ensure public health.

Award Application: Developing Scientist Award

P1-118: MYCOTOXINS IN WHEAT AND CORN: A REVIEW OF REGULATIONS AND RISK TO THE POPULATION

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract: <

Introduction: Mycotoxins pose significant risks to food safety and public health in grains (e.g., wheat and corn), where multiple mycotoxins can be found. Control and monitoring of mycotoxins is the responsibility of the producer, but advisory and action levels are not available for some mycotoxins.

Purpose: This literature review assessed current regulation and guidance levels in the US and EU, current interventions, and health risks of aflatoxins, vomitoxin, zearalenone, T-2/HT-2 toxins, ochratoxin A, and fumonisins.

Methods: Google Scholar, Elsevier, PubMed, ScienceDirect, ConnectedPapers.com, Google Search, and government websites were searched using keywords (e.g., mycotoxin, regulation, risk, intervention). Literature was included if one or more research questions were answered and if published within the last 5 years, except for regulatory or guidance references. Of the reviewed literature, 18 articles met inclusion criteria. Research questions focused on regulations and guidance in the US and EU, risks of mycotoxins, and current options for mycotoxin mitigation intervention.

Results: Mitigation strategies and regulations in the US are not directly proportional to the understanding of the risks associated with mycotoxins. All 18 articles (100%) addressed risk (e.g., cancer, genotoxicity, organ damage), but only 38.9% discussed interventions (e.g., cold plasma, polyphenols, flavonoids, natural essential oils) and 38.9% discussed regulation and guidance. The EU has regulation for 5 mycotoxins in wheat and 6 in corn, whereas the US has advisory levels for 1 mycotoxin (vomitoxin) in wheat and 1 advisory and action level in corn (aflatoxin and fumonisins). This highlights a gap in established regulation and underscores the limited availability of mitigation research.

Significance: Mycotoxin research is essential to ensuring food protection and public health. The severity of associated health risks demands further research to evaluate both acute and chronic exposure and to support the establishment of regulatory limits.

Award Application: Developing Scientist Award

P1-119: STUDY OF TWO CANDIDATE METHODS SUBMITTED FOR AOAC SMPR 2023.003 PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN PRODUCE, BEVERAGES, DAIRY PRODUCTS, EGGS, SEAFOOD, MEAT PRODUCTS, AND FEED

Primary Author:

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Ashten Noble, Nestle Quality Assurance Center

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Susan Genualdi, U.S. Food and Drug Administration

Manjula Sunkara, U.S. Food and Drug Administration

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Presentation type/Format: Technical

Category: Food Chemical Hazards

Abstract:

Introduction: An interlaboratory comparison of analytical methods developed by FDA and Nestlé for analysis of PFAS submitted to the AOAC SMPR 2023.003 call for methods.

Purpose: Both the Nestlé and FDA methods were implemented at each laboratory to evaluate method performance and laboratory influence. The interlaboratory comparison was performed on 6 samples; fish, clams, hay, tomato powder, 80% whey protein concentrate and 35% whey protein concentrate. These shared matrices represent commonly analyzed materials by each organization to compare the performance of the methods.

Methods: Two Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) methods were evaluated by both U.S. Food and Drug Administration and Nestlé Quality Assurance Center. The FDA method utilizes a QuEChERS extraction with a dispersive SPE and WAX (weak ion exchange) clean up. Analysis of 30 analytes is performed on a SCIEX 6500+ tandem mass spectrometer. The Nestlé method utilizes an acetonitrile : water extraction with a WAX and graphitized carbon black clean up. Analysis of 56 analytes is performed on a SCIEX 7500 tandem mass spectrometer.

Results: Proportional and constant bias were evaluated by both method and laboratory for the target compounds PFOA, PFOS, PFNA, and PFHxS for matrices with detections within the analytical range of each method. 5 of the matrices had detections for one or more of the target compounds. Evaluation of constant and proportional bias using Q-Stat.net statistical software demonstrates equivalency

between the 2 methods at a 95% confidence interval. Review of alternate respective methods implemented at each lab showed some instances of bias, demonstrating the impact of lab experience in result determination.

Significance: Both methods are fit for purpose and deliver equivalent results in a variety of matrix types. The implementation of each method at each facility allowed a unique approach to study the impact of laboratory influences on method interlaboratory studies.

P1-120: MICROBIOLOGICAL QUALITY AND METHOD OPTIMIZATION FOR QUANTIFICATION OF AFLATOXIN B1 IN BUCKWHEAT

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract:

Introduction: Buckwheat is a nutrient-rich pseudocereal valued for its gluten-free status. However, few studies have determined its safety and quality, which can be compromised by microbial contamination and aflatoxin B1 (AFB1) occurrence.

Purpose: To assess buckwheat microbial quality and develop a robust AFB1 quantification method.

Methods: Sixty-four buckwheat samples were purchased and microbial quality was evaluated using culture-based

Methods. Samples were enumerated for Aerobic Plate Count (APC), Enterobacteriaceae (EB), *E. coli*, coliforms, yeast, and mold. For AFB1 quantification, 50 g buckwheat flour, 10 g HPLC-grade salt, and 200 mL 80% methanol were blended for 1 minute and filtered. Filtrates were cleaned up with a combination of immunoaffinity columns, Celite and/or aluminum oxide. Eluates were evaluated by fluorometry and HPLC. For HPLC, air-dried eluates were resuspended in TFA solution and injected into a C18 column with 0.5 mL/min mobile phase flow rate and AFB1 was quantified using a fluorescence based detector. Method performance was evaluated based on recovery and matrix effect.

Results: Enumeration in buckwheat flour and groats showed, respectively, APC at 6.31 and 5.26 log CFU/g, EB at 4.99 and 5.28 log CFU/g, mold at 4.89 and 4.78 log CFU/g, coliform at 4.01 and 3.40 log CFU/g, and <1 log CFU/g for *E. coli* counts in both products. AFB1 recoveries for spiked samples with immunoaffinity clean-up ranged from 175.0-195.0% for fluorometry and 87.9-89.8% for HPLC. Using five Grams of aluminum oxide, fluorometry recoveries were 29.0-38.0%, compared to 50.9-61.5% for HPLC. The combination of aluminum oxide and Celite improved fluorometry recoveries to 49.50%, while affecting HPLC (69.3-70.2%) recoveries. HPLC was a better method due to its specificity, better recovery rates, and less matrix interference (8.7%).

Significance: This study shows that microbial contamination may occur during buckwheat milling, and HPLC with immunoaffinity column clean-up can be reliably used to quantify AFB1.

Award Application: Developing Scientist Award

P1-121: HEAVY METALS IN VARIOUS SPICES PURCHASED IN THE NEW YORK CITY AREA

Primary Author:

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Sana Mujahid, Consumer Reports

Eric Boring, Consumer Reports

James Rogers, Consumer Reports

Kristen Dorrell, Consumer Reports

Category: Food Chemical Hazards

Abstract:

Introduction: The majority of spices used in the U.S. are imported and purchased from both major retailers and from ethnic stores. Concerning levels of heavy metals in spices, particularly lead in specific spices, have been reported in published research as well as testing by the U.S. Food and Drug Administration (FDA).

Purpose: We evaluated various spices, purchased from non-mainstream and mainstream grocery stores, for the presence of arsenic, cadmium, lead, and mercury.

Methods: Three samples of 77 products (n=231) representing different spices and spice mixes were purchased from 33 stores, including multiple locations of supermarkets and ethnic stores primarily in NY, CT, NJ, and PA, as well as in VA and online. Spices common in South Asian, East Asian, African, Middle Eastern, South American, and Eastern European cuisines were included. Samples were prepared and analyzed for arsenic, cadmium, lead, and mercury using inductively coupled plasma triple quadrupole mass spectrometry (ICP-QQQ-MS) according to AOAC Method 2015.01. A mixed effects model was used to compare the lead levels of different spice categories.

Results: At least one heavy metal (arsenic, cadmium, lead, or mercury) was detected in each of the 77 spice products tested. Arsenic and cadmium were detected in 99%, mercury in 53%, and lead in all products tested. Arsenic concentrations averaged from non-detect (< 1.9 ppb) to 460 ppb. Cadmium concentrations averaged from non-detect (< 1.1 ppb) to 534 ppb. Lead averaged from 1.1 ppb to 3517 ppb. Mercury averaged from non-detect (< 3.0 ppb) to 46 ppb. The least-squares mean for all cinnamon and cinnamon blends at 861 ppb was significantly higher than for non-cinnamon spices (137 ppb) ($p < 0.01$).

Significance: Some commonly consumed spices can expose consumers to concerning levels of heavy metals.

P1-122: STABILITY OF *LISTERIA MONOCYTOGENES* IN POWDERED PEANUT BUTTER DURING ISOTHERMAL TREATMENT AND LONG-TERM STORAGE

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Presentation type/Format: Technical

Category: Low-Water Activity Foods

Abstract:

Introduction: Recalls of low-moisture foods (LMFs) due to potential contamination of *Listeria monocytogenes* have increased in recent years. These recalls highlight the need to understand this pathogen's survival and thermal inactivation kinetics in such products.

Purpose: To examine the stability and thermal resistance of *Listeria monocytogenes* in peanut butter powder (PBP) during extended storage at different water activity levels.

Methods: PBP samples (n=3; 300 g each) were inoculated with a 5-strain *L. monocytogenes* cocktail (~9.0 log CFU/g), equilibrated to 0.25 or 0.45 aw at 22 °C for 6 days, and subjected to isothermal treatments (75 and 80 °C) in a water bath using thermal death time (TDT) aluminum test cells. The remaining samples were aliquoted in moisture-barrier bags, sealed, and stored at 22 °C for up to 52 weeks. Surviving *L. monocytogenes* cells were enumerated at pre-determined sampling points using an overlay method. A Weibull model was used to describe thermal inactivation kinetics.

Results: *L. monocytogenes* population in PBP decreased gradually over the first 3 months of storage (~3.2 log CFU/g reduction) but then remained stable for up to 52 weeks at average levels of 5.8 ± 0.3 log CFU/g (0.25 aw) and 4.5 ± 0.4 log CFU/g (0.45 aw). Thermal resistance of *L. monocytogenes* was inversely related to aw. The nonlinear Weibull model satisfactorily described the *L. monocytogenes* inactivation kinetics ($R^2_{adj} > 0.95$). The D75°C and D80°C at 0.25 aw were 13.6 and 6.1 min, while at 0.45 aw were 5.7 and 0.4 min, respectively. The z-values at 0.25 and 0.45 aw were 14.3 and 4.5 °C, respectively.

Significance: This study contributes to our understanding of the survival of *L. monocytogenes* in LMFs during desiccation and thermal processing. These findings are thus important in supporting the development and validation of thermal processes to control *Listeria* contamination in powdered peanut butter.

Award Application: Developing Scientist Award

P1-123: THERMAL RESISTANCE AND LONG-TERM SURVIVAL OF *Listeria monocytogenes* IN WHOLE EGG POWDER

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: The increase in recalls of low-moisture foods (LMFs) due to *Listeria monocytogenes* contamination highlights the need to understand the survival and thermal inactivation kinetics of this pathogen in these types of products.

Purpose: To examine the stability and thermal resistance of *Listeria monocytogenes* in dried whole egg powder (WEP) during extended storage.

Methods: WEP samples (n=3; 300 g each) were inoculated with a 5-strain *L. monocytogenes* cocktail (~9.0 log CFU/g), equilibrated to 0.45 aw at 22 °C for 6 days, and subjected to isothermal treatments (75 and 80 °C) in a water bath using thermal death time (TDT) aluminum test cells. The remaining samples were aliquoted in moisture-barrier bags, sealed, and stored at 22 °C for up to 52 weeks. Surviving *L. monocytogenes* cells were enumerated at pre-determined sampling points using an overlay method. A Weibull model was used to describe thermal inactivation kinetics.

Results: *L. monocytogenes* population in WEP decreased gradually but remained detectable after 52 weeks of storage. A reduction of 7.6 ± 0.8 log CFU/g was observed in *Listeria* counts at the end of storage. The thermal inactivation curves of *L. monocytogenes* in WEP (0.45 aw) at target temperatures showed an upward concavity ($\beta < 1$), which was satisfactorily described by the Weibull model ($R^2_{adj} > 0.98$). The D75°C and D80°C were 4.4 and 1.8 min, respectively. The z-value was 13.1.

Significance: This study contributes to our understanding of the survival of *L. monocytogenes* in LMFs during desiccation and thermal processing. These findings are thus important in supporting the development and validation of thermal inactivation strategies to control *Listeria* contamination in dried whole egg powder.

Award Application: Developing Scientist Award

P1-124: COMPARISON OF TEMPERING TREATMENT TECHNOLOGIES FOR PATHOGEN REDUCTION ON WHEAT GRAIN

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Outbreaks and recalls of wheat flour due to contamination with *Salmonella* or Shiga toxin-producing *E. coli* (STEC) have raised concerns regarding food safety, and generated interest in determining mitigation treatments that can be incorporated into existing unit operations.

Purpose: Examine the efficacy of four different technologies as a tempering treatment to inactivate pathogens.

Methods: Wheat grain was inoculated with five-strain cocktails of *Salmonella* (serovars Enteritidis PT30, Typhimurium, Mbandaka, Tennessee, Montevideo) and STEC (serotypes O157:NM, O157:H7, O26:H11, O121:H19) at 8 log CFU/g, aw was equilibrated to 0.45, followed by a week of storage prior to 18h tempering. Sterile tap water, lactic acid (LA; 5% and 20%), peracetic acid (PeraGrain; 3000 and 6000 ppm), Neo-Temper (NT; I and II), and hypochlorous acid (HClO; 1200 and 2000 ppm) were applied at 4% (w/w) grain.

Results: No significant differences between *Salmonella* and STEC were observed when treated with LA and NT. Using 5% and 20% LA led to 0.99 ± 0.16 and 1.71 ± 0.22 log CFU/g reduction, respectively. NT I and II led to 1.50 ± 0.60 and 2.26 ± 0.55 log CFU/g reduction, respectively. 3000 ppm PeraGrain led to significantly greater reduction of *Salmonella* than STEC, with 2.62 ± 0.26 and 2.07 ± 0.22 log CFU/g, respectively. 6000 ppm PeraGrain had similar reductions between species, averaging 3.32 ± 0.24 log CFU/g. At 1200 ppm, HClO had significantly lower reduction of *Salmonella* at 0.98 ± 0.13 , compared to 1.60 ± 0.15 log CFU/g for STEC. HClO at 2000 ppm had similar reductions between species, averaging 1.81 ± 0.25 log CFU/g. Except for 5% LA and NT I for STEC, all technologies evaluated significantly reduced pathogen populations ($P < 0.05$) during tempering compared to the water control.

Significance: The data presented here will be useful for the milling industry considering use of an antimicrobial tempering technology. Impacts on end-use

quality and cost of implementation will also be important for decision-making.

Award Application: Developing Scientist Award

P1-125: THE EFFECT OF X-RAY IRRADIATION TECHNOLOGY ON SALMONELLA SPP. AND QUALITY PARAMETERS OF READY-TO-BAKE CHOCOLATE CHIP COOKIE DOUGH

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: in cookie dough, *Salmonella* contamination can come in many forms through eggs, flour, or even chocolate chips, and despite warnings to avoid raw consumption, many consumers still risk infection through eating raw dough.

Purpose: This study aimed to investigate the potential of X-ray irradiation as an inactivation step for *Salmonella* in one brand (Brand A) of ready-to-bake chocolate chip cookie dough and the effect of X-ray on various quality parameters in two brands of cookie dough (Brand A and Brand B).

Methods: A 4-serotype cocktail of *Salmonella* at 10 log CFU/g was prepared and inoculated into 10 g of Brand A's ready-to-bake chocolate chip cookie dough with a final concentration of 6.5 log CFU/g. The inoculated dough was then treated with 0, 0.25, 0.5, and 1 kGy doses of X-ray irradiation. The following quality parameters were evaluated for both brands: pH, aw, and colorimetry.

Results: *Salmonella* counts were decreased by 1.15, 1.79, and 2.49 log CFU/g by 0.25, 0.5 and 1 kGy, respectively. There was no statistical difference shown for pH for Brand A treated dough, while Brand B treated dough had a significant decrease from the control. For aw, it decreased significantly in the raw dough for the 1 kGy dosage in both brands from 0.78 to 0.75 (Brand A) and 0.75 to 0.72 (Brand B), while the color of the dough visually darkened in both brands as the dosage

increased.

Significance: Overall, the results conclude that X-ray irradiation could be used as an inactivation or reduction step to control pathogens in cookie dough, though its potential effect on quality should be considered.

Award Application: Developing Scientist Award

P1-126: EFFECTIVENESS OF INTENSE PULSED LIGHT, COLD PLASMA, AND UV RADIATION IN REDUCING SALMONELLA CONTAMINATION ON WHEAT GRAINS: A COMPARATIVE ANALYSIS

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Jared Rivera, Kansas State University

Kaliramesh Siliveru, Kansas State University

Snehasis Chakraborty, Kansas State University

Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Wheat is highly susceptible to microbial contamination during cultivation and processing, making it a potential carrier of pathogens like *Salmonella*. The growing number of foodborne outbreaks linked to *Salmonella*-contaminated wheat products highlights the urgent need for effective decontamination strategies.

Purpose: This study aimed to compare the effectiveness of three non-thermal decontamination techniques—intense pulsed light (IPL), cold plasma, and short-wavelength ultraviolet (UV) radiation—in inactivating *Salmonella* species on wheat grain.

Methods: Wheat grains were inoculated with a *Salmonella* cocktail (~8 log CFU/g) and dried to their pre-inoculum weight. Subsequently, 10 g wheat samples were treated with cold plasma (10–25 kV for 1–20 min), UV (265, 280, and 365 nm for 1–40 min), and pulsed light (PL, 0.2–2.59 J/cm²) for decontamination. The reduction in *Salmonella* load was assessed by plating on xylose lysine deoxycholate agar (XLD) following serial dilution and tryptic soy agar (TSA) overlay.

Results: All three treatments effectively inactivated *Salmonella* on wheat grains. However, PL (2.59 J/cm²) was the most effective, achieving a 5-log CFU/g reduction, followed by cold plasma (25 kV/20 min) and UV (265 nm/35 min), which reduced the pathogen load by approximately 2 log CFU/g. This higher efficacy of PL is attributed to its combined photochemical, thermal, and oxidative stress effects, while cold plasma primarily induced oxidative stress through reactive oxygen and nitrogen species (RONS). UV-C, on the other hand, inactivated *Salmonella* through DNA damage. SEM and FTIR analyses revealed significant structural and compositional changes in *Salmonella* cells, corroborating the inactivation mechanisms. Optical emission spectra further confirmed RONS at

wavelengths of 328–426 nm, 739 nm, and 778 nm for cold plasma generated in air.

Significance: These findings offer valuable insights into selecting effective decontamination methods to enhance food safety in wheat grains and wheat-based products, particularly for preventing *Salmonella* contamination.

Award Application: Developing Scientist Award

P1-127: SYNERGISTIC EFFECTS OF UV/VIS TREATMENT AND ACIDIC WATER TEMPERING AGAINST PATHOGENIC E. COLI CONTAMINATION IN HARD RED WINTER (HRW) WHEAT GRAINS

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Kaliramesh Siliveru, Kansas State University

Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Acidic water tempering has limitations in their efficacy as a pathogen reduction step for wheat milling. Combining this treatment with novel non-thermal treatments could improve their pathogen reduction capability.

Purpose: This study evaluated the synergistic effects of UV/Vis treatment and acidic water tempering as a pathogen reduction step against Shiga toxin-producing *E. coli* (STEC) O121 and O26 contamination on wheat grains.

Methods: Hard red winter (HRW) wheat grains were used in this study. The STEC O121 (ATCC 2219) / O26 (ATCC 2196) cocktail was inoculated into pre-dried wheat grains (5.8 ± 0.2 log CFU/g). The inoculated wheat grains were treated with UV/Vis under different wavelength (264, 280, and 366 μ m) and treatment times (0, 5, 10, and 20 mins). The most effective treatment combinations were combined with acidic water tempering (5% v/v lactic acid). Treated wheat grains were tempered (18 h) with samples taken at 2, 4, 6, 12, and 18 h. The STEC enumeration (log CFU/g) was done by plating in MacConkey agar (0.1 ml, duplicates).

Results: The UV/ Vis treatment at 264 μ m reduced (0.8 to 1.0 logs; $p < 0.05$) the STEC load of wheat grains after treatment (20 mins). Combining this treatment conditions with acidic water tempering (5% v/v lactic acid) resulted in an improved STEC reduction (2.8 to 3.1 logs; $p < 0.05$) relative to only using acidic water tempering (1.8 to 2.0 logs) after 18 h of tempering. A synergistic effect ($O/E > 1$) between the individual treatments was also observed.

Significance: The result of this study indicates that the UV/Vis has synergistic effects with acidic water tempering against STEC O121 and O26 on wheat grains. The proposed treatment can then be used as a mitigation step for wheat milling to minimize STEC contamination.

P1-128: ASCORBIC ACID SIGNIFICANTLY REDUCES THERMAL RESISTANCE OF SALMONELLA SPP. AND ENTEROCOCCUS FAECIUM IN DRY HEAT

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Modeling microbial inactivation under drying conditions is critical for controlling pathogens while maintaining product quality. While high acidity has been shown to reduce bacterial thermal resistance in fruits, its influence under high-temperature, low-humidity environments relevant to drying processes remains unclear.

Objective: This study investigates the impact of high acidity on the thermal death kinetics of *Salmonella* spp. and *Enterococcus faecium* NRRL B-2354 (a *Salmonella* surrogate) under high-temperature, low-humidity conditions representative of drying processes.

Methods: Three-strain bacterial cells of *Salmonella* cocktail (*S. Enteritidis* PT30 STCC BAA-1045, *S. Mbandaka* 69858, and *S. Tennessee* K4643) and *E. faecium* were inoculated onto sand (50 g) with or without 1 mL of 3.4% ascorbic acid (pH 2.30) to reflect high-acidity environments in fruits. The inoculated sand was dried at room temperature and RH <10%. Isothermal inactivation (80°C, 100°C, 120°C) at constant low humidity (RH <0.5%) was measured using thermal water activity cells (TAC).

Results: Initial populations of *Salmonella* and *E. faecium* on sand were 9.1 ± 0.7 and 8.6 ± 0.4 log CFU/g, respectively. Ascorbic acid reduced *E. faecium* by 1.4 log but had no significant impact on *Salmonella*. Thermal inactivation followed a linear log reduction with time, and D-values decreased exponentially with increasing temperature, consistent with the Arrhenius model. The Z-values (16–21°C) showed consistent temperature effects with or without ascorbic acid. However, D-values were significantly lower with ascorbic acid, with log D-values reduced by 1.1–1.6, corresponding to an 11–38-fold decrease. Overall, ascorbic acid significantly reduced the thermal resistance of both bacteria, with *E. faecium* serving as an effective *Salmonella* surrogate.

Significance: High-acid environments influence thermal inactivation of both

bacteria, highlighting *E. faecium* as an effective *Salmonella* surrogate. These findings suggest that high-acid food products may require less intense heat treatments for microbial safety. Incorporating product pH into microbial inactivation models can enhance process optimization and prediction accuracy.

Award Application: Developing Scientist Award

P1-129: ASSESSING VARIATION IN DESICCATION TOLERANCE ASSOCIATED PHENOTYPES AMONG SALMONELLA SEROVARS

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Teresa Bergholz, Michigan State University

Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Several strains of *Salmonella*, from multiple serovars, have proven to be persistent in low moisture environments, leading to outbreaks and illness around the world. Current data on persistence of *Salmonella* in dry environments largely involves a select few serovars and provides insight on various stress-associated phenotypes which have the potential to be associated with desiccation tolerance in *Salmonella*. These phenotypes include functionality of RpoS, the regulator of the general stress response, and production of curli and cellulose-components of the extracellular matrix which assist in water retention, adhesion, and biofilm formation.

Purpose: To assess variation in desiccation tolerance associated phenotypes over a range of serovars to better understand their contribution to desiccation tolerance. Methods: A total of 114 *Salmonella* strains from six serovars – Agona, Enteritidis, Montevideo, Tennessee, Typhimurium, and Typ1,4,[5],12:i:- were utilized for these assays. Luria Bertani (LB) agar supplemented with Congo Red and Brilliant Blue dye and LB agar supplemented with Fluorescent Brightener 28 were used to assess production of curli and cellulose, through observing colony morphology, respectively. RpoS functionality was assessed using the HPII Catalase test. Findings were analyzed using Fisher's exact test to determine what relationships exist between the phenotypes and serotypes.

Results: A significant association ($p < 0.05$) between serovar and cellulose production was observed. Serovars with the majority of strains producing cellulose included Typ1,4,[5],12:i:-, Typhimurium, and Tennessee. However, there was no significant association ($p < 0.05$) observed between serovar and curli production or serovar and RpoS functionality.

Significance: These results demonstrate variation in desiccation tolerance associated phenotypes and provide useful insights for the next steps of research, quantifying desiccation tolerance of the strains and serovars addressed.

Award Application: Developing Scientist Award

P1-130: ISOTHERMAL INACTIVATION OF ENTEROCOCCUS FAECIUM NRRL B-2354 ON APPLE CUBES AT DIFFERENT TEMPERATURES AND WATER ACTIVITIES

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: *Salmonella* is known to survive in dried fruits, and its inactivation is dependent on water activity (aw) and temperature. *Enterococcus faecium* NRRL B-2354 is commonly used as a surrogate for *Salmonella* in low-moisture foods. However, data for use in dried apples is limited.

Purpose: Evaluate the effects of temperature and aw on the inactivation of *E. faecium* on partially dried apple cubes.

Methods: Fresh-cut apple cubes, pre-dried in a home-type convection oven, were transferred into humidity-controlled chambers to equilibrate to the target aw (0.45 and 0.60) for at least 48 h. *E. faecium* NRRL B-2354 was harvested from plate-grown culture and inoculated onto pre-equilibrated apple cubes. The inoculated apple cubes were placed in humidity-controlled gloveboxes for reconditioning up to 96 h and packed into aluminum test cells for isothermal treatments in a water bath. At various time points (n=6), test cells (n=3) were removed from the water bath and immersed in ice immediately. The apple cubes were retrieved and enumerated for *E. faecium*.

Results: The starting inoculated levels of *E. faecium* for aw 0.45 and 0.60 were 8.68 and 8.54 log CFU/g, respectively. At aw 0.45, D-values at 67.5, 70.0, and 80.0°C were 12.13, 3.51, and 1.91 min, respectively (z-value 15.43°C). At aw 0.60, D-values at 60.0, 67.5, and 75.0°C were 43.66, 5.91, and 3.57 min, respectively (z-value 13.81°C). A significantly (p<0.05) higher D_{67.5°C} was observed at aw 0.45 than at aw 0.60. Compared to previously reported data, D-values for *E. faecium* at aw 0.60 were lower at 67.5°C and higher at 60 and 75°C than *Salmonella* (D_{60°C} 35.92 min, D_{67.5°C} 10.50 min, D_{75°C} 3.07 min).

Significance: The suitability of *E. faecium* as a surrogate for *Salmonella* in dried

apples is not guaranteed. This work demonstrates the need to collect the data to substantiate surrogate suitability.

Award Application: Developing Scientist Award

P1-131: AIR-DRIED BEEF: COMPARISON OF ACID-ADAPTED AND NON-ADAPTED SALMONELLA SEROVARS IN PROCESS VALIDATION TO ACHIEVE 5-LOG REDUCTION

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Air-dried beef such as biltong and droëwors do not follow traditional beef jerky processing guidelines. USDA-FSIS relies on published peer-reviewed literature on process efficacy to make regulatory decisions on process safety. The absence of such information is considered 'knowledge gap' research areas. USDA-FSIS further requests the use of 'acid-adapted' challenge cultures to provide acid-tolerant organisms for validation of processes involving acidic treatment.

Purpose: The objectives of this study were to compare microbial lethality using acid-adapted versus non-acid-adapted cultures during biltong/droëwors processing to determine the effect on process outcome and challenge current validation assumptions.

Methods: Acid adaptation was achieved by growing challenge cultures in Tryptic Soy (TS) broth with 1% glucose; non-adapted cultures were grown in glucose-free or buffered broth. Trials were conducted using beef inoculated with acid-adapted or non-adapted mixtures of five *Salmonella* serovars. Beef samples were marinated by vacuum tumbling with spices, salt, and vinegar (biltong, droëwors) or after marination, ground and encased in 17-mm casings (droëwors), and then dried at 75°F and 55% RH for 8 to 10 days. Trials were performed in duplicate with triplicate samples (n=6) enumerated on Selenite Cystine agar. For droëwors processing, a clarified and reduced-flavor liquid smoke extract was used (0.3, 0.5, 0.75%; Kerry Foods). Statistical analysis of significance ($p \leq 0.05$) of timed-series data was performed using repeated measures one-way analysis of variance (RM-ANOVA).

Results: Acid-adapted cells showed increased sensitivity during the extended biltong/droëwors drying process involving salt and desiccation than non-adapted cultures. Based on these data, non-adapted cultures were used for droëwors processing, achieving a significant 6-log reduction in *Salmonella* without compromising organoleptic qualities.

Significance: These findings challenge the assumption that acid-adapted cultures

are preferable because they are more tolerant of acidic processes and suggests that acid-adaptation may lead to stresses that can sensitize bacterial challenge cultures to inhibitory conditions in complex processes.

Award Application: Developing Scientist Award

P1-132: THERMAL INACTIVATION OF SALMONELLA IN FLAXSEED UNDER DYNAMIC ELEVATED HEAT TREATMENT

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: While *Salmonella* shows increased thermal tolerance in low-moisture food, requiring additional heat treatment to inactivate, research is needed to validate *Salmonella* lethality at elevated temperature.

Purpose: This study estimates *Salmonella* inactivation parameters in flaxseed at elevated temperatures using a one-step sequential approach and compares two secondary inactivation models for dynamic temperature conditions.

Methods: Flaxseeds were inoculated with *Salmonella enterica* Enteritidis PT30 and equilibrated to water activity 0.55 ± 0.03 . Heat treatment was conducted using a heated circulator with silicon oil at 95, 105, or 120°C for 11 equidistant time points with intervals ranging from 15 to 30 s. For each biological replicate, 0.8 g of inoculated flaxseed samples ($n=12$) were placed in two aluminum test cells per time point along with one thermocouple-equipped cell containing 0.8 g of uninoculated flaxseed. Two secondary models (Bigelow type and Weibull/Mafart) were compared using the root mean squared errors (RMSE) and the corrected Akaike information criterion (AICc).

Results: *Salmonella* survived elevated temperatures in flaxseeds, requiring a 36°C increase from the 71°C reference temperature to achieve a 10-fold increase in lethality rate ($D_{71^\circ\text{C}}\text{-value} = 3.36\text{--}3.93$ min). Statistical analyses indicated that among the two secondary models, the Weibull/Mafart model better described the thermal inactivation kinetics at elevated temperatures, with lower RMSE (0.52 log CFU/g) and AICc values at all temperatures. Experiments at higher oil bath temperatures were better for estimating $D_{71^\circ\text{C}}\text{-value}$, $z\text{-value}$, and n , because the scaled sensitivity coefficients are larger. Overall, inactivation parameters for

Salmonella at elevated temperatures under dynamic conditions can be estimated simultaneously.

Significance: These results provide insights to help food processors develop or refine *Salmonella* predictive models in low-moisture foods under elevated dynamic temperature conditions. In comparison to previous studies, *Salmonella* was more heat resistant on flaxseeds heated at elevated temperatures than at milder temperatures (<90°C).

Award Application: Developing Scientist Award

P1-133: PSEUDOMONAS AERUGINOSA AND SALMONELLA ENTERICA ENHANCE CRONOBACTER SAKAZAKII'S GROWTH IN DUAL-SPECIES IN VITRO DRY SURFACE BIOFILMS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: There is increasing evidence that pathogens can persist over extended periods in low-moisture environments such as dry or desiccated biofilms. *Cronobacter sakazakii*, an emerging opportunistic foodborne pathogen that causes life-threatening diseases in neonates and infants, was a global food security crisis in contaminated powdered infant formula in 2022.

Purpose: This study aimed to develop single- and dual-species dry surface biofilm models to better understand *C. sakazakii* adaptability and interactions with other pathogens in low moisture conditions.

Methods: Four *C. sakazakii* biofilm-forming strains (ATCC 12868 and three clinical isolates) were selected for developing multi-species biofilms with *Pseudomonas aeruginosa* ATCC 15442 and *Salmonella enterica* serovar Typhimurium ATCC 14028. Biofilms were developed in a CDC Biofilm Reactor® on borosilicate glass coupons based on the EPA MLB SOP MB-19 protocol. Biofilms were harvested and desiccated for 24, 48, and 72 hours at 21°C to form dry-surface biofilms (DSBs). DSBs were imaged with confocal laser scanning microscopy (CLSM) in triplicate; statistical analysis was completed in SAS.

Results: *C. sakazakii* ATCC 12868 and a clinical isolate had approximately one and two log₁₀ increase, respectively, when co-cultured with *Pseudomonas aeruginosa* and *Salmonella* ($p < 0.001$), suggesting that *C. sakazakii* may benefit from the interaction with both microorganisms. However, *P. aeruginosa* density decreased to 3 log₁₀ when grown with *C. sakazakii* ATCC 12868 ($p < 0.05$). On average, *Salmonella* density did not change. CLSM confirmed biofilm development and that the biofilm structure remained over time.

Significance: *C. sakazakii* can form multi-species biofilm in vitro and cells remain viable over time. This work is meaningful toward the larger goal of better

understanding low-moisture food systems.

Award Application: Developing Scientist Award

P1-134: SATURATED STEAM TREATMENT AS A KILL-STEP FOR SALMONELLA ENTERICA REDUCTION ON IN-SHELL PECANS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: On-farm harvesting and handling practices can pose risks to the safety of pecans, potentially leading to foodborne pathogen contamination. While steam treatment of in-shell pecans is a potential conditioning method that growers can use to facilitate shelling, it also holds potential as a decontamination strategy.

Purpose: This study evaluated the efficacy of steam treatments on *Salmonella enterica* inactivation on in-shell pecans.

Methods: In-shell pecans were inoculated with a five-strain cocktail of nalidixic acid-resistant mutant of *Salmonella enterica* (Anatum, Tennessee, PT9c, Oranienberg, Enteritidis PT30) to ~8 log CFU/g. The samples were steam-treated at 70, 80, and 90°C for 0–300 s using a custom-designed batch setup system after a come-up time of 20 s. After treatment, the pecans were massaged in 100 mL of 0.1% sterile peptone water, and plated on Xylose Lysine Deoxycholate agar with nalidixic acid (50 µg/mL) and enumerated after incubation at 37°C for 24 and 48 h. The water activity of the samples before and after treatment was measured. The data was analyzed using a mixed model with post-hoc Tukey test at $p < 0.05$.

Results: At 70°C, *Salmonella* was significantly reduced ($p < 0.05$) by 4.65 ± 0.01 log CFU/g within 120 s, whereas a 5-log reduction required 150 s of exposure. When the temperature was increased to 80°C, a 10 s exposure led to a 2.10 log CFU/g reduction, which increased ($p < 0.05$) to a 5.30 log CFU/g reduction at 90 s. Increasing the temperature to 90°C showed a similar reduction trend as that of 80°C. A 5-log reduction was achieved within 90 s when in-shell pecans were treated at 90°C. Steam treating the pecans for up to 5 mins at all tested temperatures increased water activity of the kernel (0.608 ± 0.002 for 70°C, 0.615 ± 0.002 for 80°C, and 0.608 ± 0.004 for 90°C vs 0.58 ± 0.006 for control).

Significance: Steam treatment of in-shell pecans can effectively reduce *Salmonella* contamination, thereby enhancing their microbiological safety.

P1-135: COMPARING THE SURVIVAL OF CO-INOCULATED *SALMONELLA* ENTERITIDIS PT 30 AND *ENTEROCOCCUS FAECIUM* NRRL B-2354 ON WHOLE AND BROKEN ALMONDS DURING DRY HEATING AT 168 °C

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: *Enterococcus faecium* NRRL B-2354 (EF) is commonly used as a surrogate for *Salmonella* in thermal process validation for whole almonds. Data are limited comparing the thermal resistance of these organisms on the common product 'broken almonds'.

Purpose: To compare survival of wild-type or rifampin-resistant *Salmonella* Enteritidis PT 30 (*Salmonella*-wt or *Salmonella*-rif, respectively) and EF on whole and broken almonds during dry heating using a novel co-inoculation method.

Methods: *Salmonella*-wt or *Salmonella*-rif harvested from agar lawns were individually mixed with an equivalent amount of EF and almonds were ;co-inoculated with EF and either *Salmonella*-wt or *Salmonella*-rif. Co-inoculated almonds (~8 log CFU/g) were dried at ambient conditions to 5.5% moisture content. Dried almonds were then heated in a gravity oven at 168 °C for 0–12 min (four technical and two biological replicates n=8). Populations were enumerated on CHROMagar *Salmonella* (ChromSal; both *Salmonella*), tryptic soy agar with rifampin (50 µg/mL, TSAR *Salmonella*-rif), and Slanetz and Bartley agar (S+B; EF).

Results: Neither *Salmonella* were recovered from S+B, and EF was not recovered from ChromSal or TSAR. Within the same almonds after dry heating for 12 min, no significant differences in reductions were observed between *Salmonella*-wt and *Salmonella*-rif or between ChromSal and TSAR (P=0.05). However, *Salmonella*-rif declines more than EF on whole almonds but similar on broken almonds. *Salmonella*-rif and EF declines of 3.4 ± 0.6 and 2.9 ± 0.6 log, respectively, were observed on whole almonds, whereas declines of 3.8 ± 0.5 and 3.9 ± 0.9 log, respectively, were observed on broken almonds.

Significance: *E. faecium* is an appropriate surrogate for *Salmonella* in both whole and broken almonds during dry heating at 168 °C. A novel co-inoculation method facilitated comparing the thermal tolerance of the two organisms.

P1-136: THERMAL INACTIVATION OF CRONOBACTER SAKAZAKII IN RECONSTITUTED POWERED INFANT FORMULA: SYSTEMATIC REVIEW AND META REGRESSION

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Presentation type/Format: Technical

Category: Low-Water Activity Foods

Abstract:

Introduction: *Cronobacter sakazakii* is an opportunistic pathogen associated with powdered infant formula (PIF) . Reconstituting PIF with water $\geq 70^{\circ}\text{C}$ is recommended, however, adherence to this advice may be inconsistent due to nutrient loss and practical difficulties. Therefore, enhanced understanding of *C. sakazakii*'s thermal inactivation over a wider temperature range is crucial for effective risk management.

Purpose: Quantify the relationship between various temperatures and the inactivation of *C. sakazakii* during the preparation of infant formula.

Methods: A systematic review was conducted across three bibliographic databases using terms reflecting concepts of *Cronobacter* spp. and thermal inactivation. Articles were screened in two phases (title/abstract and full text), and data was extracted from relevant studies. Subgroup analysis was employed based on temperature ranges. Mixed-effects model was applied to quantitatively describe *C. sakazakii* response to various temperatures over time by considering food matrix and inoculum characteristics.

Results: Of 2,197 articles screened, 14 were identified relevant for data extraction, covering temperature ranges from 48°C to 100°C . The subgroup analysis revealed distinct thermal inactivation profiles for *C. sakazakii*: at 48°C to 55°C , the pooled D-value was 17.48 min (95% CI: 12.52, 22.34); at 55°C to 60°C , it was 4.40 min (95% CI: 2.65, 6.14); and at 60°C to 66°C , it dropped to 1.39 min (95% CI: 0.75, 2.03). Temperatures of 70°C and above were most effective, achieving reduction of bacterial counts to below detection limit (1 log CFU/ml) immediately. The meta-regression highlighted non-linear temperature effect. Additionally, inocula consisting of cocktail strains tended to yield higher D-values compared to single strains, emphasizing the variability in thermal resistance among different strains.

Significance: This study emphasizes the need to adhere to recommended PIF preparation temperatures to prevent infections from *C. sakazakii* particularly considering strain diversity, and provides a thermal inactivation profile to better assess risks in real practices.

Award Application: Developing Scientist Award

P1-137: QUANTIFYING PATHOGEN TRANSFER FROM LOW-MOISTURE FOOD PERSISTENT BACTERIAL POPULATIONS (LMF PBP) TO NON-CONTAMINATED PRODUCTS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Cross-contamination in low-moisture food (LMF) processing poses significant risks for bacterial transfer when pathogens are harbored on equipment or tools. Cross-contamination is likely impacted by environmental conditions, such as relative humidity (RH). Quantifying these risks is crucial for implementing effective risk management strategies.

Purpose: This study quantified LMF persistent bacterial populations (PBP) of *Salmonella* Tennessee transferred from surfaces to foods through cross-contamination impacted by RH.

Methods: Stainless steel coupons were inoculated with high (7-8 log CFU) or low (3-4 log CFU) concentration of *Salmonella* Tennessee to establish an LMF PBP by two

Methods to simulate different contamination scenarios: 1) premixed slurry of powder and inoculum (0.5 mL:0.4 g) or 2) powder pressed on top of the inoculum (0.1 mL:0.4 g). Coupons were then stored at 30 or 70% RH (25°C). The percent transfer (PT) of LMF PBP to powder was calculated on days 3 or 7 by placing a coupon into a sterilized 250-mL conical tube with 10 g of non-inoculated NFDM and vortexing for 2 minutes. The coupon was then submerged into 20 mL of 0.1% peptone, while the cross-contaminated NFDM powder was sampled by diluting and plating. Data were analyzed with ANOVA and Tukey's HSD to determine significant differences.

Results: The PT of *Salmonella* increased as RH decreased, ranging from 67–84% at 30% RH to 28–50% at 70% RH. Each RH level, matrix, and method significantly influenced the PT ($p < 0.05$) and displayed a three-way interaction (RH:Matrices:Method). The highest observed log CFU transfers for the low and high inocula were 3.5 and 5.3 log CFU, respectively.

Significance: Quantifying cross-contamination risks and bacterial transfer in LMF

systems could help establish proper hygienic breaks. This study revealed complex relationships between environmental factors and subsequent cross-contamination, which should be further investigated to inform targeted risk mitigation strategies in food manufacturing environments.

Award Application: Developing Scientist Award

P1-138: EVALUATION OF REAL-TIME PCR METHODS FOR THE DETECTION OF LISTERIA MONOCYTOGENES IN SPICES, SEASONINGS, AND FLAVORINGS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Infection via *Listeria monocytogenes*, which causes listeriosis, is most threatening to the pregnant, elderly, and immunocompromised population. *L. monocytogenes* is commonly found in deli meat and unpasteurized dairy products. However, contamination of the pathogen in food additives like spices, seasonings, and flavors is possible due to external factors like product environment and handling. Due to anti-bacterial characteristics of these food products and their likeliness to interfere with PCR, there is relatively little historical data on the growth and detection of *L. monocytogenes*.

Purpose: To evaluate two real-time PCR methods (GENE-UP LMO and LIS) to rapidly detect *L. monocytogenes* in challenging low moisture matrices.

Method: A variety of spices, seasonings, and flavorings containing inhibitory elements like vanillin, propylene glycol, vinegar powder, or heat treatment were tested for *L. monocytogenes* using PCR. Thirty samples of each spice were tested in 25 g portions, seasonings in 125 g, and ten samples of each flavoring at 25 g or less. Products were enriched with LPT broth at their appropriate dilution, inoculated with ≤ 10 CFU of *L. monocytogenes*, and incubated at $35 \pm 1^\circ\text{C}$. K_2SO_3 salt was added to spice enrichments to ensure recovery of organisms following the Food and Drug Administration Bacterial Analytical Manual (FDA BAM) guidelines. After incubation, all products were tested by PCR with inhibition mitigation techniques when needed and confirmed using the FDA BAM Chapter 10 methodology.

Results: PCR was successfully performed, giving a positive or negative result in less than an hour on all products. *L. monocytogenes* was recovered, and there were no discrepancies between the PCR result and the confirmation method.

Significance: Under the appropriate enrichment conditions, the rapid PCR methods are suitable for detection of *L. monocytogenes* when present in challenging food additives.

P1-139: INVESTIGATING THE EFFICACY OF HOT AIR-DRYING ON COLOR AND SALMONELLA AND E. COLI POPULATIONS ON MICROGREENS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Microgreens, seedlings of edible vegetables and herbs, are a growing health and aesthetic food trend. However, the short shelf life of microgreens often leads to spoilage and losses for small growers – incentivizing methods to extend product stability.

Purpose: This study evaluated the effectiveness of hot air-drying in reducing *Salmonella* and *E. coli* populations in radish and alfalfa microgreens while retaining visual quality.

Methods: Radish and alfalfa seeds (50g) were planted in a 1020 tray containing 600 Grams of soilless peat mix and watered daily with sterile deionized water (500 mL). Microgreens were harvested at 75 Grams and 30 Grams were spot-inoculated with 100 μ L of a rifampicin-resistant pathogen cocktail per 5 Grams (*S. Javiana*, *S. Cubana*, *S. Saintpaul* and two strains of *E. coli* O157:H7) at 7.0-log CFU/mL. Hot air drying (4h at 60°C) and positive and negative controls were sampled in triplicate (N=12 per microgreen). 5-Gram samples were collected, diluted, and spiral-plated onto Modified Oxford Agar and Sorbitol MacConkey Agar (with 50 μ g/mL rifampicin). ΔE values and log reductions were calculated with pre- and post-treatment counts and analyzed using Tukey tests.

Results: For radish, dehydration exhibited average log reductions of 1.0 ± 0.5 log CFU/g ($p = 0.006$, significant at $\alpha = 0.05$) and 1.3 ± 2.1 log CFU/g ($p = 0.1$) for *Salmonella* spp. and *E. coli* spp. respectively. Alfalfa exhibited average log reductions of 0.7 ± 3 log CFU/g ($p = 0.4$) and 0.8 ± 3 log CFU/g ($p = 0.4$) for *Salmonella* spp. and *E. coli* spp. respectively. Color changes observed were $\Delta E = 20.8$ for radish and $\Delta E = 22.9$ for alfalfa.

Significance: These findings highlight the limitations of hot air-drying in achieving substantial log reductions for pathogens commonly associated with microgreens and offer insights on how hot air-drying affects color.

Award Application: Developing Scientist Award

P1-140: ENHANCING STRAWBERRY SHELF-LIFE WITH ENCAPSULATED CURCUMIN AND QUERCETIN

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Strawberries are a highly valuable crop desired for fresh and fresh-cut consumption. However, their delicate skin and ripeness at harvest result in reduced shelf-life, with fungal spoilage accounting for 50% of postharvest losses.

Purpose: This study evaluates the functionality of nanoencapsulated curcumin (Cm) and quercetin (Q) as sustainable antimicrobial agents to extend the shelf-life of strawberries against fungal pathogens.

Methods: Nanoencapsulates were fabricated using an antisolvent layer-by-layer method, encapsulating curcumin or quercetin in a zein protein core with a chitosan shell. The encapsulates were characterized by hydrodynamic diameter, zeta potential, polydispersity index (PDI), encapsulation efficiency, and antifungal activity (MIC and MFC) against *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Botrytis cinerea*. Nanoencapsulates were incorporated into gum-arabic films, applied to strawberries, and stored at 4°C for 15 days, with periodic monitoring of physiological parameters (weight loss, disease incidence, color, firmness, pH, and microbial counts).

Results: Nanoencapsulates were spherical, positively charged, and exhibited high antifungal activity. Hydrodynamic diameters were 284.0 ± 151.0 nm (Cm) and 358.6 ± 128.4 nm (Q), with PDIs of 0.232 ± 0.003 (Cm) and 0.184 ± 0.031 (Q), zeta potentials of 53.0 ± 9.5 mV (Cm) and 46.3 ± 7.3 mV (Q), and encapsulation efficiencies of 56% (Cm) and 65% (Q). MIC and MFC values demonstrated potent antifungal activity, with Cm and Q inhibiting fungal growth within 24–48 hours. Gum-arabic films containing nanoencapsulates reduced yeast and mold counts and disease incidence by 80% ($P < 0.05$), while maintaining fruit firmness, color, and pH. Shelf-life was extended to at least 15 days at 4°C.

Significance: Encapsulated curcumin and quercetin offer a sustainable, broad-spectrum antimicrobial solution for reducing postharvest fungal spoilage in fresh produce, supporting global efforts to reduce food loss and waste.

P1-141: NANO-CURCUMIN AND -QUERCETIN FOR ENHANCED FOODBORNE BACTERIAL INHIBITION

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: *Pseudomonas aeruginosa*, *Salmonella* Montevideo, and *Listeria monocytogenes* pose significant challenges to food safety and preservation. Although curcumin (Cm) and quercetin (Q) possess potent antimicrobial properties, their poor solubility and instability limit their application in food systems. Encapsulation using zein and chitosan nanoparticles may overcome these obstacles by improving bioavailability and antimicrobial performance.

Purpose: Establishing efficacy of nanoencapsulated Cm and Q against key spoilage and pathogenic bacteria for food quality and safety applications.

Methods: Nanoparticles were prepared by encapsulating 300 µg/mL of Cm and Q singly and in combination in a zein protein core and chitosan outer layer using the layer-by-layer antisolvent technique. Their antimicrobial efficacy was determined against *P. aeruginosa*, *L. monocytogenes* cocktail, and *S. Montevideo* by microbroth dilution and optical density determination at 630nm. Bacterial cultures were treated with nanoparticles at their minimum bactericidal concentration (MBC: 75 µg/mL) in Mueller Hinton Broth. Cell concentrations were assessed on AC petrifilms at 0, 4, 8, 12, and 24 hours. Scanning Electron Microscopy (SEM) was used to observe cell surface structural effects.

Results: The encapsulated Cm, Q and CmQ had strong antibacterial effects in broth. At the MBC, significant log reductions ($P < 0.001$) up to 6.5 log CFU/mL were observed at each sampling point during 24 h incubation. Bacterial inhibition was concentration ($P < 0.001$) and time-dependent ($P < 0.05$). *L. monocytogenes* exhibited higher inhibition by Cm compared to Q encapsulates ($P < 0.001$). The SEM images showed nanoparticles attached to bacterial cell surfaces, facilitating the release of Cm or Q into cells for enhanced inhibition.

Significance: Encapsulating Cm and Q in zein-chitosan nanoparticles overcome

solubility and stability challenges, and significantly enhances antimicrobial efficacy, providing a sustainable solution for improving food safety and shelf-life in diverse food systems.

P1-142: CLEAN-LABEL NATURAL FLAVOR-BASED PRESERVATION SYSTEM TO EXTEND THE SHELF-LIFE OF FRESH NOODLES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Microbial spoilage of fresh noodles is primarily caused by the growth of bacteria, yeasts, and molds due to their high moisture content and neutral pH, which provide an ideal environment for microbial activity. Even with storage at $\leq 4^{\circ}\text{C}$, noodles show sign of spoilage such as discoloration (yellowing, green, or black spots due to molds), off-odors, and slimy texture.

Purpose: Extend the shelf-life of fresh noodles beyond 3 weeks of storage under refrigerated conditions using dry natural flavor-based antimicrobial, Flavoset D5400.

Methods: Fresh noodles, packed and stored at $\leq 4^{\circ}\text{C}$ for 3 weeks, were observed closely for any signs of spoilage, like discoloration, and sampled every week on different isolation media such as potato dextrose for yeasts and molds, MRS for lactic acid bacteria, and Tryptic Soy agar for mesophilic bacteria, to enumerate and isolate the spoilage microorganisms. in-vitro screening of the isolated microbes were conducted against different doses of Flavoset D5400 (0.4%-0.8%) at pH 9.0. The microbes were tested at 30°C for 5-14 days using selective broth depending on the organism. Optical density of each well was measured every 30 minutes at 600nm to monitor microbial growth. Growth curves were analyzed, and the lag phase (days) and maximum growth rate (μ_{max}) were estimated using the Gompertz model. Differences were compared using one-way ANOVA ($P < 0.05$).

Results: The noodles showed increasing counts of APC, LAB, yeasts, and molds after 2 weeks of storage. Higher pH of the product affected the efficacy of the antimicrobial at a lower dosage of 0.4%, however at higher dosage of 0.6% and 0.8%, Flavoset D5400 was able to inhibit the growth of all isolated spoilage microorganisms.

Significance: For higher pH products, where most antimicrobials fail, natural flavored-based preservation system was able to inhibit spoilage microorganisms and extend the shelf-life of fresh noodles.

P1-143: EFFECTIVENESS OF NATURAL FLAVOR IN CONTROLLING LISTERIA MONOCYTOGENES IN ALMOND MILK

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Almond milk, a nutrient-rich environment, is prone to spoilage due to microbial growth including potentially harmful pathogens like *Listeria monocytogenes*. With near-neutral pH (6.5–7.0), it is prone to *Listeria* growth as witnessed in the recent outbreak in almond milk in Canada.

Purpose: Effectiveness of natural flavor system (Flavoset® 5400L) in controlling *Listeria monocytogenes* in almond milk.

Methods: In-vitro screening of a natural flavor-based antimicrobial system, Flavoset® 5400L, was conducted at different doses (0–1%) against a five-strain cocktail of *L. monocytogenes* (serotypes 1/2a, 1/2b, 4a, and 4b). The treatments were prepared in Brain Heart Infusion broth at pHs 7.0 and 7.5, along with a no antimicrobial control. The analysis was conducted using a Bioscreen-C instrument at 30°C for 3 days. Optical density of each well was measured every 30 minutes at 600 nm to monitor microbial growth. Growth curves were analyzed, and the lag phase (days) and maximum growth rate ($\mu_{\max}$) were estimated using the Gompertz model. Furthermore, for in-application testing, almond milk was inoculated with 2.5 log CFU/mL of *Listeria* cocktail along with different doses (0.1–0.5%) of Flavoset® 5400L. Samples were plated onto Modified Oxford Agar at 0, 4, 8, and 24 hours to check the cidal efficacy of Flavoset® 5400L. All differences were compared using one-way ANOVA ($P < 0.05$).

Results: In-vitro analysis demonstrated that 0.4% Flavoset® 5400L was able to completely inhibit the growth of *Listeria monocytogenes* cocktail at both pH levels. The application study showed that Flavoset® 5400L at a dosage as low as 0.2% was able to completely inhibit the growth of *Listeria* starting at 4 hours, thus exhibiting strong cidal effects.

Significance: Natural flavor system is a highly effective solution in inhibiting

pathogenic *Listeria* in almond milk and thus extending its shelf life.

P1-144: EFFICACY OF VINEGAR AND NATURAL FLAVOR FOR EXTENDING THE SHELF LIFE OF FLAVORED SAUCE AGAINST ZYGOSACCHAROMYCES BAILII

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: The yeast *Zygosaccharomyces bailii* is known for its high tolerance to acidic environments and resistance to common preservatives like sorbic and benzoic acids, making it challenging to control in low-pH food products. Its resilience often leads to spoilage, as it produces off-flavors such as sour and bitter notes. As consumer demand for natural and minimally processed ingredients grows, clean-label antimicrobials derived from natural sources are gaining prominence. These alternatives are considered safer and healthier compared to synthetic preservatives traditionally used in the food industry

Purpose: Effectiveness of a vinegar and natural flavor system (NouriShield® D4040) in controlling the acid-tolerant yeast *Z. bailii* in flavored sauce

Methods: A flavored sauce was prepared with a commercial control containing 7% vinegar (T1) and tested against a clean-label antimicrobial system comprising 0.7% vinegar and a natural flavor-based solution combined with 7% vinegar (T2). Both formulations were inoculated with *Z. bailii* adjusted to pH 3.5 at an initial concentration of 2.5 log CFU/g. Uninoculated samples served as negative controls. Duplicate samples were analyzed weekly for the first four weeks and then monthly for six months. Data were statistically analyzed using one-way ANOVA at $P < 0.05$

Results: Results showed that *Z. bailii* remained below detectable levels (< 1 log CFU/g) in negative control samples throughout the study. In both treatments, *Z. bailii* counts dropped below detection within nine days. However, in T1 (commercial control), regrowth occurred after two months, reaching 4 log CFU/g. In contrast, T2 maintained *Z. bailii* levels below detection for the entire six-month duration

Significance: Clean-label antimicrobial solution offered enhanced efficacy in inhibiting the growth of acid-tolerant yeasts like *Z. bailii*. This approach provides a more effective solution than low pH alone, supporting the development of safer, longer-lasting food products that align with consumer preferences for natural

ingredients

P1-145: SHELF-LIFE EXTENSION OF FRESH READY-TO-EAT MEAT AND CHEESE BOWLS AGAINST SPOILAGE MICROORGANISMS AND LISTERIA MONOCYTOGENES

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: Refrigerated products often retain a more natural taste and texture compared to frozen products. However, fresh products typically have a shorter shelf life compared to frozen products, and thus are more susceptible to microbial spoilage.

Purpose: Extending the shelf-life of refrigerated Ready-To-Eat (RTE) cooked meat and cheese bowls using preservation systems.

Methods: RTE bowls (8 oz) with cooked sirloin meat and cheese were prepared with three antimicrobial systems, namely, T1 – sodium acetate/diacetate and natural flavor-based system, T2 – vinegar and natural flavor-based system, and T3 – vinegar and cultured dextrose-based system and stored at 4°C for 60 days. A no antimicrobial treatment (T4) was also prepared for comparison. All the RTE bowls were MAP packaged with 60% N₂ and 40% CO₂. One set of the bowls was sampled every two weeks for up to 60 days to enumerate spoilage lactic acid bacteria and mesophiles on MRS and TSA plates. Another set of bowls was inoculated with 2.5 log CFU/g of *Listeria monocytogenes* cocktail (serotype 1/2a, 1/2b, 4a, and 4b) and sampled at the same frequency on Modified Oxford Agar for *Listeria* enumeration. Treatment differences were compared using one-way ANOVA ($p < 0.05$).

Results: All three antimicrobial treatments (T1, T2, and T3) were effective in slowing the growth of spoilage lactic acid bacteria and mesophilic microbes for up to 60 days, compared to the no antimicrobial control, which reached 6 log CFU/g within 20 days. Similarly, all treatments inhibited the outgrowth of *Listeria monocytogenes* over 60 days, while the untreated control reached 6 log CFU/g in 35 days.

Significance: Clean-label/conventional antimicrobial systems are highly effective in significantly extending the shelf-life of fresh RTE products.

P1-146: SHELF-LIFE EXTENSION OF READY-TO-EAT MAC & CHEESE BOWLS

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: Mac & Cheese is a popular Ready-To-Eat (RTE) moisture-rich product, which supports microbial growth, thus making preservation critical. Despite modified atmosphere packaging (MAP), preservatives are required to provide a combined protection against spoilage microorganism which can cause bloating, off-taste, and odor in the product.

Purpose: Shelf-life extension of RTE mac & cheese bowls using preservation systems.

Methods: RTE bowls of mac & cheese were produced and packaged with 60% N₂ and 40% CO₂. The products were stored at 4°C and were analyzed for uninoculated shelf-life via signs of bloating and off-odor. The spoilage microorganisms were isolated by sampling the product on different isolation agar such as PDA for yeasts and molds, MRS for lactic acid bacteria (LAB), and TSA for mesophilic spoilage organisms (APC). in-vitro screening of three antimicrobial systems, namely, T1 – sodium acetate/diacetate and natural flavor-based system, T2 – vinegar and natural flavor-based system, and T3 – vinegar and cultured dextrose-based system, was conducted with all the isolated spoilage microbes in either MRS broth or TSB, depending on the spoilage organism at 30°C for 5 days, along with a no antimicrobial control. Optical density of each well was measured every 30 minutes at 600nm to monitor microbial growth. Growth curves were analyzed, and the lag phase (days) and maximum growth rate (μ_{max}) were estimated using the Gompertz model. Differences were compared using one-way ANOVA ($p < 0.05$).

Results: Spoiled mac & cheese showed the presence of two different strains each of LAB and APC. in-vitro analysis demonstrated that T1 and T2 preservation systems successfully inhibiting the growth of both the strains of LAB and APC, thus exhibiting cidal effects, as compared to the no antimicrobial control.

Significance: Natural flavor-based antimicrobial systems are highly effective in extending the shelf-life of RTE mac & cheese products by controlling spoilage and thus reducing food wastage.

P1-147: ANALYSIS OF ANTI-GERMINATION ACTIVITY OF SUCROSE ESTER OF PALMITIC ACID ON BACILLUS SUBTILIS SPORES

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Because bacterial spores are ubiquitously distributed in environments and easily contaminate in foods, the inactivation procedure of spores has been attracting widespread attention from food industries.

Purpose: To establish a new strategy for food hygiene and food quality control, we examined the mode of action of sucrose ester of palmitic acid (SEPA), which has been used as a food emulsifier known to have bacteriostatic effect on spore-forming bacteria. The

Purpose of this study is to analyze the mode of action of SEPA against spores and try to obtain a clue to maximize its inhibitory effect in foods.

Methods: SEPA (P-1670) is a gift from Mitsubishi Chemical Co. *Bacillus subtilis* is used as a model organism of a spore former. The germination-to-outgrowth process of the bacterial spores was visualized under microscopy using Live Cell Imaging system (Nikon). The spores were incubated at 37C in nutrient broth with or without P-1670. The sequential images taken every one minute during the process were analyzed (three independent experiments) and the time when the germination was completed and the time when the outgrowth started were identified for each spore (n= ca. 100).

Results: The spores' germination process was not significantly ($p>0.3$) affected even in the presence of P-1670. in contract, the outgrowth process was completely inhibited in its presence. Next the P-1670-treatment was limited within the period that the spores stayed at pre-germination status. These spores revived and started outgrowth after SEPA's removal. in contrast, when the spores at post-germination status were exposed to SEPA, the spores' outgrowth was not observed even if SEPA was removed after treatment.

Significance: The results suggest that SEPA acts selectively on the germinated spores and disturbs spores' entry into outgrowth phase. Our study clearly determined the mode action of SEPA's bacteriostatic effect at a single cell basis.

P1-148: APPLYING A DIGITAL SOLUTION TO ASSESS FOOD AND BEVERAGE E-COMMERCE DISTRIBUTION SYSTEMS

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: E-commerce enhances food purchasing convenience but risks temperature fluctuations that promote bacterial growth and compromise the quality of perishable products.

Purpose: This project aimed to develop a user-friendly app to simulate growth of bacteria in HTST milk delivered via e-commerce and traditional channels, predicting product shelf-life.

Methods: A spoilage prediction tool was developed to simulate bacterial growth in 100 lots of HTST milk (each containing 100 containers) during transportation. Dairy processing plants were classified by post-pasteurization contamination frequencies into Long (0–33%), Medium (34–66%), and Short Shelf-life (67–100%) categories using Voluntary Shelf-Life data from New York State dairy processors. The shelf-life was defined as the day when the predicted spoilage bacteria exceeded 6 log₁₀ CFU/mL in ≥25% of containers in a lot. Simulated e-commerce scenarios included direct delivery from processor to consumer (e-commerce1), processor-to-retail-to-consumer delivery via an intermediary (e-commerce2), and the traditional (processor-to-retail) scenario.

Results: The model-predicted shelf-life for HTST milk delivered via e-commerce was comparable to traditional methods within each plant category. For the Long-Shelf-life category, the median shelf-life for e-commerce1 was 24 days (5th–95th percentile: 18–28 days), e-commerce2 was 23 days (5th–95th percentile: 18–28 days), and traditional was 25 days (5th–95th percentile: 19–28 days). In the Medium-Shelf-life category, the median shelf-life was 13 days for products delivered via all methods (5th–95th percentile: 10–18 days for e-commerce1, 9–17

days for e-commerce2, and 10–17 days for traditional). For the Short-Shelf-life category, e-commerce1 had a median shelf-life of 9 days (5th–95th percentile: 8–11 days), e-commerce2 had 8 days (5th–95th percentile: 7–10 days), and traditional had 8 days (5th–95th percentile: 7–10 days).

Significance: This project provides a digital solution to predict spoilage and support actions preserving the quality of e-commerce food and beverage purchases. Similar approaches assessing pathogen growth could offer further competitive advantages for these platforms.

P1-149: RAPID DETECTION OF BACILLUS SPECIES SPORES IN CHOCOLATE-FLAVORED PUDDING CUPS USING HYGIENA'S INNOVATE™ SYSTEM

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: *Bacillus* spores are a risk organism identified in spoilage due to their ability to survive high temperature processing in ESL and UHT products. The detection of these spoilage organisms and other contaminants is done through microbiological testing. Traditionally, products are plated for growth, which can take several days. Rapid screening methods can provide the industry with clear presence/absence results for a faster product release.

Purpose: The objective of this study was to evaluate chocolate pudding cups inoculated at a fractional level of *Bacillus* spores with the innovate Rapid Microbial Screening System compared to traditional plating.

Methods: Chocolate pudding cups were incubated and tested with the RapiScreen™ Dairy assay to establish baseline RLU values. After establishing a threshold to differentiate contaminated products, chocolate pudding cups were inoculated with *Bacillus cereus* spores (n=20) and *Bacillus spizizenii* spores (n=15) at a low fractional level. Amylase was added to cups and shaken for 5-10 minutes to initiate product liquification. Samples were incubated at 35 °C and tested over a 7-day period. Each day, products were shaken to redistribute the sample, then aliquots were taken for parallel testing using the innovate System and plating.

Results: Fractional detection was achieved for *Bacillus cereus* spores in 6/20 (30%) cups and for *Bacillus spizizenii* spores in 12/15 (80%) cups with the RapiScreen Dairy assay after 24 hours of incubation. RLU values remained over the threshold throughout all 7 days of testing. All plating results were in agreement but required an additional 24 hours of incubation for colony development.

Significance: The utilization of ATP bioluminescence for the rapid screening of *Bacillus* spores in chocolate pudding cups after a 24-hour incubation can result in faster, more efficient product release times compared to plating.

P1-150: IMPROVING THE SPOILAGE STABILITY OF MILD AND SPICY SAUCES WITH ARTIFICIAL AND NATURAL PRESERVATIVES

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: Driven by consumers' preference for clean labels, food scientists are seeking natural preservative alternatives. Challenge tests investigate a product's stability over shelf-life and reveal important evidence in screening effective preservatives.

Purpose: To investigate the effect of artificial or natural preservatives in inhibiting spoilage microorganisms in Mild and Spicy sauces.

Methods: Five lactic acid bacteria (LAB) strains were revived in De Man, Rogosa and Sharpe broth for 3 days at 35 °C and pooled to heterofermentative and homofermentative LAB cocktails. Six yeast and *Zygosaccharomyces bailii* strains were cultured on potato dextrose agar for 5 days at 25 °C and harvested before pooling to respective cocktails. Mild or Spicy samples (500 g), with or without artificial (0.1% potassium sorbate) or natural (0.5% rowanberry extract, 0.5% cultured sugar and vinegar) preservatives, were inoculated with each cocktail (5 mL) to achieve 10²–10³ CFU/g. After storing at 30 °C for varied intervals, aliquots (11 g) of inoculated samples were plate-counted using BAM methods. Tasting evaluation was conducted with a panel of 6 people.

Results: Without preservatives, *Z. bailii* growth was observed in both Mild (1.3 log increase) and Spicy (1.1 log increase) after 1 week of storage at 30 °C, indicating the failure of the formulas in preventing spoilage. For samples supplemented with 0.5% cultured sugar and vinegar, Mild failed at week 1 (1.4 log increase) while Spicy failed at week 2 (1.0 log increase) with *Z. bailii* growth. On the contrary, 0.1% potassium sorbate and 0.5% rowanberry extract effectively inhibited the growth of all inoculated microorganisms throughout the 52-week shelf-life for both Mild and Spicy. The addition of these preservatives did not cause significant deviation from the no-preservative formulas based on taste evaluation.

Significance: The study investigated the feasibility and effectiveness of artificial and natural preservatives to improve the spoilage stability of Mild and Spicy sauces.

P1-151: EFFECT OF MAIN INGREDIENTS OF CREAM ON THE BEHAVIOR OF STAPHYLOCOCCUS AUREUS IN CREAM-FILLED BREAD

Primary Author:

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KI SUN YOON, Kyung hee

Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: The growing consumption of cream-filled bread has heightened concerns about the risk of *Staphylococcus aureus* food poisoning, emphasizing the need for effective control measures during production and distribution.

Purpose: The study aimed to evaluate the impact of key cream ingredients on *Staphylococcus aureus* growth in cream-filled bread, with the ultimate objective of developing a formulation that enhances shelf life while ensuring product safety.

Methods: Cream formulations were prepared with varying ingredient ratios, including milk-based to oil-based cream (10:0, 6:4, 4:6, 0:10), lemon juice concentrations (0, 2.5, 5%), and sweeteners such as sugar, allulose, and erythritol (0, 15.9%). Two types of bread, with cream either exposed to air or sealed, were also prepared. The cream was inoculated with *Staphylococcus aureus* (2 log CFU/g) and stored under various temperatures. The effects of individual and combined ingredients on *Staphylococcus aureus* growth in cream were evaluated.

Results: The growth of *Staphylococcus aureus* was faster in creams with a higher proportion of milk-based cream. At 10°C, no growth of *Staphylococcus aureus* was observed in creams containing 5% lemon juice or 15.9% erythritol. At 25°C, the individual addition of erythritol or lemon juice resulted in *Staphylococcus aureus* growth exceeding 7 log CFU/g, indicating that neither ingredient alone was sufficient to inhibit growth. However, significant growth inhibition was observed when erythritol and lemon juice were combined. Among sweeteners, allulose extended the lag phase longer than sugar, while the growth of *Staphylococcus aureus* remained largely unaffected by air exposure, regardless of temperature.

Significance: The risk of *Staphylococcus aureus* in cream-filled bread can be mitigated through the appropriate formulation of key cream ingredients.

Award Application: Undergraduate Student Award

P1-152: ANTIFUNGAL PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM MALTESE SHEEP MILK AND CHEESE

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Sholeem Griffin, University of Malta

Vasilis Valdramidis, University of Athens

Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Lactic acid bacteria (LAB) produce potent antifungal activity through production of organic acids and bacteriocins, providing natural solutions to fungal spoilage and mycotoxin risks in dairy. This study investigates the antifungal potential of LAB from Maltese raw sheep milk and cheese against fungal spoilers common in dairy, highlighting their role in enhancing safety and shelf life.

Methods: A total of 129 LAB isolates from raw sheep milk and cheese were screened for antifungal activity against *Aspergillus niger* and *Penicillium expansum*. Strains with strong antifungal properties underwent further broad-spectrum analysis against eight additional fungal species. LAB strains with the strongest antifungal activity were characterised by using 16S rRNA sequencing. Hereafter, HPLC analysis quantified their acid production, and the most effective strains were tested in a cheese matrix and real cheese ecosystem.

Results: Eight LAB isolates, including *L. plantarum* YW11 and *L. pentosus* ZFM94, exhibited strong antifungal activity against fungi like *A. flavus* and *C. Albicans*. Their antifungal effects remained stable after heat and catalase treatments but decreased with protease or neutralization. Lactic acid and phenyl lactic acid in CFS remained stable at 48 hours. In a simulated cheese matrix, *L. plantarum* YW11 and A9 when combined with *L. pentosus* ZFM94 effectively inhibited *P. expansum*, enhancing shelf life.

Significance: This study is the first to isolate microbes from Maltese raw sheep milk and cheese, showcasing LAB strains' potential as natural bioprotective agents to improve cheese quality and shelf life.

Award Application: Developing Scientist Award

P1-153: SHELF-LIFE AND MICROBIAL COMMUNITY DYNAMICS OF REDUCED OXYGEN PACKAGED SEAFOOD PRODUCTS

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Reduced oxygen packaging (ROP) has emerged as an innovative solution to limiting microbial growth, slowing spoilage, and extending the shelf life of food products, though its efficacy for seafood products remains completely underexplored.

Purpose: To evaluate the shelf life and identify microbial community dynamics of the reduced oxygen packaged seafood products during refrigerated storage.

Methods: Reduced oxygen-packaged seafood samples (i.e., flounder, mahi, perch, salmon, scallop, shrimp, snapper, swai, tilapia, and tuna) were collected from a large seafood processor in Florida, transported frozen, and stored at -20°C. Samples were thawed for 48 hours at 4°C, chopped, mixed, and 25 Grams of each sample was homogenized with 225 mL peptone water and serially diluted for enumerating aerobic bacteria, coliform, and lactic acid bacteria counts using standard FDA BAM protocols. The FDA and FAO guidelines to evaluate the acceptability of seafood samples. Microbial DNA was extracted using DNeasy Blood & Tissue Kit. Bacterial community profiling was performed by PCR amplification of the V4 region of the 16S rRNA gene, followed by library purification, barcoding, and sequencing on an Illumina MiSeq platform. The sequencing data was analyzed using the CLC Genomics Workbench.

Results: on day 1, the average aerobic plate count (APC) was 3.5 log CFU/g, which increased to 6 log CFU/g by day 5 and reached 7.5 log CFU/g by day 9. These

Results indicate that the seafood samples exceeded the spoilage thresholds established by the FDA and FAO by day 5 under refrigerated conditions. Sequencing data revealed that the dominant bacterial genera across most seafood species were *Photobacterium*, *Psychrobacter*, *Pseudomonas*, and

Brochothrix. Notably, *Pseudomonas* emerged as the predominant genus by day 5, surpassing all other bacterial genera in abundance.

Significance: Comprehensive data generated in our study can be used by seafood processors to decide the shelf life of seafood products, enable retailers to decide product storage conditions, reduce spoilage and product loss, and protect brand names.

Award Application: Developing Scientist Award

P1-154: AI-ENABLED APPROACH FOR THE RAPID AND SPECIFIC DETECTION OF BACTERIA FROM DIVERSE FOOD MATRICES

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Contamination of food with pathogenic bacteria poses significant public health risks, highlighting the need for rapid and accurate detection methods. Recently, AI-based rapid microbial detection methods have been suggested, but challenges remain in detecting bacteria in complex food matrices.

Purpose: This study aimed to develop a robust deep learning-based detection model capable of accurately identifying and classifying bacteria in complex food matrices.

Methods: *Escherichia coli*, *Listeria monocytogenes*, and *Bacillus subtilis* were used as representative bacterial species. Microcolonies were prepared by incubating bacteria on soft TSA plates at 37°C for 3 hours. White light microscopy images of bacterial microcolonies and food debris from chicken breast, spinach, and Cotija cheese were captured using a 60x phase contrast objective. Images were annotated with bounding boxes and divided into training (60%), validation (10%), and test (30%) datasets. A Faster R-CNN model with a ResNet50 backbone was trained and evaluated. A GFP-producing *B. subtilis* strain was selected to validate the model with the mixture of food debris and bacterial microcolonies.

Results: The model trained only on bacterial microcolonies showed strong performance, with a mean precision of 98.9% and mean recall of 99.1%. However, it misclassified food debris as bacteria, resulting in a false positive rate of 24.2%. Incorporating food debris images into training enabled the model to learn their distinct morphological features, reducing the false positive rate to 0% across food matrices while maintaining high classification performance on bacterial microcolonies (mPrecision and mRecall were 100% and 94.4%, respectively). Validation using GFP-producing *B. subtilis* confirmed the model's robustness, achieving a mean precision of 94.6% and a mean recall of 92.5%.

Significance: This study presents a rapid, cost-effective AI-based detection model

that accurately classifies bacteria in complex food matrices within 3 hours, offering practical applications for food safety.

P1-155: MICROBIOLOGICAL QUALITY AND SAFETY OF FROZEN BLUEBERRIES SOLD IN KOREA

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: Fresh and frozen blueberries contaminated with *Salmonella*, *Listeria*, or *Escherichia coli* have been linked to foodborne outbreaks and recalls around the world.

Purpose: This study evaluated the microbiological qualities of frozen blueberry in online market in Korea.

Methods: A total of 20 frozen blueberry products purchased from online market were tested for aerobic mesophilic (AE), coliforms and *Escherichia coli* (CE), yeast and mold (YM), and foodborne pathogens (*Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7).

Results: Among the frozen blueberry products purchased from online market, most products were produced in the United States (35%), followed by Chile (25%), Canada (20%), Korea (10%), Peru (5%), and China (5%). More than half of products provided washing instruction of frozen blueberry (55%). Among these products, four products (20%) recommended “washing with tap water before consumption” and 7 products (35%) indicated “washing not required”. Populations of AE in frozen blueberry for washing required, washing not required, or no instruction were 1.00, 1.00, or 2.33 log CFU/g, respectively. Contamination levels of YM in frozen blueberry ranged from 2.35 log CFU/g to 2.95 log CFU/g. CE and pathogenic microorganisms were not detected in frozen blueberry products evaluated in this study.

Significance: Although this study shows relatively low microbiological contamination of frozen blueberries, preventive control measure should be considered to avoid possible foodborne disease outbreaks related to frozen blueberries.

P1-156: EVALUATION OF UV-C AND ORGANIC ANTIMICROBIAL TREATMENTS TO CONTROL *PENICILLIUM EXPANSUM* ON POSTHARVEST APPLES

Primary Author:

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: *Penicillium expansum* causes blue mold rot, one of the most economically significant postharvest diseases in tree fruits. The current reliance on chemical fungicides is increasingly unsustainable, necessitating alternative strategies.

Purpose: To develop and optimize a sustainable approach combining ultraviolet-C (UV-C) light treatment and organic antimicrobials to mitigate *P. expansum* infections in postharvest apples.

Methods: A bench-top UV-C (254 nm) chamber was used to apply dosages up to 2.16 kJ/m² to *P. expansum* spores. Spores were suspended in water, exposed to UV-C, and plated on potato dextrose agar to assess viability after 72 h at 25°C. Whole Gala apples inoculated with *P. expansum* were treated with UV-C (30 s / 1.62 kJ/m²), hydrogen peroxide (H₂O₂, 3%), or Tsunami 100™ (215 ppm and 575 ppm), or a combination of UV-C and antimicrobials. Decay severity was assessed over one month at room temperature. Screening also identified the most effective organic fungicides.

Results: UV-C treatment alone reduced *P. expansum* counts by >1 log CFU/mL. On fruit, UV-C effectively reduced surface infections compared to controls. Hydrogen peroxide delayed internal infection at stem/calyx sites, while Tsunami 100™ at 575 ppm significantly reduced decay on surface wounds and outperformed other treatments. No visual or textural changes were observed in any treated apples.

Significance: This study offers a sustainable strategy for postharvest decay management in apples using UV-C and organic antimicrobials. The approach supports organic fruit production by reducing losses due to fungal decay without compromising fruit quality.

P1-157: EVALUATING THE MICROBIAL DYNAMICS OF DIFFERENT PACKAGING TECHNOLOGIES OF STEAKS UNDER PROLONGED STORAGE

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: Conventional packaging techniques for fresh meat often involve the use of polyvinyl chloride (PVC) film and rollstock film, primarily intended to influence the meat's surface color. Another method used is modified atmosphere packaging (MAP), which offers various advantages such as desirable meat qualities and extended shelf-life potential.

Purpose: This study assessed the microbial load of steaks stored at 4°C under different packaging technologies, including high oxygen (HiOx), carbon monoxide (CO), nitric oxide (NO), Rollstock, and PVC, to evaluate the influence of packaging type on spoilage organisms.

Methods: Steaks were allocated to each packaging type and displayed in a retail case for 96 hours. Spoilage organisms were evaluated at 0, 48, and 96 hours. Pre-hydrated 25 mL swabs were taken using a 50 cm² template on the steak surface. Samples were stomached, homogenized, diluted as needed, and evaluated for Aerobic Counts (AC), Enterobacteriaceae (EB), Lactic Acid Bacteria (LAB), and Psychrotrophic Aerobic Counts (P-AC) using the TEMPO® enumeration system. TEMPO cards were incubated at 35°C for 24 h (AC and EB), 35°C for 48 h (LAB), and 7°C for 6 days (P-AC). Data were analyzed using Kruskal-Wallis and Pairwise Wilcoxon tests, with significance set at $P < 0.05$.

Results: Microbial counts increased significantly over time for all bacteria ($P < 0.05$). LAB showed no significant difference between packaging treatments ($P = 0.12$). AC and P-AC exhibited differences only at the 96 h timepoint ($P = 0.003$ and $P = 0.04$, respectively). EB showed a difference between treatments at the 0 h timepoint ($P = 0.022$).

Significance: Results indicate that microbial counts were not consistently

influenced by packaging type. Different packaging technologies can be applied to extend the shelf-life of ground beef, as evaluated by multiple microbial indicators.

P1-158: SHELF-LIFE EXTENSION OF POMEGRANATE ARILS USING THE SYNERGISTIC COMBINATION OF AQUEOUS OLIVE POMACE EXTRACT (OPE) AND MILD HEAT

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Category: Microbial Food Spoilage

Abstract:

Introduction: Preserving the microbial quality and shelf life of ready-to-eat pomegranate arils while maintaining their nutritional and sensorial attributes poses a significant challenge.

Purpose: This study aims to develop an intervention strategy that can reduce the risk of microbial spoilage of ready-to-eat pomegranate arils using the synergistic combination of OPE and mild heat (MH).

Methods: The aqueous OPE was obtained from dried olive pomace using water-based, ultrasound-assisted extraction (UAE). An in vitro antifungal test was performed to evaluate the potential synergisms between OPE and MH (50°C and 60°C) using *Alternaria alternata* as a model spoilage microbe. An in vivo shelf-life study was conducted to monitor the effects of the combined treatment in preserving the microbial quality of ready-to-eat pomegranate arils during 4-week storage at 4°C. The nutritional and sensorial quality of pomegranate arils treated with OPE and MH were monitored during 3 weeks of storage at 4°C. The potential membrane damage and oxidative stress induced on *A. alternata* by the combined treatment were assessed.

Results: The OPE (2.0 mg GAE/mL) obtained using water-based UAE exhibited strong synergistic antifungal potential with MH (60°C) and achieved > 3.5 log reduction of *A. alternata* conidia within 2 min. Based on the in vivo assay, pomegranate arils treated with OPE + MH showed ca. 2.8 and 2.9 logs lower total plate and yeast and mold counts, respectively, after 21 days of cold storage. The combined treatment synergistically delayed the discoloration and phenolic content loss of the arils during the storage. The combined treatment of OPE and MH synergistically disrupted the membrane integrity of *A. alternata* hyphae and the redox balance of *A. alternata* conidiophores, respectively.

Significance: These findings provide a novel insight into developing a practical strategy that can enhance the microbial quality of ready-to-eat pomegranate arils while preserving their nutritional and sensorial qualities using the synergistic combination of plant-derived extracts and mild heat.

Award Application: Developing Scientist Award

P1-159: ANTIFUNGAL ACTIVITY OF LACTOBACILLUS PLANTARUM KM2 AGAINST CLADOSPORIUMSPP. AND FUSARIUM SPP. AND ITS POTENTIAL APPLICATION AS BIOPROTECTIVE CULTURE

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Lactic acid bacteria with antifungal activity have shown potential as bioprotective cultures for controlling fungal spoilage of dairy products.

Purpose: The purpose of this study was to investigate antifungal activity of *Lactobacillus plantarum* KM2 and its application in yogurt to control fungal spoilage.

Methods: The antifungal activity of *L. plantarum* KM2 was evaluated by using the overlay agar method against *C. pini-ponderosae*, *C. colombiae*, *C. oxysporum*, and *F. annulatum*, previously isolated from yogurt production environments.

Furthermore, cytotoxicity of various concentrations of CFS from *L. plantarum* KM2 was assessed by MTT assay. The stability of the CFS against thermal (60, 80, 100, 121°C) and pH (3–7) treatment was evaluated. After the yogurt production by fermentation of UHT milk with *L. plantarum* KM2 and commercial starter (YoFlex® Mild 1.0), the antifungal activity of the yogurt was evaluated by spot assay. Finally, the challenge assay was assessed by inoculating the three *Cladosporium* spp. and one *Fusarium* spp. to the yogurt, respectively, and the growth of fungi was monitored at 10°C and 28°C for 120 h.

Results: *L. plantarum* KM2 exhibited an inhibitory effect on the growth of *C. pini-ponderosae*, *C. colombiae*, *C. oxysporum*, and *F. annulatum*. The CFS from *L. plantarum* KM2 significantly decreased the cell viability of fungi in a dose-dependent manner with minimum inhibitory concentration at 25 mg/mL. Under thermal (60–121°C) and pH (3–4) conditions, the antifungal activity of the CFS was maintained up to 80%. Moreover, the mycelial growth of three *Cladosporium* spp. and one *Fusarium* spp. was inhibited in yogurt plate compared to the control (without *L. plantarum* KM2). Moreover, *L. plantarum* KM2 effectively inhibited the growth of *Cladosporium* and *Fusarium* for 72 h at 10°C and for 48 h at 28°C in

yogurt.

Significance: *L. plantarum* KM2 and its CFS have the potential as bioprotective culture in yogurt.

Award Application: Developing Scientist Award

P1-160: INVESTIGATION OF ELUTION CHARACTERISTICS BY MATERIAL FOR FOOD CONTACT MATERIALS IN SOUTH KOREA

Primary Author:

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Presentation type/Format: Poster

Category: Packaging

Abstract:

Introduction : There are various additives, such as antioxidants, polymerization initiators, and coating agents, used to improve functionality in food equipment and container packaging. However, there are relatively insufficient on the usage or compliance with banned substances in food contact materials (FCM) in South Korea.

Purpose : This study aimed at conducting as a preliminary study for fact-finding survey on domestic distribution of FCM.

Methods: A total of 100 samples were selected on data analyzing inspection performance by referring to the Annual report on inspection of imported foods (Ministry of Food and Drug Safety in South Korea, 2021-2023). We investigated the Potassium permanganate (KMnO₄) consumption and Overall Migration Values (OMV) of items specified in the Korean Standards and Specification for Utensils, Containers, and Packages for 100 utensils made by synthetic resins for food.

Results: Monitoring samples were comprised of 34 Polypropylene (PP), 26 polyethylene (PE), 12 polyethylene-terephthalate (PET), 12 polylactide (PLA), 9 polyamide (PA), and 7 polystyrene (PS). The

Results showed that the average of KMnO₄ consumptions and OMVs detected in a total of 100 samples were low compared with the migration specification of MFDS. The maximum level of KMnO₄ consumptions in PA corresponded to 9.13% of the migration limit. The highest levels of OMVs varied differently for materials using simulating conditions: PE (9.6% in distilled water), PET (24.3% in 4% acetic acid), and PS (8.29% in n-heptane) respectively.

Significance: This study was conducted as the preliminary research to identify the current status of substances that are restricted or prohibited for use in manufacture of FCMS. According to this study, it can be concluded that the investigation of elution characteristics by FCMS was safely managed in South Korea.

P1-161: DEVELOPMENT OF CARBOHYDRATE-BASED PACKAGING FILMS INCORPORATED WITH CAM-21 BACTERIOPHAGE FOR BIOCONTROL OF E. COLI O157:H7 ON BABY SPINACH

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Presentation type/Format: Poster

Category: Packaging

Abstract:

Introduction: The incorporation of bacteriophage in active packaging has gained attention due to advancements in materials science and the continued challenges in foodborne disease outbreaks.

Purpose: This study aims to replace synthetic plastics with biodegradable films to mitigate contamination of food by *Escherichia coli* O157:H7 via incorporation of a novel *Escherichia* phage, CAM-21.

Methods: Three different films made with carboxymethyl chitosan-gelatin (CG), carboxymethyl chitosan-gelatin-sodium alginate (CGSA), and pullulan-gelatin-sodium alginate (PGSA), each incorporated with phage CAM-21, were developed. The films were evaluated for physicochemical properties including moisture content, water vapor permeability (WVP), and opacity. Morphological structures were analyzed using Scanning Electron Microscopy, and phage distribution was assessed by confocal fluorescence microscopy. Antimicrobial potential was tested on baby spinach stored for one week at 4 °C.

Results: CGSA film had the highest moisture content (5.14%). WVP differed significantly ($P \leq 0.05$) across films, with PGSA exhibiting the best barrier properties at $2.505 \times 10^{-10} \text{ g} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$. PGSA also had the highest opacity (2.26), while CGSA had the lowest (0.573). Confocal microscopy showed uniform CAM-21 distribution in all films. Reductions in *E. coli* O157:H7 on spinach leaves after one week of storage were 1.94 log (CG), 1.78 log (PGSA), and 1.42 log (CGSA), compared to the control.

Significance: This research demonstrates the potential of incorporating bacteriophages into biodegradable packaging films as an effective biocontrol strategy against *E. coli* O157:H7 in fresh produce.

Award Application: Developing Scientist Award

P1-162: ASSESSING THE BIODEGRADABILITY OF BACTERIAL CELLULOSE PROCESSED WITH DIFFERENT DRYING TECHNIQUES IN SIMULATED ENVIRONMENTS FOR PACKAGING SUSTAINABILITY

Primary Author:

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Presentation type/Format: Poster

Category: Packaging

Abstract:

Introduction: Bacterial cellulose (BC) is a sustainable material with applications in food packaging and other industries. Its environmental impact is closely tied to its biodegradability, which can be influenced by drying techniques that alter its physical and chemical properties.

Purpose: This study examined the effect of drying methods on the biodegradability of BC in simulated environmental trials mimicking natural conditions, focusing on soil burial and environmental variables.

Method: BC was produced via fermentation of *Komagataeibacter xylinus* (ATCC 53524) in Hestrin-Schramm medium at 30°C for 21 days under static conditions. After purification, BC was dried using room temperature (RT), freeze-drying (FD), or oven-drying (OD) methods. Biodegradation trials were conducted in a greenhouse (GH) and growth chamber (GC) with controlled conditions (25°C, 65% relative humidity, 12-hour light/dark cycle). Soybean plants were grown in the soil, which was monitored for moisture and pH. BC samples were retrieved weekly and assessed for degradation through visual inspection and weight measurement.

Result: The soil moisture was maintained at $40\pm5\%$ with a pH of 6.2-6.7 for both treatments. BC thickness varied by drying method: OD (0.061 ± 0.01 mm), RT (0.492 ± 0.01 mm), and FD (0.350 ± 0.03 mm). Visible degradation of BC began after the first week, with significant breakdown within 2 weeks. After 4 weeks, remaining BC in the GC was 0.062 g (OD), 0.086 g (RT), and 0.016 g (FD), while no visible remnants were recovered from the greenhouse (GH) soil. Soil organic matter content decreased from 2.04% to 1.48% (GH) and 1.18% (GC), indicating BC decomposition and interaction with soil environmental.

Significance: Bacterial cellulose's biodegradability positions it as a promising material for sustainable packaging applications. While drying methods affected BC thickness, they did not significantly impact its overall degradation behavior in soil environments.

Award Application: Developing Scientist Award

P1-163: FOOD CONTAMINATION BY FOREIGN MATERIALS DETECTED IN COMMERCIAL LABORATORIES IN JAPAN, 2015-2019

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Akira Terashima, Japanese Consumers' Co-operative Union

Goro Tajika, Ikari Shodoku, Co., Ltd.

Jun Murasugi, Ikari Shodoku Co., Ltd.

Akira Fujimura, Ikari Shodoku Co., Ltd.

Yuko Kumagai, Wayo Women's University

Yoshinori Mizoguchi, Hiroshima Jogakuin University

Shizunobu Igimi, Food Safety Research Center, Tokyo University of Agriculture

Kunihiro Kubota, National Institute of Health Sciences

Presentation type/Format: Poster

Category: Physical Hazards and Foreign Materials

Abstract:

Introduction: In Japan, complaints on food contamination by foreign materials (FMs) are reported to the municipal health centers. We presented archived data for the complaints from health centers at IAFP2023. However, not all FM-contamination events are reported to health centers. As a different information source, complaints detected in commercial laboratories were aggregated and analyzed in this study. Nationally aggregated data are necessary to guide food business operators (FBOs) in properly conducting control measures against food contamination by FMs.

Purpose: To widely analyze the situation and the risk factors for food contamination by FMs in Japan.

Methods: Complaints from the consumers on food contamination by FMs were provided by Ikari Shodoku Co., Ltd. (period: 2015–2018) and by Japanese Consumers' Co-operative Union (period: Fiscal Year 2016–2018). We compiled the data for Ikari Shodoku and CO-OP, respectively, by FMs, food items, occurrence of health damage, and hard object contaminants.

Results: There were 131,389 (Ikari Shodoku) and 4,299 (CO-OP) complaints on

food contamination by FMs during the data collection period. Most frequently reported FMs to Ikari Shodoku were plastics followed by plants and metals. Raw materials followed by plastics and dirt were reported to CO-OP. Lunch box/various side dishes, bread/confectionery, and frozen processed products were the most frequently reported food items in Ikari Shodoku data. Frozen foods, confectionery, and frozen vegetables were the most frequently reported food items in CO-OP data. Thirty-two events with injury and five events with unwellness were reported in CO-OP data. The FMs that caused injury were raw materials (81.3%), plastics (9.4%), stones (6.3%) and plants (3.1%).

Significance: These data, together with the municipal data, enable us to recognize the situation of food contamination by FMs in Japan, and to guide FBOs in preventing food contamination by FMs.

P1-164: TRANSFER OF SALMONELLA ENTERICA, ESCHERICHIA COLI O157:H7 AND LISTERIA MONOCYTOGENES TO MICROGREENS AND SOIL FROM CONTAMINATED IRRIGATION WATER

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Abani Pradhan, University of Maryland

Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Similar to leafy greens, microgreens may be contaminated at the pre-harvest level. There are recalls associated with microgreens, leading to growing food safety concerns amidst increased consumption due to beneficial bioactive compounds.

Purpose: Irrigation water has been identified as a source of several foodborne pathogens in leafy greens. This study aims to determine the transfer of enteric pathogens to microgreens irrigated with contaminated water.

Methods: Municipal(MW) and rainwater(RW) inoculated with low and high inoculum-level cocktails of *S. enterica* and *E.coli* O157:H7 or *L. monocytogenes* was used to irrigate daikon, red cabbage, broccoli, and mustard, grown on soil beds on days 0 and 3. On days 7 and 14, microgreen and soil samples (n=288) were harvested, pummeled in 5X TSB, and subjected to MPN or spiral plating on selective media. Seeds and soil samples were confirmed to be pathogen-free, prior to use. Results from three independent replicates were analyzed for significant differences due to microgreens, contamination level and source of water.

Results: There was a significant difference in the numbers of pathogens recovered over days 7 and 14, irrespective of the microgreen or water type. At 5 log CFU/g, broccoli, daikon and red cabbage irrigated with RW-*Salmonella* showed significant reduction by day 14. Daikon, mustard and red cabbage irrigated with MW-*Listeria* showed significant changes over 14 days. Similar results were obtained for *E. coli* O157:H7 in Daikon and red cabbage irrigated with RW-*E.coli*. At 3 log CFU/g, only broccoli irrigated with *E.coli*-RW showed significant changes in pathogen populations over 14 days and a significant reduction by day 14 when irrigated with *Salmonella*-RW.

Significance: Pathogen transfer from irrigated water to edible portion of microgreens and potential internalization pose significant risk to human health.

Stringent control of microbial quality of irrigation water is required in Controlled environment agriculture to minimize pathogen contamination.

P1-165: ALTERNATIVE IRRIGATION WATER WITH DIFFERENT MICROBIAL PROFILE AFFECTS THE BIOACTIVE COMPONENTS OF MICROGREENS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: The consumption of microgreens has increased globally due to the awareness of the beneficial effects attributed to the presence of antioxidant bioactive compounds such as glucosinolates. Alternative irrigation water containing different microbial populations may alter the metabolite profiles of microgreens.

Purpose: The microgreen industry relies on municipal water as the primary irrigation source. This study compares the differences in nutritional profiles of microgreens irrigated with two different water sources.

Methods: Daikon, red cabbage, mustard and broccoli microgreens were grown in a controlled environment chamber using municipal and roof-harvested rainwater as irrigation sources. Methanolic extracts of freeze-dried microgreens were analyzed using ultra-high-performance-liquid-chromatography-high-resolution-mass-spectrometry (UHPLC-HRMS). A Vanquish UHPLC with an Orbitrap Fusion ID-X Tribrid mass spectrometer with an Agilent Eclipse Plus C18 column was used with specific settings to establish the metabolite profiles. The analyses were done in triplicates and the significant differences in the relative abundances of each of the marker bioactive compounds were determined. The chemical and microbial fitness of the water sources was established using YSI probes and IDEXX testing, respectively.

Results: The two irrigation water sources were free from pathogens but their microbial and chemical parameters were different. The effects of the type of irrigation water on the glucosinolates was found to be species dependent and differed between microgreens. Neoglucobrassicin was found in all microgreens except daikon. Red cabbage microgreens irrigated with both sources of water had profiles that were distinctly different from the other microgreens and showed the presence of glucoraphanin, 4-methoxyglucobrassicin, glucoiberberin among

others. Results indicated that the type of water did affect the metabolite profiles of microgreens.

Significance: Alternative sources of irrigation water are required for sustainability and during adverse climate conditions. Irrigation water source affects beneficial bioactive compounds of microgreens.

P1-166: SURVIVAL OF LISTERIA MONOCYTOGENES ON THE SURFACE OF BLUEBERRIES AND RASPBERRIES STORED AT -18°C AND 4°C

Primary Author:

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Claire Murphy, Washington State University

Miriam Ruiz, Washington State University - Irrigated Agriculture Research and Extension Center

Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: *Listeria monocytogenes* poses a significant food safety concern for raw and minimally processed berries, due to its ability to survive and multiply at low temperatures.

Purpose: This study aimed to determine the growth and survival of varying levels of *L. monocytogenes* on blueberries and raspberries stored at refrigeration and freezer temperatures over time.

Methods: Fresh blueberries and raspberries were inoculated with 20µg of a five-strain cocktail of rifampicin-resistant *L. monocytogenes*. The initial inoculum target was 6.5 log CFU/g for high inoculation and 3.5 log CFU/g for low inoculation. Berries were stored at 4 ± 2°C and -18 ± 2°C. Samples were enumerated at 0, 0.167 (4 h), 1, 2, 3, 5, 7, 10, and 14d for 4°C and 0, 1, 3, 7, 14, 28, 56, 84, 112, 140, and 168d for -18°C. *L. monocytogenes* populations were determined by duplicate plating of samples onto tryptic soy agar supplemented with rifampicin. A two-way ANOVA with Tukey's post-hoc test was performed to determine significant differences ($p < 0.05$) between means for sampling times and temperatures.

Results: Raspberries stored at 4°C showed no significant changes ($P > 0.05$) in *L. monocytogenes* populations over 10d, regardless of the initial inoculation level. In contrast, high-inoculated blueberries at 4°C experienced a significant reduction in *L. monocytogenes* populations ($p < 0.05$) of 1.6 log CFU/g over 14d, while low-inoculated blueberries showed no significant differences ($p > 0.05$ between 0d and 14d). While *L. monocytogenes* populations at -18°C significantly decreased ($p < 0.05$) over time for both blueberries and raspberries, regardless of inoculation level, populations were still detectable at 168d.

Significance:

Results suggest that blueberries and raspberries support the survival of *L. monocytogenes* under refrigeration and freezer temperatures, regardless of starting population level. This has critical implications for the produce industry, emphasizing the need for stringent pre- and postharvest practices to limit

contamination.

Award Application: Developing Scientist Award

P1-167: REDUCTION OF LISTERIA MONOCYTOGENES ON BLUEBERRIES AND POSTHARVEST WATER BY UV-C LIGHT

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: The persistence of *Listeria monocytogenes* on fresh and minimally processed blueberries poses a significant food safety challenge, necessitating innovative, non-thermal interventions to reduce contamination while preserving product quality.

Purpose: The objective of this research was to determine the reduction of *L. monocytogenes* on inoculated blueberries treated with three dosages of UV-C 254 nm (232, 478, and 843 J/m²), both with and without postharvest water at varying turbidity levels.

Methods: Fresh blueberries were inoculated with a five-strain cocktail of *L. monocytogenes* to reach a starting population of 4.5 log CFU/g. An enclosed conveyor belt operated with an integrated UV-C 254nm bulbs system was used to irradiate 10 Grams of blueberries at 232, 478, and 843 J/m². Trials included inoculated blueberries not in water and inoculated blueberries submerged in turbid water at 0, 150, 300, and 450 NTU. Two trials of three samples per treatment (n=6) were conducted. Samples were serially diluted in peptone water and plated in duplicate on tryptic soy agar and modified oxford agar with rifampicin. A log-linear model was used to characterize how each combination influenced *L. monocytogenes* reductions.

Results: *L. monocytogenes* populations were significantly reduced ($p < 0.05$) by 1.10.3, 1.90.7, and 2.71.4 log CFU/g after irradiating blueberries without water at 232, 478, and 843 J/m², respectively. When inoculated blueberries were placed in simulated postharvest water, ~2.9 log CFU/mL of *L. monocytogenes* was transferred to water prior to UV-C treatment, regardless of water turbidity level. As UV-C dosage increased, significantly greater reductions of *L. monocytogenes* on blueberries and in water were observed ($p < 0.05$). Although reductions did not differ significantly at turbidity levels of 0, 150, and 300 NTU ($P > 0.05$), a turbidity of 450 NTU led to significantly lower *L. monocytogenes* reductions ($p < 0.05$).

Significance: These findings highlight the importance of optimizing postharvest

treatments and emphasize that UV-C irradiation is a promising non-thermal intervention for improving the microbial safety of blueberries.

Award Application: Developing Scientist Award

P1-168: IMPACT OF PRE-HARVEST WATER STRESS ON ENTERIC PATHOGEN PERSISTENCE ON BABY ROMAINE LETTUCE IN STORAGE

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Donald Murphy, University of Maryland, Upper Marlboro Facility

Guy Kilpatric, University of Maryland, Terp Farm

Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: The impact of pre-harvest water stress on the persistence of enteric pathogens on baby Romaine lettuce leaves is not well understood.

Purpose: To explore how different irrigation treatments during lettuce cultivation affect enteropathogen persistence on harvested lettuce and during subsequent refrigerated storage.

Methods: Romaine lettuce 'Rouge d'Hiver' was direct seeded along 60 cm wide beds in a high tunnel at the Central Maryland Research and Education Centre, Upper Marlboro. Two irrigation treatments were set up in a randomized design based on regular watering (High, n=3) and water restriction (Low, n=4) and volumetric soil water content (VWC) recorded continuously prior to harvesting at 26 days post-germination. On harvest day, 3 sets of 2 leaves per treatment replicate were placed into sterile bags and transported to the lab on ice for bacterial inoculations with rifampicin-adapted *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* inocula of 5–6 log CFU/mL. Leaves were spot inoculated with 100 µL either on the abaxial or adaxial side and incubated for 18 h at room temperature (RT) or for 3 or 6 days at 4°C. Leaves were then stomached for 1 min and serial dilutions plated on tryptic soy agar with rifampicin for colony counting.

Results: Soil moisture ranged from 0.08 m³/m³ (low) to 0.14 m³/m³ (high) (p<0.01). When incubated at RT, 0.2 log CFU fewer *E. coli* counts were detected on leaves grown under low irrigation (p=0.01), while *Listeria* and *Salmonella* were unaffected. Under refrigerated storage, counts from low irrigation leaves inoculated with *Listeria* decreased by 0.4 log CFU from day 3 to day 6 (p≤0.05), while those inoculated with *E. coli* O157:H7 and *Salmonella* were unaffected.

Significance: Despite different watering regimes, the soil moisture did not vary

widely between high and low watering plots. Pathogen persistence on lettuce differed only slightly with soil moisture levels during cultivation and post-harvest storage time.

P1-169: SPECIALIZED METABOLITE RESPONSES TO INTERNALIZED ENTEROPATHOGENS IN BABY ROMAINE LETTUCE 'CARLSBAD' PRE- AND POST-HARVEST

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Claire Hudson, University of Maryland

Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: How *Escherichia coli* O157:H7 (EcO157) and *Salmonella enterica* affect lettuce metabolite levels at different stages of harvest is unknown, hindering effective strategies for improving food safety.

Purpose: Explore how EcO157 and *Salmonella* affect plant specialized metabolites in pre-harvest and post-harvest baby romaine lettuce.

Methods: Romaine lettuce (*Lactuca sativa*) 'Carlsbad', grown for 4 weeks under controlled conditions was used as pre-harvest plants, or leaves cut, washed with SaniDate15, dried, bagged and stored for 7 days at 4°C for post-harvest leaves. Using a needleless syringe, pre- and post-harvest leaves were infiltrated with 100 µL of deionized water (DI), or live or heat-killed inocula of rifampicin-adapted EcO157 and *Salmonella* Enteritidis (~7 log CFU/mL). Pre-harvest samples were incubated for 4 and 24 h, and post-harvest samples were incubated for 24 h, then homogenized in 0.1% peptone water and enumerated by plate counting. Lyophilized leaf tissue was extracted in aqueous methanol for spectrophotometric analysis. Standard curves of phenolic compounds apigenin, catechin, gallic acid and quercetin were used to calculate equivalent concentrations at specific wavelength absorbances.

Results: Pre-harvest leaves inoculated with EcO157 showed greater log reductions at 4 hpi compared to post-harvest leaves at 24 hpi, 1.16 and 0.59 log CFU/leaf, respectively ($p < 0.05$). Similarly, counts from pre-harvest leaves at both 4 and 24 hpi with *Salmonella* (2.2 and 2.0 log CFU/leaf, respectively, $p < 0.001$ for both time points), were lower than post-harvest leaves 24 hpi (1.33 log CFU/leaf). For all metabolites tested, phenolic compound equivalent (PCE) values were higher ($p < 0.01$) in pre-harvest than post-harvest leaves. PCEs were elicited in pre-harvest leaves 4 hpi with live and dead *Salmonella*, which were reversed at 24 hpi. PCE levels in pre-harvest leaves were unchanged following EcO157. We observed no differences overall for post-harvest leaves.

Significance: Changes in secondary metabolites in response to inoculations with both live and dead pathogens are most notable in pre-harvest leaves compared to

post-harvest leaves.

Award Application: Developing Scientist Award

P1-170: GALACTURONIC AND QUINIC ACID LEVELS ON TOMATO FRUIT SURFACES VARY BY CULTIVAR AND RIPENESS AND SHOW DIFFERENTIAL ASSOCIATION WITH SALMONELLA NEWPORT

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Tomato fruit surface metabolomes, containing sugars, organic and phenolic acids, shift with ripening. *Salmonella* associates positively with simple sugars on the fruit surface; however, the interaction between organic acids and *Salmonella* has not been described.

Purpose: To identify organic acids on tomato fruit and assess any modulatory effects on *Salmonella* Newport.

Methods: Tomato fruits of 'Heinz-1706', 'Black Icicle', 'Purple Bumblebee', 'Emerald Evergreen', and 'Amish Paste' were harvested and washed in sterile distilled water for 90 minutes with shaking to collect surface metabolites. Washes were lyophilized and reconcentrated in 1.5 mL HPLC water. Organic acids were separated using an Agilent HPLC1260 on an infinity LabPoroshell EQ-C18 column with an isocratic mobile phase of 0.1% phosphoric acid and 2.5% methanol, and detected with a diode array detector. Standards of galacturonic acid (GA) and quinic acid (QA) were used to identify retention times and quantify compounds based on peak area. Data were analyzed using the Least Square Means model and Tukey HSD. Multivariate NMDS and ANOSIM analyses were used to identify associations between organic acids and *Salmonella* growth parameters in extracts.

Results: GA was higher (151.43 mg/mL) than QA (11.28 mg/g) across all cultivars and ripeness stages ($p < 0.001$). Cultivar was a significant factor for GA ($p < 0.05$) and QA ($p = 0.001$). GA in 'Emerald Evergreen' (390.4 mg/mL) was significantly higher than in 'Heinz-1706' (84.60 mg/mL; $p < 0.05$). QA levels in 'Emerald Evergreen' and 'Amish Paste' (24.4, 20.1 mg/mL) were higher than in 'Heinz-1706' (2.81 mg/mL; $p < 0.05$). GA correlated with *Salmonella* growth rate ($r = 0.38$, $p < 0.05$) and final population ($r = 0.28$; $p = 0.1$). QA correlated with lag phase ($r = 0.42$, $p < 0.05$) and growth rate ($r = 0.60$, $p < 0.001$). NMDS plotting (stress=0.08) and ANOSIM statistics showed clustering by cultivar ($R = 0.12$, $p < 0.05$) and ripeness ($R = 0.11$, $p < 0.05$).

Significance: Galacturonic and quinic acid levels were quantified for the first time

on the tomato surface. Organic acids may contribute to modulation of *Salmonella* dynamics on the fruit surface.

P1-171: TOMATO EXOCARP POLYPHENOLIC PROFILES SHOW CULTIVAR VARIATION AND SHIFT DIFFERENTIALLY WHEN INOCULATED WITH EIGHT *SALMONELLA* ENTERICA SEROVARS ON THE FRUIT SURFACE

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: *Salmonella*-tomato interactions have been studied to assess metabolite influence on *Salmonella* but fruit responses to the pathogen need investigation.

Purpose: To assess polyphenolic shifts in tomato exocarp when inoculated with *Salmonella*.

Methods: *Solanum lycopersicum* var. *cerasiforme* (cherry tomato; Slc) and cultivar 'Orange Peach' (peach colored, fuzzy heirloom tomato; OP) were grown under high-tunnel conditions and harvested at red-ripeness. Tomatoes (n=5/serovar) were inoculated with ~5 log CFU/fruit of *Salmonella* Newport, Montevideo, Javiana, Anatum, Braenderup, Poona, Saintpaul and Senftenberg. Uninoculated and distilled water (DI) inoculated fruit were used as controls. After 24 h, fruits were flash-frozen and peels removed and lyophilized. Polyphenolics were extracted from 25 mg of peel with 2% glacial acetic acid in 80% methanol and profiles obtained on UV-Vis scanner at 210-400 nm. Standard curves of gallic acid (GAE), catechin (CE), quercetin (QE) and apigenin (AE) were made to quantify polyphenols. The sum of all equivalent measures gave total phenolic (TP) values for each treatment.

Results: No difference in TP was detected between uninoculated fruit of Slc and OP (43.7 and 45.0 mg/g TPE, respectively), but Slc recorded higher apigenin ($p < 0.001$) and quercetin ($p < 0.01$) than OP. UV-Vis profiles and TP showed *Salmonella*-inoculated tomatoes had lower absorbance than uninoculated ($p < 0.05$, Kruskal-Wallis test) and DI-inoculated tomatoes ($p < 0.05$, OP only). A higher *Salmonella*-induced TP reduction was detected in OP ($p < 0.05$; Brown-Forsythe test). Quercetin, catechin and gallic acid in OP were lower than uninoculated fruit for all serovars ($p \leq 0.05$) except *S. Saintpaul* and *Poona*. Slc inoculated with *S. Javiana* (4.2 mg/g QE) and *Saintpaul* (4.33 mg/g QE) recorded lower quercetin than uninoculated tomatoes (6.80 mg/g QE) ($p < 0.05$). In Slc, *S. Braenderup* induced higher catechin than *S. Javiana* and *Senftenberg* ($p < 0.05$)

and higher gallic acid than S. Senftenberg ($p < 0.05$).

Significance: Data show that polyphenolic profiles in tomato fruit vary by cultivar and shift in response to *Salmonella* in a serovar-dependent manner.

P1-172: EFFECT OF FLAVONOIDS FROM TOMATO EXOCARP EXTRACTS ON *SALMONELLA* GROWTH DYNAMICS IN VITRO

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Tomato fruit exocarp contains flavonoid compounds with antioxidant and antimicrobial effects, whose potential antimicrobial properties against *Salmonella* have not been investigated.

Purpose: Investigate flavonoid levels and profiles in exocarp of different tomato cultivars and their potential antimicrobial effects on *Salmonella* Newport and Montevideo.

Methods: Flavonoids were extracted from 20 mg of lyophilized fruit exocarp of tomato cultivars 'Black Icicle', 'Midnight Roma', 'Dixie Red', 'Indigo Rose', 'Indigo Cherry', 'Heinz-1706', and 'Charger' in 5% methanol for polyphenolic, total sugar, and microbial analyses (n=5/cultivar). Total flavonoids were measured using a colorimetric assay (at 510 nm absorbance), quantified using a catechin standard curve and expressed as mg catechin equivalent (CE)/g. Flavonoid profiling was performed on a UV-Vis spectrophotometer at 240–400 nm. One mL aliquots of extracts were inoculated with ~6 log CFU/mL *Salmonella* Newport or Montevideo, incubated with shaking at 35°C and sampled at 2, 8, and 20 h post-inoculation for plating enumeration (n=5/timepoint). Microbial data were analyzed using a Least-Squares Mean model with Tukey HSD.

Results: *Salmonella* counts increased over time across all cultivars and serovars except for *S. Montevideo* on 'Heinz-1706'. Timepoint was a significant effect for microbial growth in extracts for both *S. Newport* (<0.001) and *Montevideo* (<0.05). Significant differences in *Salmonella* counts were detected only at 20 h for *S. Montevideo* in 'Heinz-1706' (lower, <0.001), 'Indigo Rose' and 'Dixie Red' (higher, <0.05). Moreover, multiple significant differences were detected across timepoints for *S. Newport* (<0.05). *S. Newport* counts were higher than *Montevideo* overall in 'Black Icicle', 'Charger' and 'Indigo Cherry' extracts (p<0.05). 'Indigo Rose' had higher total flavonoids (85.38 mg CE/g) than 'Charger' (17.98 mg CE/g, <0.01), 'Heinz-1706' (20.61 mg CE/g <0.01), and 'Black Icicle' (27.25 mg CE/g, <0.05). A weak positive correlation between microbial growth and total catechin content was observed (r = 0.11, <0.1).

Significance: Tomato exocarp flavonoid levels and profiles, which varied by cultivar, had a weak effect on *Salmonella* growth that differed between two serovars.

P1-173: COMPARING THE SURVIVAL OF LISTERIA INNOCUA ON ORGANIC AND CONVENTIONAL PEARS STORED UNDER COMMERCIAL RA AND CA CONDITIONS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Pears are typically stored under commercial storage conditions for extended periods. While fresh whole pears have not been directly linked to listeriosis outbreaks, the potential for contamination and subsequent survival of *Listeria monocytogenes* during postharvest handling and storage remains a concern. Understanding the factors influencing *Listeria* survival on pears is crucial for developing effective mitigation strategies to ensure safety.

Purpose: This study examined the survival of *L. innocua*, a surrogate of *L. monocytogenes*, on organic and conventional Bartlett and d'Anjou pears stored under two common commercial conditions: refrigerated air (RA) and controlled atmosphere (CA) storage for up to 36 weeks.

Methods: Organic and conventional Bartlett and d'Anjou pears were inoculated with or without $\sim 6.5 \log_{10}$ CFU/mL of 3-strain *L. innocua* cocktail and subjected to RA and CA storage for up to 36 weeks. Sampling occurred in weeks 3, 6, 12, 18, 24, 30, and 36 for microbial enumeration. The firmness, soluble solids, and titratable acidity of the pears were further measured at weeks 24 and 36 using uninoculated fruits.

Results: *L. innocua* population on the surface of conventional and organic d'Anjou pears declined by 2.1-2.2 and 2.5-2.6 $\log_{10}$ CFU/pear, respectively, over the storage period. On Bartlett pears, *L. innocua* declined by ~ 1.6 and 2.1-2.6 $\log_{10}$ CFU/pear for conventional and organic pears, respectively, after 12 weeks of

storage. The levels of aerobic bacteria also decreased by 0.81-1.36 log₁₀CFU/pear across the varieties. Yeast and molds increased by 0.69-1.42 log₁₀CFU/pear. Additionally, the firmness of the pears decreased under all storage conditions, while SS and TA remained unchanged.

Significance: This study provides insights into the survival dynamics of *Listeria* on organic and conventional pears under commercial conditions, highlighting the resilience of *L. innocua* on pears, regardless of cultivation practice, and underscoring the need to effective interventions to ensure pear safety.

Award Application: Developing Scientist Award

P1-174: THE FATE OF LISTERIA INNOCUA ON BARTLETT, D'ANJOU, AND BOSCH PEARS OVER 36 WEEKS OF COMMERCIAL STORAGE

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: *Listeria monocytogenes* is a significant foodborne pathogen associated with fresh produce. Understanding the behavior of *Listeria monocytogenes* is critical for evaluating food safety risks during the prolonged storage of pears.

Purpose: This study aimed to evaluate the survival of *L. innocua*, a surrogate for *Listeria monocytogenes*, on Bartlett, d'Anjou, and Bosc pears stored under commercial Regular atmosphere (RA) and Controlled atmosphere (CA) conditions for up to 36 weeks.

Methods: Conventional Bartlett, d'Anjou, and Bosc pears were inoculated with or without ~6.5 log CFU/mL of 3-strain *L. innocua* cocktail. The pears were coated with PrimaFresh Carnauba wax and stored under commercial CA or RA conditions for up to 36 weeks. Pears under storage were sampled at 0, 3, 6, 12, 18, 24, 30 and 36 weeks for microbial enumeration. The quality of uninoculated pears was evaluated at 24 and 36 weeks of storage by assessing firmness, soluble solids content (SS), and titratable acidity (TA).

Results: After 36 weeks of storage, *L. innocua* population on d'Anjou and Bosc pears decreased by 2.44-2.47 and 2.02-2.05 log₁₀ CFU/pear under both CA and RA conditions, respectively. *L. innocua* on Bartlett pears exhibited a reduction of

1.47-1.74 log₁₀ CFU/pear over 12 weeks of storage. Aerobic bacteria levels declined by 1.58-2.25, 1.38-1.52 and 1.06-1.11 log₁₀ CFU/pear on Bartlett, d'Anjou and Bosc respectively. Meanwhile yeast and mold counts increased by ~1.5 log₁₀ CFU/pear on pears, regardless of the storage condition or variety. Quality assessments revealed no significant changes in SS or TA across all varieties; however, firmness significantly declined during storage.

Significance: This study provides valuable insight into fates of *L. innocua* and other resident microbial population on pears during extended commercial storage, thereby assisting the industry in implementing practical measures to mitigate the food safety risks.

Award Application: Developing Scientist Award

P1-175: VALIDATING SANITIZER INTERVENTIONS IN APPLE DUMP TANKS TO CONTROL LISTERIA MONOCYTOGENES WITH ENTEROCOCCUS FAECIUM NRRL B-2354

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Cross-contamination of *Listeria monocytogenes* during the postharvest dump tank practices presents a major food safety risk. Chlorine and peroxyacetic acid (PAA) are commonly used in commercial apple dump tanks to prevent cross-contamination, but their practical efficacy against *L. monocytogenes* remains underexplored.

Purpose: To assess the effectiveness of chlorine and PAA in reducing *L. monocytogenes* cross-contamination in commercial dump tanks at four Washington apple packinghouses, using *Enterococcus faecium* NRRL B-2354 as a surrogate.

Methods: in-plant sanitizer testing was conducted at the end of the dump tank water's usage life. In each facility, a half bin of apples (200 inoculated, 800 uninoculated) was processed in the commercial dump tank for 2-5 minutes. At each time point, ~ 100 inoculated and 400 uninoculated apples were collected for microbial survival analysis. Water samples were also collected in duplicates from

three different locations for microbial enumeration and physiochemical property measurement.

Results: The efficacy of chlorine, PAA, and water parameters varied significantly across facilities. Both sanitizers reduced cross-contamination but could not completely prevent it. A 2-5 min processing using 50ppm chlorine and 60ppm PAA, respectively, reduced *E. faecium* by 0.9-1.9 and 1.2-2.2 log on inoculated apples, regardless of apple variety. This led to 1.7-3.6 and 1.6-3.3 log transfers to the uninoculated apples, with 0.1-0.8 log residual in the dump tank water.

Significance: This study provides valuable insights into the apple industries on the anti-*Listeria* efficacy of chlorine and PAA in commercial dump tank washing systems, offering practical guidance for improving food safety protocols in postharvest processing.

Award Application: Developing Scientist Award

P1-176: COLONIZATION AND INTERNALIZATION OF SALMONELLA IN PEACH FRUIT

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: An outbreak of *Salmonella* associated with the consumption of contaminated fresh peaches occurred in 2020. While *Salmonella* is not typically associated with peaches, it was suspected that fugitive dust from a nearby poultry farm could have served as the route of contamination.

Purpose: To investigate the ability of *Salmonella* Enteritidis to colonize and internalize peach fruit when applied to blossoms during pre-harvest as an initial proof-of-concept study.

Methods: Peach trees (*Prunus persica*) of two varieties, Florida Prince (semi-freestone, yellow flesh, grown in mild winter areas) and Babcock (semi-freestone, white flesh, lower acid, grown in California), of fruiting size (1.25" caliper) were purchased commercially and maintained in two controlled environmental chambers within the NC State pHytotron for up to three seasons. Each blossom was manually pollinated 1-4 hours prior to inoculations. *Salmonella* Enteritidis contamination was introduced via blossoms at ca. 6.6 log CFU/blossom. Peaches were analyzed for *Salmonella* by enrichment in accordance with modified FDA-BAM methods. Data were analyzed for prevalence of contamination (surface and inside).

Results: of the total yellow cling peach fruit harvested from *Salmonella*-inoculated blossoms (n=107), 16.8% (18/107) were contaminated, and of those *Salmonella*-positive, 38.9% (7/18) had *Salmonella* internalized into the fruit. For white flesh peaches collected from *Salmonella*-inoculated blossoms (n=25), 12.0% (3/25) were contaminated, and of those *Salmonella*-positive, 0.0% (0/3) had *Salmonella* internalized into the fruit.

Significance: These

Results from a controlled growing environment identified blossoms as a possible route by which *Salmonella* Enteritidis can colonize and internalize mature peach fruit when exposed to contamination during pre-harvest. Future work will examine poultry litter dust as a vehicle for *Salmonella* contamination through the flower.

P1-177: AN INVESTIGATION OF SALMONELLA AFRICANA AND BRAENDERUP INFECTIONS LINKED TO CUCUMBERS FROM SOUTHEAST FLORIDA - UNITED STATES, 2024

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Between 2012 and 2018, six multistate outbreaks of *Salmonella* Javiana, Saintpaul, Poona, infantis, and Oslo were likely associated with cucumbers in the United States; resulting in 1,098 illnesses, 248 hospitalizations, and six deaths.

Purpose: in 2024, an outbreak investigation of one *S. Africana* strain and four *S. Braenderup* strains, grouped into a single outbreak cluster, were linked to cucumbers grown in Southeast Florida, resulting in 551 ill people in 34 states and the District of Columbia with 155 hospitalizations reported; this was the first known outbreak of *S. Africana* in the U.S. Methods: An illness was defined as infection with *S. Africana* or *S. Braenderup* with an isolate related by whole genome sequencing to the outbreak strains and illness onset from March 11 to

July 26, 2024. Traceback analysis and farm inspections were conducted per standard FDA practices.

Results: of those illnesses with known information, 68% (155/226) reported consuming cucumbers. FDA's traceback investigation included 19 ill people from nine states with cucumber exposure associated with 17 points of service (POS). Two growers in Southeast Florida were identified as the likely sources of illness; however, they did not account for all illnesses. Three environmental samples from Grower 1 and six from Grower 2 yielded isolates of 39 *Salmonella* strains. Two samples, one from each grower, yielded two of the S. Braenderup outbreak strains in canal water used by the farms. Three environmental samples from public land yielded isolates of eight *Salmonella* strains; one of which matched a 2015 cucumber sample from Grower 2.

Significance: The epidemiologic, traceback, and laboratory evidence confirmed that cucumbers were the vehicle for this outbreak, and two farms in Southeast Florida supplied cucumbers to nearly 60% of the POS in the traceback. Traceback and laboratory findings supported a potential regional contamination issue in Southeast Florida.

P1-178: PULSED LIGHT AND COLD PLASMA SYNERGISTICALLY INACTIVATES E. COLI IN LETTUCE

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Leafy greens such as Romaine lettuce has been continually involved in foodborne illness outbreaks. industry employs chlorine-based antimicrobial wash which has limited efficacy. Novel

Methods are needed.

Purpose: The objective of this study was to determine the efficacy of combined pulsed light (PL) and cold plasma (CP) treatments against *E. coli* O157:H7 on Romaine lettuce as an alternative strategy to current chlorine wash method.

Methods: Romaine lettuce was spot inoculated with a bacterial cocktail containing three outbreaks' strains of *Escherichia coli* O157:H7 to a level of about 6.2 log CFU/g. Two nonthermal treatments combinations were explored, namely, PL followed by CP (PL-CP) and CP followed by PL (CP-PL). Briefly, inoculated lettuce was treated for 45 s with CP either before or after PL treatment for 30 s (31.5 J/cm²). 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 (37°C). When populations were below detection limit, pathogen absence in the sample was confirmed by enrichment

Methods. Experiments were conducted in triplicates and data were analyzed with ANOVA to assess

Significance of treatment effects using statistical analysis system (SAS institute, Cary, NC).

Results: Significant ($p < 0.05$) increases in pathogen inactivation was achieved by combination treatments of PL and CP compared to individual treatments. PL (30 s) and CP (45 s) treatments individually inactivated 2.7 ± 0.65 and 2.1 ± 0.43 logs of *E. coli*, respectively on Romaine lettuce. Both combination treatments (PL-CP and CP-PL) resulted in synergistic inactivation as no *E. coli* cells were detected after combination treatments, indicating > 5 log reductions on produce.

Significance: These results demonstrate that PL and CP combination is an

efficacious treatment strategy which can ensure the microbial safety of Romaine lettuce.

P1-179: LISTERIA SPP. PREVALENCE ON FOOD CONTACT SURFACES IN CHERRY PACKINGHOUSES

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Tree fruits have increasingly been identified as a product of concern for *Listeria monocytogenes* contamination, with numerous recalls and outbreaks linked to cross-contamination between food contact surfaces (FCS) in packing environments. Currently, research is lacking on the prevalence of *Listeria* spp., a surrogate for *L. monocytogenes*, on FCS specific to cherry packing operations.

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

Methods: 40 FCS were sampled in four commercial cherry packinghouses in Washington during 20224 to detect *Listeria* spp. Samples were collected prior to and five hours into packing during each sampling event. Each packinghouse was visited at the beginning, middle, and end of the 6-10 week packing season. The isolation, detection, and confirmation of *Listeria* spp. were conducted following a modified FDA bacteriological analytical manual method with PCR confirmation.

Results: of the 960 samples collected, 1.9% (18) were positive for *Listeria* spp. The FCS with the highest prevalence of *Listeria* spp. were bristle flaps (5/72; 6.9%) and solid conveyor belts (3/72; 4.2%). *Listeria* spp. was found at both sampling times in one case (5.9%), with 29.4% of positives detected only before packing and 64.7% only during packing. The prevalence of *Listeria* spp. on FCS increased throughout the cherry packing season, with 1, 1, and 16 positive samples at the beginning, middle, and end of the packing season, respectively

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

Award Application: Developing Scientist Award

P1-180: EFFECT OF FUNGICIDES AND/OR LATEX ON CHLORINE-BASED DISINFECTANTS IN POSTHARVEST PAPAYA WASH-WATER, AND PATHOGEN PERSISTENCE

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Antimicrobial treatment of water used for postharvest wash is recommended to prevent cross-contamination of papayas and to prevent outbreaks of pathogens, including *Salmonella*. The wash-water treatments of fungicides with chlorine-based disinfectants are used to increase the safety, quality, and shelf-life of fresh produce including papayas. Papaya latex exudates are released in the wash-water during postharvest washing.

Purpose: This study focuses on determining whether chlorine-based antimicrobials' efficacy is compromised in the presence of fungicide and/or papaya latex exudates during postharvest washing of fresh papayas.

Methods: Dose-response assays (using 24-well plates) were carried out using bleach/sodium hypochlorite (1–50 ppm) with fungicide, Switch 62.5WG (70–2800 ppm) using a *Salmonella* outbreak strain S. Agona SL-1486. In addition, papaya latex (exudates) was also tested in the same assay both with and without fungicide. Pathogen persistence was assessed on fresh papayas that were washed with water, (<10 ppm NaOCl) treated with fungicide, and then contaminated with the *Salmonella*. Bacterial enumeration/persistence of *Salmonella* was carried out on papaya surfaces and wash-water using enrichment culture, selective media and colony-count techniques.

Results: Bleach/NaOCl (1–50 ppm) by itself, fully retained *Salmonella*-reduction activity against 10^6 CFU of bacteria (5 minutes, at RT). When fungicide and/or latex was added with bleach solution, the bactericidal activity was significantly ($P = 0.04$) diminished allowing *Salmonella* persistence. Fungicide alone showed no effect, and fungicide used with bleach dose-dependently antagonized the effect of NaOCl in the wash-solution. Fresh papayas washed with *Salmonella*-contaminated post-harvest wash-water (treated with bleach and fungicide) resulted in *Salmonella* detection for >2 weeks (at 30°C).

Significance: These results show that during postharvest washing, the effectiveness of chlorine-based disinfectant against *Salmonella* is reduced when fungicide is used in the wash solution. Moreover, the presence of papaya latex with fungicide can further antagonize the effect of bleach allowing pathogen persistence on the papayas.

P1-181: STEC GENOMIC DIVERSITY WITHIN LETTUCE RESEARCH PLOTS IN THE US SOUTHWEST

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: A survey of research plots was conducted near a cattle feedlot and composting operation located in the southwestern agricultural region of the US between 2022 and 2024 to assess Shiga-toxin producing *Escherichia coli* (STEC) prevalence and diversity in romaine lettuce and environmental samples.

Purpose: The genomic diversity of STEC recovered from lettuce and environmental samples was investigated in terms of sequence types, serotypes, virulence profiles, and antibiotic resistance genes.

Methods: More than 3000 lettuce and environmental (soil, drag swabs, irrigation canal water, sediment, and air) samples were analyzed. STEC isolates (n=522) were recovered using the FDA BAM 4a method. DNA extraction was performed with Maxwell Cultured Cell kits and DNA libraries were made with Illumina Nextera DNA prep kits. Whole genome sequencing was performed using an Illumina NextSeq 2000 (600 cycles). Sequence data, after removing clonal assemblies, representing each serotype found per sample (n=140), were analyzed with bioinformatic tools such as MicroRunQC_v1.2 workflow and ONT-genome-profiling workflow on GalaxyTrakr.

Results: A total of 42 different serotypes was identified from 140 isolates. Among them, O168:H8 (17.1%) was the most common. Members of the Top Seven STEC serogroup, O26:H11 (2.9%), O103:H2 (2.9%), and O145:H28 (1.4%) were also

recovered. Multilocus Variable Number Tandem Repeat Analysis identified 39 different sequence types among these isolates with ST-718 being the most common (17.1%). A total of 40 unique AMR genes were identified among isolates, with *emrE* (13.4%), *blaEC* (13.3%), and *mdtM* (12.9%) most common. The *stx2a* gene, typically associated with severe human illness, was found in 40 isolates (28.6%), including O168:H8 (32.4%), O26:H11 (10.8%), and O145:H28 (5.4%).

Significance: This work elucidates the genetic diversity of pathogenic STECs associated with research plots located in proximity to a feedlot in the southwestern US. The data obtained can be incorporated into risk assessments to improve food safety.

P1-182: ASSESSING THE MICROBIOLOGICAL SAFETY OF LEAFY GREENS: INSIGHTS FROM FARMERS MARKETS AND GROCERY STORES IN EDMOND, OKLAHOMA

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Leafy greens are essential for healthy diet but are also a common source of foodborne illnesses due to their susceptibility to microbial contamination. Assessing microbiological safety of leafy greens is critical for ensuring public health.

Purpose: This study aimed to assess the microbiological safety of leafy greens sold at farmer's market and grocery stores in Edmond, Oklahoma by quantifying microbial loads and identifying potential food safety risks.

Methods: Leafy greens, including kale, lettuce, curly mustards, collard greens, spinach, and turnip greens, were collected from farmer's markets and grocery stores in Edmond. Samples were homogenized in PBS, diluted, and tested for generic *E.coli*, coliforms, and aerobic bacteria using Petrifilm. For *E.coli* O157:H7, *Salmonella*, and *Streptococcus*, samples were enriched in TSB for 24hrs, plated on selective agars, and incubated at 37°C for 24 hours before colony enumeration (log CFU/g). Pathogen confirmation was performed using qPCR. Two bunches of each leafy green were collected, and duplicates from each bunch were analyzed.

Results: Microbial contamination was observed in all leafy greens at varying levels. Coliform contamination ranged from 1.76 - 3.95 log CFU/ml, with curly mustard showing the highest levels. Aerobic bacterial counts were also highest in curly mustard (3.81 log CFU/ml), followed by lettuce and kale (3.39 and 2.72 log CFU/ml, respectively), while turnip greens exhibited the lowest (2.28 log CFU/ml). *E. coli* was less prevalent, with detectable levels in curly mustard and collard greens (2.15 and 1 log CFU/ml, respectively). Streptococci levels were highest in curly mustard (3.99 log CFU/ml). *E.coli* O157:H7 and *Salmonella* were undetectable. Overall, curly mustard exhibited contamination across most microbial groups, while turnip greens showed the lowest, highlighting varying risks associated with different types of leafy greens.

Significance: This study underscores the importance of field studies in assessing the safety of leafy greens using real-world data to develop targeted food safety measures.

Award Application: Developing Scientist Award

P1-183: COLONIZATION POTENTIAL OF SALMONELLA NEWPORT ON BULB ONIONS WHEN GROWN IN SPIKED SOIL IN CONTROLLED ENVIRONMENT AGRICULTURE

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Several recent multistate outbreaks in the United States were linked to bulb onions. However, there is limited knowledge on the ability of *Salmonella* to colonize bulb onions grown in contaminated soil.

Purpose: The aim of the study was to assess colonization potential of *Salmonella* Newport on bulb onions when grown in spiked soil.

Methods: Bulb onions (red) were started from seed and resultant seedling was transplanted into a pot. Soil was spiked with *S. Newport* during transplant and again two weeks before harvest. Three inoculum levels of *Salmonella* Newport were evaluated [10^2 CFU (low), 10^4 (medium), 10^6 (high) CFU in 200 g soil] in triplicate and three unspiked pots served as controls. Three different trials were performed using two different soil types. After lifting, onions were cured for one week and then tested for *Salmonella* presence by plating following the BAM method. Metagenomic analyses were performed on the bulb onions and soil from the high inoculum group in trial 1 (field topsoil) at 0 h, 1 h, and 24 h.

Results: in trial 1 (field topsoil), one bulb onion was positive for *Salmonella* in the high inoculum group. in trial 2 (Miraclegro), all three bulb onions were positive for *Salmonella* in the high and medium inoculum groups. in trial 3 (Miraclegro), all three bulb onions were positive for *Salmonella* in the high inoculum group and one bulb onion was positive for *Salmonella* in the medium inoculum group. *Salmonella* was not detected in bulb onions in any of the low inoculum group. Metagenomic analyses showed that bacterial diversity was reduced after 24hr enrichment as compared to 0 h and 1 h.

Significance: These data will help to understand colonization capability and changes in the microbial community in soil type and onion after *S. Newport* contamination.

P1-184: ASSESSING THE EFFICACY OF ASCAROSIDE #18 TREATMENT IN THE CONTROL OF ESCHERICHIA COLI O157:H7 IN SPINACH AT HARVEST AND POSTHARVEST STAGES

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Outbreaks of *E. coli* O157:H7 infection linked to spinach consumption have occurred in the United States in 2006, 2012, and 2021. The recurring nature of these outbreaks highlights the need for effective antimicrobial interventions.

Purpose: This study 1) assessed the efficacy of spinach seed treatment with ascaroside (ascr) #18 or Ca(OCl)₂, and 2) evaluated the combined effect of ascr #18 seed and foliar spray on *E. coli* O157:H7 populations in spinach during harvest and postharvest storage.

Methods: Spinach seeds (2 g per sample per treatment) were treated with 20,000 ppm Ca(OCl)₂ or 1 mM ascr#18 for 15 or 20 min, and control seeds were treated with sterilized water or 10% ethanol, respectively. The treated seeds were grown for 4 and 6 weeks. In separate experiments, spinach grown from 1 mM ascr#18-treated seeds were foliar sprayed with ascr#18 solutions (0.1 µM, 1 µM, 10 µM) or a control solution (0.1% ethanol) at three intervals: 1 week, 2 weeks, and 2 days before each harvest in weeks 3, 5, and 7. Following harvest in both experiments, spinach stems (10 g per sample) were inoculated with ca. 6 log CFU of *E. coli* O157:H7. The inoculated samples were stored at 4°C for 4 weeks. *E. coli* O157:H7 populations were enumerated weekly.

Results: The average cell counts in spinach grown from two types of treated seeds were significantly ($P \leq 0.05$) or insignificantly ($P > 0.05$) different from their respective controls on non-selective and selective agar, respectively. Compared to spinach treated with seed treatment only, adding ascr#18 foliar spray resulted in lower *E. coli* O157:H7 populations, with lower populations observed in mature leaves than in younger leaves, which was not shown in seed-only treated spinach.

Significance: This study may assist those aiming to control *E. coli* O157:H7 on

spinach with ascr#18 seed and foliar treatments.

Award Application: Developing Scientist Award

P1-185: SURVIVAL OF SALMONELLA TYPHIMURIUM, LISTERIA MONOCYTOGENES, AND THEIR POTENTIAL SURROGATES ON CANTALOUPE AFTER TRIPLE-WASHING WITH PEROXYACETIC ACID

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: In October 2023, an outbreak of *Salmonella*, where cantaloupes were implicated, took place across 44 states and a total of 407 illnesses, 158 hospitalizations, and 6 deaths were reported due to this occurrence.

Purpose: As a rapid response to the most recent *Salmonella* cantaloupe outbreaks, this study aims to evaluate the survival of *Salmonella* Typhimurium, *Enterococcus faecium*, *Listeria monocytogenes*, and *Listeria innocua* on cantaloupes during storage and after triple-wash exposure (water-water-antimicrobial) with peroxyacetic acid (PAA).

Methods: Bacteria were spot-inoculated onto cantaloupe surfaces prior to triple-washing for 45 s at each washing step using PAA at 0, 27, 54, and 96 ppm. Spread-plating and a modified most-probable-number (MPN) method were utilized for enumeration of cells. The inoculated cantaloupes were also stored in the biosafety cabinet for 0, 1, 3, 6, 9, 12, 18, 24h (day 1), 72h (day 3), 120h (day 5), and 168h (day 7) at 21°C and 40.6% relative humidity. The Tukey-Kramer test for pairwise comparison was conducted at a significant level of $\alpha = 0.05$. The pathogenic and surrogate bacteria survival on cantaloupe surfaces were calculated using USDA-integrated Pathogen Modeling ProGram for Predictive Microbiology software.

Results: D-values for *S. Typhimurium* were lower ($P < 0.05$) than *E. faecium* and were similar ($P > 0.05$) between *L. monocytogenes* and *L. innocua*. Though all

tested concentrations of PAA mixture resulted in significant ($P < 0.05$) reduction of bacteria cells compared to the inoculated unwashed control, the greatest ($P < 0.05$) reduction of *S. Typhimurium* and *E. faecium* (4.55 vs 3.23 log₁₀ CFU/cm² and 3.38 vs 2.55 log₁₀ MPN/mL, respectively) occurred at 96 ppm. This concentration was also the most effective against *L. monocytogenes* and *L. innocua* population (3.76 vs 3.38 log₁₀ CFU/cm² and 3.34 vs 2.90 log₁₀ MPN/mL, respectively).

Significance: *E. faecium* and *L. innocua* could be used as surrogates of *S. Typhimurium* and *L. monocytogenes*, respectively, for antimicrobial washing validation studies on cantaloupes.

Award Application: Developing Scientist Award

P1-186: PLASMA ACTIVATED WATER-MEDIATED INACTIVATION OF E. COLI K12 IN LETTUCE WASH TANK WATER WITH HIGH SUSPENDED ORGANIC MATTER

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: In the fresh produce industry, cross contamination from pathogens suspended in wash tank water represents an ongoing food safety risk. Cold plasma can be injected into wash tank water as a continually renewed sanitizing agent. However, the impact of high levels of suspended organic matter is poorly understood.

Purpose: This study aimed to evaluate the effectiveness of cold plasma-activated water (PAW) as a nonthermal intervention to reduce *E. coli* in simulated wash water containing high organic load.

Methods: Three test solutions were prepared: sterile water (H₂O), buffered peptone water (BPW), and simulated wash tank water (SWT). SWT was prepared by blending 10 g of fresh green leaf lettuce in 500 ml sterile water, creating a suspension of high particulates and suspended organic matter. Cold plasma was injected into 500 ml samples of each liquid with a plasma jet at a distance of 2 cm above the liquid surface. Temperature and pH were monitored during processing. The nonpathogenic *E. coli* K12 was inoculated into H₂O and SWT to create a suspension of ~10⁶ CFU/ml. Microbial counts were assessed immediately after processing (n=9, performed in triplicate, ANOVA with P>0.05).

Results: Cold plasma treatment lowered pH for all solutions: from 6.8–7.0 (untreated control) to 3.8 (H₂O), 4.4 (BPW), and 4.7 (SWT) after 10 minutes of plasma treatment. In inoculated trials, cold plasma reduced the recoverable *E. coli* in H₂O to below detection (a ~6 log (99.999+%) reduction) after 3 minutes in H₂O and after 10 minutes in SWT. The suspended organic matter in the SWT thus somewhat reduced the efficacy of the PAW treatment. Maximum temperature reached after plasma injection (49.2–52.1°C after 10 minutes) is below the level of thermal kill for *E. coli* K12, confirming this form of PAW as a nonthermal antimicrobial process.

Significance: Injection of cold plasma into wash water represents a possible “green chemistry” option to eliminate suspended bacteria, even under conditions

of high organic load.

P1-187: COMPARISON OF TEMPERATURE AND HUMIDITY FROM 2001, 2012, AND 2023 IN THE SURVIVAL OF ESCHERICHIA COLI O157:H7 IN ROMAINE LETTUCE (LATUCA SATIVA) FROM CALIFORNIA AND TEXAS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Outbreaks of *E. coli* O157:H7 linked to romaine lettuce vary from year to year; factors like temperature and humidity may contribute to this irregularity. In most cases, outbreaks linked to romaine or leaf lettuce are primarily attributed to *E. coli* O157:H7, with irrigation water as the principal source of contamination.

Purpose: The study aimed to evaluate whether temperature and humidity varied between years with and without outbreaks (2001, 2012, 2023) and whether these differences affected the survival of *E. coli* O157:H7 in romaine lettuce grown in Salinas Valley, California, and Lubbock, Texas.

Methods: Temperature and humidity data were collected for three years: 2001 (no outbreaks), 2012 (seven outbreaks), and 2023 (one outbreak). *Escherichia coli* O157:H7 survival was evaluated post-inoculation over 28 days in California and 23 days in Texas. Samples were collected and analyzed to determine microbial persistence and its correlation with environmental conditions.

Results: In California, 2023 had significantly higher average temperatures than 2001 and 2012 by 2.5 and 2 °C, respectively ($P < 0.05$), while relative humidity remained similar across years ($P > 0.05$). In Texas, no statistically significant differences in temperature or humidity were observed ($P > 0.05$). The highest *E. coli* O157:H7 survival in California occurred in 2023 ($P < 0.05$), with 1–2 log CFU/g higher levels at day 28 post-inoculation compared to earlier years. In Texas, microbial counts persisted up to 23 days in all years, with 2012 showing higher survival than 2001 ($P < 0.05$), and 2023 resembling both.

Significance: Higher temperatures may increase the survival of *E. coli* O157:H7 in romaine lettuce, particularly in California. These findings suggest that warmer conditions near harvest may heighten the risk of contamination, emphasizing the need for mitigation strategies under changing climate patterns.

Award Application: Developing Scientist Award

P1-188: INVESTIGATING THE PHYSICAL AND MICROBIAL QUALITY OF PEA MICROGREENS ENRICHED WITH PROBIOTICS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Microgreens are a category of emerging food products with increasing popularity due to their ease of growth and health benefits. However, the yield and the microbial quality of microgreens need to be further improved to meet the increasing demand. Probiotic bacteria is used frequently in the food industry for their positive effects on human health. However, the impact of probiotic bacteria on the physical and microbial quality of microgreens remains unknown.

Purpose: This study aims to investigate the effect of a probiotic bacteria *Lactobacillus plantarum* on the yield, appearance, and microbial quality of pea microgreens.

Methods: in this study, 15 pea seeds were soaked in 15 mL of *Lactobacillus plantarum* culture (10^7 CFU/mL) for 18 hours. The controls were soaked in DI water. Then the treated seeds were germinated to pea sprouts in the dark for 3 days and further grown to pea microgreens on coconut pads. The concentration of *Lactobacillus* at different stages of pea plants was evaluated. The seed germination rate, the length, weight, and microbial quality of pea sprouts and microgreens were measured. Three biological replicates and three samples per replicate were conducted for each treatment ($n=9$).

Results: The results showed that seed soaking is an effective approach to enrich the seeds with probiotic bacteria. The treated seeds had significantly more *Lactobacillus* than the control seeds ($p \leq 0.05$). No significant morphology changes were observed for treated sprouts or microgreens when compared to controls ($p > 0.05$). The microbial quality of sprouts and microgreens was found to be slightly improved after the probiotic bacteria treatment ($p \leq 0.05$).

Significance: The findings of this study will be helpful to the sustainable production of microgreens with high quality and function. The treated microgreens could also be used as ingredients for a broader application.

P1-189: OZONE-BASED TREATMENTS FOR INACTIVATING SHIGA TOXIN-PRODUCING ESCHERICHIA COLI O157:H7 ON ALFALFA SEEDS

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a major contributor to foodborne illnesses, frequently linked to contaminated sprouting seeds, including alfalfa.

Purpose: Evaluating gaseous ozone and ozonated water treatments for reducing STEC on alfalfa seeds and their impact on seed viability for safe sprout production.

Methods: Pesticide-untreated alfalfa seeds were inoculated with STEC O157:H7 EDL 933 (10^6 CFU/mL) and incubated for 30 minutes at $\sim 22^\circ\text{C}$. After incubation, the soaked seeds were dried for 2 hours in a laminar flow cabinet. Three treatments were applied: (i) a stream of 5% ozone gas for 25 minutes, (ii) rinsing with ozonated water at a concentration of 5 ppm O_3 for 15 minutes under continuous stirring, and (iii) a sequential combination of gaseous ozone followed by ozonated water. The populations of STEC on untreated and treated seeds were determined by spread-plating diluted samples on tryptic soy agar. Additionally, untreated and treated seeds were analyzed for germination rates. Data were analyzed in GraphPad software.

Results: The population of STEC was significantly reduced ($P < 0.001$) by 1.29 ± 0.10 and 1.25 ± 0.15 log CFU/g seeds following individual treatment with ozone gas and ozonated water, respectively. A combined treatment of ozone gas followed by ozonated water resulted in an additive lethal effect, reducing STEC population on the seeds by 2.45 ± 0.16 log CFU/g ($P < 0.001$). Treatment with ozonated water (5 ppm) for 15 minutes mildly but significantly reduced seed germination (77.19%) compared to untreated seeds (86.96%, $P = 0.001$), whereas ozone gas alone had no significant impact on germination (88.89%, $P = 0.318$). Furthermore, no significant differences in shoot and root lengths were observed between treated and control seeds ($P = 0.212$).

Significance: The combination of ozone gas and ozonated water effectively inactivates STEC on alfalfa seeds with minimal effect on seed germination and no detrimental effect on sprout quality.

Award Application: Developing Scientist Award

P1-190: MEASURING CROSS-CONTAMINATION DURING MECHANICAL BLUEBERRY HARVEST AND THE IMPACT OF CLEANING AND SANITIZING

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Blueberry mechanical harvesters (MHs) are large, complex machines with various hard-to-clean surfaces, posing a potential cross-contamination risk for fresh berries.

Purpose: Quantify bacterial transfer from contaminated berries to MHs and to evaluate the efficacy of a wet cleaning and sanitation procedure to reduce cross-contamination of a subsequent blueberry harvest.

Methods: Blueberry clusters in a field plot (n=96 clusters/plot) were spray-inoculated with rifampicin-resistant *Escherichia coli* (~6.8 log CFU/g) and harvested with a MH (Harvest1). The contaminated MH was used to harvest berries from an uninoculated plot (Harvest2). MH was cleaned and sanitized (C&S; peroxyacetic acid) and used to harvest berries from a third uninoculated plot (Harvest3). Before and after each harvest, MH surfaces (beater bars [A], bottom conveyor belts [B], plastic catcher plates [C], top conveyor belts [D]) were sampled by swabbing (n=4/surface) and enumerated for *E. coli* on Eosin Methylene Blue Agar+rifampicin (37°C, 24h). Harvested berries (100 g/sample; 2 samples/harvested bin; n=30) were also analyzed. The experiment was repeated using two different strains of *E. coli*.

Results: Following Harvest1, surfaces had 4.5, 6.5, 5.7 and 5.7 log CFU/swab for A, B, C and D, respectively, while blueberries averaged 4.4 log CFU/g. After Harvest2, 3.0, 4.8, 4.1 and 3.4 log CFU/swab were recovered on these surfaces, respectively, whereas an average of 1.1 log CFU/g was on blueberries. Following Harvest3, 0.3, 1.4, 1.9 and -0.1 log CFU/swab were recovered from surfaces, and -2.0 log CFU/g from blueberries. Cross-contamination from MH to berries was significantly reduced by C&S (p<0.05, mixed model with Tukey HSD). B and C had significantly higher *E. coli* loads across sampling stages compared with A and D (p<0.05).

Significance: Cross-contamination between blueberries and MH was demonstrated. C&S of the MH significantly reduced, but did not eliminate, cross-contamination. Catcher plates and bottom conveyor belts are more heavily contaminated and require more thorough C&S.

P1-191: VALIDATION OF SANITIZER CONCENTRATION TO REDUCE CROSS-CONTAMINATION RISK DURING WASHING OF APPLES

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Peroxyacetic acid (PAA) and sodium hypochlorite (NaOCl) are both commonly applied in the dump tank washing of apples. However the antimicrobial efficacy of the sanitizers can be influenced by chemical oxygen demand (COD) present in the wash water.

Purpose: This study evaluated the efficacy of 80 ppm PAA and 80 ppm NaOCl to control *E. coli* TVS353 on freshly harvested apples and reduce cross-contamination in a simulated dump tank wash (SDTW) in the presence of low (500 mg/L) and high (3000 mg/L) COD levels.

Methods: One apple was spot inoculated with a 24 h overnight culture of rifampicin-resistant *E. coli* TV353. The inoculated apple was washed in a simulated dump tank wash (SDTW) containing 14 uninoculated apples with 2 min contact time. Three treatments (control, 80 ppm PAA, and 80 ppm NaOCl), and two COD conditions 500 and 3000 mg/L)) were used. Bacterial enumeration was carried out using the most probable number (MPN) method modified using lactose peptone broth.

Results:At low and high COD levels, respectively, 3.8 ± 0.5 and 3.0 ± 0.6 log MPN/apple and 0.2 ± 0.0 and 0.5 ± 0.7 log MPN/apple were detected on inoculated apples for NaOCl and PAA, respectively. NaOCl was significantly influenced by organic matter compared to PAA ($p > 0.05$). For the control, NaOCl, and PAA treatment, the log percent transfer from inoculated to uninoculated apples was -2.5 ± 0.2 , -3.3 ± 0.0 , and -4.5 ± 0.0 log MPN/apple, respectively, at low COD and -3.5 ± 0.3 , -4.3 ± 0.0 , and -4.4 ± 0.1 log MPN/apple, respectively, at high COD. Both sanitizer treatments significantly reduced the transfer of bacteria compared to the control ($p < 0.05$).

Significance: Eighty ppm of either PAA or NaOCl was effective in reducing cross contamination during washing of apples in a wide COD range.

Award Application: Developing Scientist Award

P1-192: SURVIVAL OF LISTERIA MONOCYTOGENES, ESCHERICHIA COLI O157:H7 AND SALMONELLA ENTERICA ON WORK-IN-PROCESS (WIP) FRESH-CUT RED CABBAGE

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Work-in-process (WIP) fresh-cut ingredients, such as cabbage, often stored temporarily at refrigerated temperatures before final food product mixing, represent a critical yet under-explored food safety hazard.

Purpose: The objective of this study was to evaluate the survival of *Listeria monocytogenes* (LM), *Escherichia coli* O157:H7 (EC) and *Salmonella enterica* (SE) on WIP red cabbage, as affected by holding time, temperature, sanitizer treatment, and microbiota.

Methods: WIP red cabbage was inoculated with five-strain composites, each, of LM, EC, and SE, isolated from fresh produce outbreaks. The inoculated samples were washed with 100 ppm chlorine (pH 6.5), 80 ppm peracetic acid (PAA), or water, and stored at 4, 8, and 12°C for 7 days. Populations of inoculated pathogens, total mesophilic aerobic bacteria, aerobic psychrotrophs, and yeast and mold were monitored on days 0, 1, 4, and 7. Quadratic response surface regression models were employed to predict pathogen growth and survival, based on holding temperature, time, sanitizer treatment, and background microbiota.

Results: Results showed that EC and SE did not grow at 4 and 8°C but increased by up to 1.7 log CFU/g at 12°C within 7 days, with no significant ($P>0.05$) differences among sanitizing treatments. In contrast, LM populations increased by up to 2.4 log on samples washed with chlorine and PAA at both 8 and 12°C. Population of psychrotrophic bacteria, and yeast and mold increased over time at all temperatures for all sanitization treatments, but samples treated with PAA often showed the greatest increases, despite large variability in populations. Statistical analysis indicated that holding time and temperature had a significant ($p<0.05$) effect on the growth of inoculated pathogens, and background microbiota, regardless of the sanitization method.

Significance: These findings emphasize the importance of controlling the storage temperature for WIP fresh-cut produce, to reduce the risk of bacterial pathogen growth, highlighting the need for stringent time-temperature management.

Award Application: Developing Scientist Award

P1-193: REDUCTION OF NOROVIRUS ON TOMATOES IN AN OVERHEAD SPRAY AND BRUSH ROLLER SYSTEM

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Addition of sanitizer to single pass water (i.e. overhead spray) used during produce packing can reduce bacterial foodborne pathogens on the surface of fruits and vegetables; less is known about reduction of viral pathogens.

Purpose: The purpose of this work is to evaluate the reduction of MS2 bacteriophage, a norovirus surrogate, on tomatoes in an overhead spray and brush roller system.

Methods: Unwashed, mature green tomatoes were inoculated with 10, 10 µl spots of ca. 9 log PFU/ml MS2 bacteriophage, acting as a surrogate for norovirus. The inoculated tomato was placed onto a model overhead spray brush roller system, with one uninoculated tomato on either side. The wash system was run for 30 s, with different concentrations (0, 20, 60, and 80 ppm) of a peracetic acid (PAA) mixed into well water. For each PAA concentration, five technical replications with three biological replicates were conducted (n=15 tomatoes). MS2 reductions were evaluated on the inoculated tomatoes. MS2 was recovered from the tomatoes using phosphate buffered saline with 1% sodium thiosulfate and a 30 s shake, rub, shake method. Serial dilutions were conducted, and plaque assays were performed to enumerate MS2.

Results: MS2 reductions occurred with only well water and at each concentration of PAA. Well water resulted in a 2.69 ± 0.07 log PFU/tomato reduction, 20 ppm PAA resulted in a 4.95 ± 0.08 log PFU/tomato reduction, 60 ppm PAA resulted in a 5.85 ± 0.03 log PFU/tomato reduction, and 80 ppm PAA resulted in a 3.71 ± 0.26 log PFU/tomato reduction.

Significance: Greater concentrations of PAA lead to higher reduction of viral surrogates, up to 60 ppm PAA, suggesting its ability to also control viral foodborne pathogens during produce washing in an overhead spray and brush roller system.

P1-194: ESCHERICHIA COLI TRANSFER ONTO AND INTERNALIZATION INTO GRAPEFRUIT DROPPED ON FLORIDA SANDY SOILS

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Michelle Danyluk, University of Florida

Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: The US FDA Produce Safety Rule prohibits the distribution of dropped-covered produce due to contamination risks from the ground and the potential for impact damage to facilitate pathogen internalization.

Purpose: Evaluate *Escherichia coli* transfer and internalization potential to grapefruit dropped onto Florida sandy soil from various heights.

Methods: Unwashed and unwaxed red grapefruit (n=240) were randomly selected, weighed, and dropped from five heights (15.24, 30.48, 60.96, 121.92, and 182.88 cm) using PVC pipes onto two sandy soils (A and B) adjusted to 3.5% w/w moisture. Soils were spot-inoculated with gfp-tagged *E. coli* (ca 8 log CFU) and dried for 1 h. Bacterial transfer (BT) to the fruit surface was quantified using plate counts. The bacterial internalization (BI) was assessed by sterilizing and peeling each fruit; both peel and inner tissue were further analyzed by plate counts and enrichment to quantify or identify *E. coli* internalization. Welch's ANOVA identified significant differences ($p < 0.05$) among scenarios, while linear regression examined BT/BI correlations with fruit weight. Three independent experiments with four replicates per condition (N=12) were conducted.

Results: *E. coli* populations significantly increased in sandy soils after inoculation and drying (A=8.40 $\pm$ 0.19 log CFU/soil; B=8.36 $\pm$ 0.15 log CFU/soil). BT onto grapefruit surface ranged from -1.70 $\pm$ 1.38 to -0.87 $\pm$ 0.84 log % CFU/fruit (soil A) and -2.12 $\pm$ 0.47 to -1.25 $\pm$ 0.66 log % CFU/fruit (soil B). Welch's ANOVA indicated that height did not significantly ($p = 0.10$) affect BT. BI was not detected, and no correlation was observed between BT or BI and fruit weight.

Significance: A consistent BT across dropping heights was observed. Despite the absence of any bacterial internalization, these findings underscore the importance of avoiding the harvest of dropped grapefruit to mitigate food safety risks.

P1-196: INFLUENCE OF SYNTHETIC AND ORGANIC NUTRIENT FERTILIZERS ON THE SURVIVAL AND PERSISTENCE OF ESCHERICHIA COLI IN NFT HYDROPONICS SYSTEMS DURING MINT PRODUCTION

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Hydroponics, a soil-free farming method that has the potential to address challenges such as urban growth and limited land, may present food safety risks from potential microbial contamination.

Purpose: This study examined the effects of five synthetic and organic nutrient fertilizer solutions on the persistence and distribution of *E. coli* ATCC 25922 in Nutrient Film Technique (NFT) hydroponic systems during mint production.

Methods: *E. coli* ATCC 25922 (5 log CFU/mL) was inoculated into sweet mint (*Mentha x suaveis* Guss)-grown NFT hydroponic systems (n=6) with three synthetic and two organic (fish-based) nutrient solutions. The nutrient solutions and the mint plants were collected, and the survival, persistence, and distribution of *E. coli*, along with changes in pH and effects on plant growth, were determined over up to 25 days. Each treatment was performed in triplicate, and the *E. coli* levels were enumerated using 3M Petrifilm™ plates. Tukey's honestly significant difference test was used to determine significant differences ($p < 0.05$) in *E. coli* levels based on treatments.

Results: *E. coli* levels decreased over time, dropping from 5 log CFU/mL to below the detectable level in synthetic nutrient solutions in the hydroponic systems 10 days post-inoculation. The level of reduction was significantly different ($P < 0.05$) between static and dynamic hydroponic settings. However, the microbial population increased significantly in fish-based solutions, reaching up to 8.51 log CFU/mL within 24 hours, and the reduction rate was significantly lower in subsequent days compared to the synthetic nutrient solution, maintaining a level of up to 4.18 log CFU/mL until day 10. Mint plants grown in fish-based nutrient

solutions had significantly higher *E. coli* levels in the edible parts and the roots than those grown in synthetic fertilizers.

Significance: This study highlights the greater food safety risks associated with fish-based organic nutrient solutions compared to synthetic ones in the event of microbial contamination.

Award Application: Developing Scientist Award

P1-197: EFFECTIVENESS OF UV-C IN CONTROLLING BLUEBERRY SPOILAGE BY BOTRYTIS CINEREA CONIDIA AND HYPHAE

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: *Botrytis cinerea* causes significant postharvest spoilage, and while UV-C shows promise as a postharvest sanitizer, its efficacy against fungal hyphae is understudied.

Purpose: This study aimed to 1) evaluate UV-C efficacy against *B. cinerea* spores and hyphae and 2) assess the impact of antifungal UV doses on blueberry phenolics and antioxidant content.

Methods: Conidia spores of *B. cinerea* were harvested from 10-day old colonies, while hyphal fragments (HF) were obtained by homogenizing mycelia from 5 to 7-day old colonies and suspended in deionized water. Both spores and HF were exposed to 254 nm UV-C (0, 1.36, 2.72, 4.08, and 5.44 kJ/m²) in a benchtop chamber, and surviving populations were quantified by standard plate counts (performed in triplicate). Kinetics were modeled using JMP Pro 18. Spores and HF were also exposed for the same time planktonically; treated suspension was added to PDA and the OD 595 nm was read over 96 hrs in a microplate reader. UV-C effects on blueberry phenolics and antioxidants were evaluated using Folin-Ciocalteu assay and DPPH (2,2-diphenyl-picrylhydrazyl) assay performed in duplicate.

Results: UV-C treatment reduced spores and HF. Inoculum type was found to have no significant impact on the percent log reductions achieved with UV-C treatments (Student's t-test $p = 0.98$). A mechanistic growth model was used to predict surviving inoculum kinetics against UV-C dosage and indicated strong fits for both spores and HF (spore kinetics: $y = 1.126 * (1 + 3.34e^{-0.692x})$, $R^2 = 0.96$; HF kinetics: $y = 1.056 * (1 + 2.227e^{-1.395x})$, $R^2 = 0.94$). UV-C exposure up to 5.44 kJ/m² did not significantly affect blueberry antioxidant (ANOVA $p = 0.24$) or phenolic content (ANOVA $p = 0.98$).

Significance: This study demonstrates that UV-C effectively reduced both *B. cinerea* spores and *B. cinerea* hyphae—which is less investigated in filamentous

fungi research—and is important to broadening UV's potential as a postharvest antifungal treatment. Importantly, at the doses required to reduce *B. cinerea*, UV-C showed minimal effect on berry quality, supporting its practical application for industry.

P1-198: SURVIVAL OF LISTERIA MONOCYTOGENES ON FROZEN BANANA, CANTALOUPE AND SWEET CORN

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Produce-associated outbreaks and recalls have been linked to *Listeria monocytogenes* contamination.

Purpose: This study sought to quantify *Listeria monocytogenes* survival on banana, cantaloupe, and sweet corn under frozen storage.

Methods: Ten-Gram samples of each fresh produce commodity were spot inoculated with 10 μ L (8-10 drops) of a six-strain rifampicin-resistant *L. monocytogenes* cocktail at ca. 5 log₁₀ CFU/g and frozen at $-20 \pm 2^\circ\text{C}$ for 0, 0.02, 1, 3, 6, 10, 15, 30, 60 and 90 d. Duplicate trials were conducted with five replicates per commodity per time-point. At each time-point, frozen samples were mixed with 20mL of peptone water and macerated for 90 s at low-speed. Subsequently, serial dilutions were surface plated in duplicate onto selective (modified oxford agar) and non-selective media (tryptic soy agar) + rifampicin (80ppm) + 0.1% sodium pyruvate. *L. monocytogenes* die-off was modeled using linear regression models, and Tukey's HSD test was used to determine significant differences in die-off rates ($p \leq 0.05$) in R-Studio (V.4.3.3).

Results: *L. monocytogenes* starting concentrations were at 5.34 ± 0.24 , 5.38 ± 0.17 and 5.38 ± 0.18 log CFU/g in frozen banana, cantaloupe, and corn, respectively, with reductions of 0.25 ± 0.36 , 0.18 ± 0.23 and 0.13 ± 0.25 by the end of the 90 d storage. Based on Tukey's HSD test, no significant differences in die-off rates were found between cantaloupe and corn, while *Listeria monocytogenes* die-off on frozen banana differed from cantaloupe and corn.

Significance: Although no growth was observed, *L. monocytogenes* was still detectable up to 90 d of storage.

Results from this study support the need for effective measures to reduce potential contamination of *L. monocytogenes* prior to freezing.

Award Application: Developing Scientist Award

P1-199: EFFICACY OF POSTHARVEST CHEMICAL SANITIZER TREATMENTS ON SALMONELLA TYPHIMURIUM IN HYDROPONICALLY GROWN LIVING BASIL

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Hydroponic farming is sustainable for producing high-quality fresh produce, including living basil, harvested and sold with intact roots. Roots extend freshness but also accumulate pathogens, thereby increasing postharvest cross-contamination.

Purpose: This study assessed the efficacy of postharvest root dipping in sanitizer solutions—sodium hypochlorite (NaOCl, 100 ppm), peracetic acid (PAA, 40 ppm), and lactic acid (LA, 1%)—on *S. Typhimurium* loads.

Methods: Basil roots were inoculated with 6 log CFU/mL of *S. Typhimurium* and dipped in the sanitizer solutions for 30 and 60 seconds, then rinsed in Dey-Engley neutralizing broth for 60 seconds. Pathogen survival on roots and leaves was assessed immediately after rinsing (0h) and periodically (6h, 12h, 24h, 48h, 5d, 10d, 14d) at 8°C.

Results: Results revealed that storage and sanitizers had significant interactive effects (p -value < 0.05) on the survival of *S. Typhimurium*. After 14 days of storage, the average recovery of *S. Typhimurium* on the control samples was 0.59 log CFU/g. For the treatments, recovery was 3.32 log CFU/g (LA), 2.61 log CFU/g (NaOCl), and 2.47 log CFU/g (PAA).

Significance: These findings suggest that storage time and sanitizer interactions are critical in controlling *S. Typhimurium* during the storage period at 8°C, and highlight the need to optimize storage conditions and postharvest sanitizer

treatments to improve the safety and shelf life of hydroponically grown living basil

Award Application: Developing Scientist Award

P1-200: SURVIVAL OF SALMONELLA AND LISTERIA MONOCYTOGENES ON FIVE PACKINGHOUSE FOOD CONTACT SURFACES

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Presentation type/Format: Poster

Category: Produce

Abstract: Introduction: Food contact surfaces (FCS) in packinghouses may pose a food safety risk due to the persistence of microorganisms in areas that are difficult to clean and sanitize. Purpose: The objective was to examine the survival of Salmonella and Listeria monocytogenes concentrations on polycarbonate, polypropylene, PVC, rubber, and stainless steel FCS. Methods: FCS coupons (5x5 cm) were surface sterilized, and spot inoculated with a five-strain cocktail of 80ppm rifampicin-resistant Salmonella or Listeria monocytogenes at ca. 7 log CFU/coupon. Inoculated coupons were stored at 22°C and 45-55% relative humidity. Coupons were enumerated in duplicate at including 0, 0.06, 0.25, 1, 2, 3, 7, 10, 14, 21, 30, 60, and 90d post-inoculation (dpi), and plated in duplicate on non-selective and selective agars for both pathogens. When counts fell below the limit of detection (LOD, <1.30 log CFU/coupon), enrichments were performed using standard methods. Significant differences over time were evaluated using ANOVA and Tukey's HSD Test ($P \leq 0.05$). Results: By 14 dpi, Salmonella populations declined significantly by 2.2–2.8 log CFU/coupon, ($P < 0.05$) across all material types. By 60 dpi, reductions of >4 log CFU/coupon were observed on all FCS surfaces other than rubber. At 90 dpi, Salmonella counts fell below the LOD except for rubber and stainless steel (2.591.11 and 1.500.64 log CFU/coupon, respectively). L. monocytogenes populations declined significantly by 14dpi (2.19 – 4.01 log CFU/coupon, $p < 0.05$), and by 60 dpi, L. monocytogenes reductions of > 5 log CFU/coupon were observed on all FCS. For both Salmonella and L. monocytogenes, die-off rates were fastest on polycarbonate and slowest on rubber. Significance: Survival of foodborne pathogens varied across FCS, with rubber demonstrating the greatest survival. Since pathogens survived on FCS for up to 90d, establishing frequency of sanitation is key to limiting potential cross-contamination risk.

Award Application: Developing Scientist Award

P1-201: EVALUATING WASTEWATER SEQUENCING METHODS IN FOOD SAFETY SURVEILLANCE

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Wastewater-based epidemiology has emerged as a valuable non-invasive tool for monitoring the prevalence of pathogens, including SARS-CoV-2, in communities. However, the optimal sequencing strategies for this application remain to be fully elucidated, particularly in the context of food safety surveillance.

Purpose: This study aimed to compare high quality long and short read sequencing approaches for the identification of SARS-CoV-2 and other pathogens in wastewater samples, and to evaluate the advantages and limitations of each method for wastewater metagenomics in food safety applications.

Methods: Wastewater samples collected from May to June 2022 across three states were sequenced using both short (150–400 bp) and long (1400 bp) amplicon approaches within the FDA Covidtracr project. Sequence reads were processed by the CFSAN Wastewater Analysis Pipeline to detect point mutations, identify subvariants, and quantify relative abundance. Statistical analysis in R was used to compare the results between the long and short read data.

Results: Comparison of 84 sequenced samples revealed that long read sequencing is more sensitive to wastewater composition than short read sequencing. While short read sequencing provided higher coverage of SARS-CoV-2 genes, long read sequencing detected significantly more ($p < 0.001$) mutations including characteristic mutations of circulating variants, while retaining higher sequence quality (Q80+ versus Q30+). Variant prevalence also differed between the two approaches, with short reads detecting rare subvariants (below 0.5% relative abundance) that were undetected by long reads.

Significance: The complementary strengths of short and long read sequencing approaches identified in this study can inform the design of optimal wastewater metagenomics strategies for food safety surveillance, not only SARS-CoV-2, but

also for other pathogens of concern, such as norovirus, hepatitis A, or antimicrobial resistant bacteria.

P1-202: EVALUATION OF INDICATOR BACTERIA CONCENTRATION IN DIFFERENT HOLDING TIMES

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Presentation type/Format: Technical

Category: Water

Abstract:

Introduction: Maintaining accurate water quality assessments through microbial indicator monitoring hinges on understanding the impacts of holding times on water samples.

Purpose: This study investigates the interacting factors influencing holding times for fecal indicator bacteria (FIB) in water samples held for 0–216 hours under three storage conditions: controlled cold ($\sim 4^{\circ}\text{C}$), controlled ambient ($\sim 21^{\circ}\text{C}$), and changing conditions (thawing ice pack).

Methods: Four separate trials were conducted using Sacramento River water ($n = 2$) and irrigation canal water ($n = 2$). For each trial, 50-liter samples were divided into 315 aliquots of 500 mL and placed under the three treatment conditions. Samples were processed using membrane filtration to quantify two primary FIB: *E. coli* (on MmTEC and CHROMagar ECC) and fecal coliforms (on mFC and ECC). Linear regression with autocorrelation and Dunnett's test was used to analyze the effects of holding time on FIB concentrations.

Results: Across all conditions, FIB concentrations remained statistically similar for the first 24 hours post-collection. In canal water, concentrations remained consistent for 30 hours under ambient, 48 hours under changing conditions, and up to 72 hours under cold storage. In river water, consistency extended to 36 hours under ambient, 72 hours under changing, and up to 180 hours under cold storage. These results suggest that water source significantly influences FIB stability, in addition to storage condition.

Significance: Samples stored under controlled cold conditions were stable well beyond the commonly accepted 6–8 hour holding times cited in regulatory statutes. These findings highlight the complexity of setting universal holding time guidelines for microbial water quality monitoring and underscore the importance of context-specific recommendations.

Award Application: Developing Scientist Award

P1-203: RISK RANKING OF PRE-HARVEST AGRICULTURAL WATER ASSESSMENT FACTORS BY ACADEMIA AND INDUSTRY

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: In May 2024, the FDA published final revisions to the Produce Safety Rule Subpart E, shifting requirements from a testing-based to a systems-based approach: Pre-harvest agricultural water assessment or 'AGWA'. The outcomes of this risk assessment introduce challenges, as potential hazards are deemed risky or not by the farm, allowing for subjective management.

Purpose: To gather produce experts in Academia and industry to assess risks related to Subpart E and identify areas for future education efforts.

Methods: Subject matter experts in Academia with expertise in produce food safety and water quality were distributed an online Qualtrics™ survey. The survey was also modified to a Mentimeter™ presentation to accommodate live participation by food safety industry professionals in Yuma, Arizona. Survey questions focused on ranking factors of an agricultural water assessment as identified in Subpart E rule language (i.e., agricultural water source, agricultural water distribution system, agricultural water degrees of protection, agricultural water use practices, crop characteristics, and environmental conditions) through conceptual (N=13) and scenario-based (N=14) questions. Predominantly focusing on the top three factors, mean ranking analysis was conducted using RStudio (V.4.4.2).

Results: Nine Academia (N=9) and thirteen industry (N=13) responses were recorded. At least one of the top factors for 100% (27/27) of questions aligned between Academia and industry, with agricultural water use practices, agricultural water source, and agricultural water distribution system most often selected. In 40.7% (11/27) of questions, all top factors aligned. Top-ranked factors in scenario-based questions with surface water (7/7; 100%) varied more than those with groundwater (1/7; 14.3%). When the top three factors did not align, agricultural water degrees of protection were primarily ranked by academia (13/16; 81.3%) while environmental conditions were top-ranked by industry (7/16; 43.8%).

Significance: Survey results identified agricultural water degrees of protection and

environmental conditions as areas to focus education efforts to aid in risk assessment and regulatory compliance.

Award Application: Developing Scientist Award

P1-204: CHARACTERIZATION OF MULTISEROVAR *SALMONELLA* POPULATIONS RECOVERED FROM TWO RIVERS IN ECUADOR

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Surface water in Ecuador is used for multiple purposes including drinking water for animals, crop irrigation, and even washing clothes. Previous work has demonstrated that many bacteria, including pathogenic species such as *Salmonella*, can survive and persist in freshwater, so inhabitants are subject to daily exposure risk.

Purpose: This study sought to measure *Salmonella* recovery and compare serovar diversity along two rivers in Ecuador embedded in urban and agricultural areas, respectively.

Methods: Samples were collected at four points on each river for four consecutive days across Río San Pedro and Río Machángara (n=32). For each sample, 10 liters of water were filtered through modified Moore swabs attached to a peristaltic pump. After pre-enrichment, samples were enriched in tetrathionate and Rappaport-Vasilliadis broths and on MSRV plates and then plated on Xylose Lysine Deoxycholate agar to isolate presumptive *Salmonella* colonies for confirmation and whole genome sequencing (WGS). Deep serotyping with CRISPR-SeroSeq was performed on all *Salmonella*-positive enrichments.

Results: *Salmonella* was recovered in 87.5% (14/16) and 62.5% (10/16) samples from Río Machángara and Río San Pedro, respectively. Eight serovars were identified in total by WGS, with some serovars represented by up to three different core genome sequence types (cgMLST) collected from the same river, suggesting there may be multiple

Introduction events. The number of serovars detected by CRISPR-SeroSeq differed between Río San Pedro (six serovars) and Río Machángara (18 serovars) according to the Shannon diversity index using Hutcheson t-test ($p < 0.05$). Serovars Typhimurium and infantis were persistent throughout the four sampling days in Río San Pedro, while Typhimurium, infantis, Corvallis, and Muenster were persistent in Río Machángara.

Significance: This is the first study to demonstrate the presence of *Salmonella* in these rivers, which serve as effluents and tributaries of the water system in Quito, the Ecuadorian capital.

Award Application: Developing Scientist Award

P1-205: RAPID DETECTION OF SALMONELLA SPP. IN WATER SAMPLES BY REAL-TIME PCR

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: A rapid and sensitive protocol for detecting *Salmonella* spp. in water samples was evaluated to ensure the safety of the water supply and minimizing impacts of public health.

Purpose: The study evaluates methods using a real-time PCR kit, the iQ-Check *Salmonella* II, combined with plate confirmation, to detect *Salmonella* spp. in water samples.

Methods: Two methods were used to prepare water samples, with filtration (method 1) and without filtration (method 2), before enrichment. To verify the capacity of the method to accurately detect the presence of *Salmonella* in water samples, the estimated level of detection (eLOD) protocol described in the ISO 16140-3 was used.

Results: The culture-based method produced negative results for the unspiked sample and seven positives out of seven of the spiked samples in both studies. For method 1, water sample assessed by PCR was negative for *Salmonella* spp. in the unspiked sample. Six out of seven of the spiked samples were positive for *Salmonella* spp. For method 2, water sample evaluated by PCR produced a negative result for the unspiked sample. Seven positives out of seven of the spiked samples were positive. Therefore, these results are in the acceptable range to pass ISO 16140-3 eLOD criteria (≥ 6 out of 7).

Significance: Results obtained from this study indicated that using a method including a real-time PCR kit provide useful tools for routine water quality monitoring and for rapid screening during outbreaks. It could help identify potential threats posed by *Salmonella* contamination and allow for an immediate response to a potential public health issue.

P1-206: EVALUATING TREATMENT EFFECTS ON HARVESTED RAINWATER QUALITY ON A PERI-URBAN FARM IN UPPER MARLBORO, MARYLAND

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Harvested rainwater could offer urban and peri-urban farmers a supplemental irrigation water source, but the microbial quality of rainwater requires assessment.

Purpose: To evaluate the microbial quality of harvested rainwater and the efficacy of water treatment technologies at a peri-urban farm in Upper Marlboro, Maryland.

Methods: Rainwater was collected from an asphalt roof using a gutter system with a first flush diverter. Harvested rainwater was filtered through a zero-valent iron (ZVI) sand filter (50% ZVI / 50% sand) or treated with Sanidate® 12.0 peracetic acid (PAA) at a target concentration of 8 ppm to reduce microbial contaminants. Municipal water, first flush rainwater, untreated rainwater, ZVI:sand-filtered rainwater, and PAA-sanitized rainwater (n = 13 per water type) were collected and analyzed for *Escherichia coli* using IDEXX Colilert assays. A Brassica mix irrigated with municipal water, untreated rainwater, ZVI:sand-filtered rainwater, and PAA-sanitized rainwater (n = 9 per water type) was analyzed for *E. coli* by culturing PBS rinses on MI agar. The association between water type and *E. coli* concentration was analyzed using repeated measures ANOVA. Results were compared to the Maryland Department of Agriculture's Good Agricultural Practices (GAP) standard for irrigation water (≤ 126 MPN *E. coli*/100 mL).

Results: *E. coli* was not detected in municipal or PAA-sanitized rainwater. Average *E. coli* concentrations in first flush, untreated, and ZVI:sand-filtered rainwater were 214, 833, and 15 MPN/100 mL, respectively. First flush and untreated rainwater exceeded the GAP standard. *E. coli* levels significantly differed among water types ($p < 0.05$), with ZVI:sand-filtered rainwater ($p < 0.05$) and PAA-sanitized rainwater ($p < 0.001$) showing significantly lower *E. coli* levels than untreated rainwater. No *E. coli* was detected on produce.

Significance: Rainwater could be a viable supplemental irrigation source. Treatment with PAA or ZVI:sand filtration may reduce *E. coli* contamination in water and help improve produce safety.

Award Application: Developing Scientist Award

P1-207: REMOVAL AND INHIBITION OF VIRUSES AND PROTOZOA FROM AGRICULTURAL WATER BY MYCOREMEDIATION WITH A COMPLEX FILTER MATRIX

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Mycoremediation can be combined with filtration for microbe removal from water. Activation of ligninolytic activity, combined with chemical inhibition, and physical removal by biochar/zero-valent iron (BC/ZVI), can reduce pathogens from agricultural water.

Purpose: This study evaluates removal of hepatitis A virus (HAV), Tulane virus (TV), *Eimeria tenella* and *Cryptosporidium parvum* by activated phanerochaete chrysosporium (PC) grown on woodchips (WC) combined with ZVI and biochar.

Methods: Filters were constructed utilizing PC-treated WC (100g), 35%:65% ZVI:sand (100g) and BC (100g). Filters were primed with 3 pore volumes (pvol) of artificial ground water (AGW) with a flow rate of 0.5L/min and retention time of 0.8min. inoculated (3pvol) AGW, containing HAV (10 log-genome-copies/L) (log-gc/L), TV (8 log-gc/L), or 1×10^5 -oocysts of *E. tenella* or *C. parvum* was pumped through the filter. Filters were flushed 2x with 2pvol of AGW. Effluent and filter matrices were collected after each run. infectious parasites were quantified using cell culture with qPCR. Viruses were detected using dPCR. Control filters did not include PC. Data, including pH and H₂O₂, were analyzed using one-way ANOVA and student T-test across 8 trials (n=16 per/treatment).

Results: Virus removal was significant in the presence of PC compared to controls (p<0.001). PC-filters contained 0.5mg/L H₂O₂, indicative of ligninolytic activity. PC-filtration removed 3.3 and 2.2 log-gc/L of TV and HAV, respectively, compared to 1.7 log-gc/L without PC. Reduction of *E.tenella* and *C.parvum* oocysts was 6.2×10^5 and 4.0×10^5 and without PC 1.4×10^5 and 8.5×10^5 , respectively. Among filter matrices, ZVI retained significantly (p<0.0001) greater HAV (8.8 log-gc/L), and TV (5.5 log-gc/L) compared to WC or BC. For *E. tenella*, ZVI (2.83×10^4 -oocysts) and WC (2.40×10^4 -oocysts) retained more oocysts than BC (7.02×10^3 -oocysts); however, BC retained more *C. parvum* (2.4×10^4 -oocysts)

than ZVI or WC.

Significance: PC grown on WC combined with ZVI/BC removed significantly more HAV and protozoan oocysts, creating an advantageous filtration system for irrigation water.

Award Application: Developing Scientist Award

P1-208: ENCAPSULATED POSTBIOTICS OF BIFIDOBACTERIUM LACTIS BPL1 APPLIED TO ENHANCE MICROBIOLOGICAL QUALITY IN PORK MEAT

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Food safety is a priority to prevent outbreaks caused by pathogenic bacteria, thus avoiding serious health and economic problems. An innovative strategy is to use postbiotics as natural antimicrobial agents

Purpose: This study aimed to evaluate the antimicrobial effect of encapsulated postbiotics from *Bifidobacterium lactis* BPL1 applied to pork meat, against *Escherichia coli* ATCC 25922, and *Listeria monocytogenes* Scott A

Methods: Postbiotics were prepared using *B. lactis* BPL1 inoculated into a culture medium based on whey and inulin. The fermented culture was inactivated through thermo-sonication (55 min at 55°C); pea protein (0, 1%, or 2% w/v) and maltodextrin (to adjust solids to 15%) were added as encapsulation agents for spray drying (170°C and 10 g/min). The *in vitro* antimicrobial activity of the encapsulated postbiotics was evaluated using the agar diffusion well method

Results: The pea protein maintained the antimicrobial activity after the spray-drying process. Pork meat was immersed in reconstituted encapsulated postbiotics, and results showed a significantly reduced microbial population ($p < 0.05$) of *E. coli* and *L. monocytogenes* after 13 days of storage at 4°C. The final microbial populations of postbiotic samples were 3.81 ± 0.17 and 2.77 ± 0.17 log CFU/g, respectively, compared to 6 log CFU/g in the samples without postbiotic immersion

Significance: *B. lactis* BPL1 encapsulated postbiotics were effective as antimicrobial agents with the potential to enhance food safety and extend the shelf life of fresh meat products

P1-209: UTILIZATION OF BIOCHAR IN REMOVING NANO-MICROPLASTIC FROM FOOD AND AGRICULTURAL WATER

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: The emerging contaminants in food and agricultural water pose high risks to public health and the environment that require new treatment strategies.

Purpose: To use biochars as filter materials to adsorb and remove contaminants (e.g. micro-microplastic) from water sources.

Methods: Biochars were produced via pyrolyzing the corn cobs at 350oC, 550oC and 700oC. The obtained biochars were characterized by the production yields, main components, proportions, and physicochemical properties. in the application of contaminants treatment, the ground biochars (average diameter: $781.08 \pm 3.27 \mu\text{m}$) were used as filtering materials against flowing water that contained nano-microplastic (polystyrene-based, i.e. PS-MNPs), which has been recognized as a new type emerging contaminants in the food and agricultural water sources. The adsorption effectiveness will be investigated by quantifying the content levels of PS-MNPs before and after filtration.

Results: The biochars demonstrated the capacity of adsorbing and filtering PS-MNPs from the water source in different conditions. in the treatment of PS-MNPs, the biochar could adsorb the microplastic particles with the size of 0.10, 0.50, 0.95, and 2.10 μm at different concentrations (from 105 beads/mL to 107 beads/mL), the maximum removal rate could reach to $(97.99 \pm 1.04)\%$. The studies also showed that the biochar could adsorb biochar through different mechanisms, including cation binding, porous retention, and electrostatic attraction.

Significance: This study provides a practical approach to producing sustainable filtering materials to achieve effective adsorptions and removal of emerging contaminants from water sources.

P1-210: UTILIZATION OF BIOCHARS IN THE TREATMENT OF EMERGING CONTAMINANTS IN FOOD AND AGRICULTURAL WATER SOURCES

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Emerging contaminants in food and agricultural water pose high risks to public health and the environment and require new treatment strategies.

Purpose: To use biochars as filtering materials to adsorb and remove contaminants (for example, nano-microplastic) from water sources.

Methods: The biochars were produced via pyrolyzing the corn cobs at 350, 550 and 700 degree celsius. The obtained biochars were characterized by the production yields, main components and proportions, physicochemical properties, etc. in the application of contaminants treatment, the ground biochars (average diameter: $781.08 \pm 3.27 \mu\text{m}$) were used as filtering materials against flowing water that contained nano-microplastic (polystyrene-based, i.e. PS-MNPs), which has been recognized as a new type of emerging contaminant in the food and agricultural water sources. The adsorption effectiveness will be investigated by quantifying the content levels of PS-MNPs before and after filtration.

Results: The biochars demonstrated the capacity of adsorbing and filtering PS-MNPs from the water source in different conditions. in the treatment of PS-MNPs, the biochar could adsorb the microplastic particles with the size of 0.10, 0.50, 0.95 and $2.10 \mu\text{m}$ at different concentrations (from 10^5 beads/mL to 10^7 beads/mL), the maximum removal rate could reach to $(97.99 \pm 1.04)\%$. The studies also showed that the biochar could adsorb biochars through different mechanisms, including cation binding, porous retention and electrostatic attraction.

Significance: This study provides a practical approach to producing sustainable filtering materials that effectively adsorb and remove emerging contaminants from water sources.

P1-211: EVALUATION OF THE MICROBIAL AND PHYSIOCHEMICAL QUALITY OF WATER AND PECAN NUTMEAT IN A PROCESSING FLOAT SYSTEM

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Presentation type/Format: Technical

Category: Water

Abstract:

Introduction: Many pecan producers utilize a recirculated float system to remove shells and debris from pecan nutmeat. Utilizing sanitizers in the recirculated float water is used to reduce the risk of microbial contamination from the water to the nutmeat.

Purpose: The objective of this study is to determine the influence of physiochemical factors on the microbial quality of water and pecan nutmeat from a processing float system.

Methods: Recirculated water and nutmeat samples from a float system were collected at a pecan shelling facility. Samples were collected at the beginning of operation and every 20 minutes until the end of processing (~220 minutes). Water and nutmeat samples were analyzed for heterotrophic plate count (HPC), total coliforms (TC), and *E. coli* levels using MPN and standard plating methods. Water samples were analyzed for total chlorine, free chlorine, pH, and COD. Pearson correlations were evaluated between physiochemical water parameters and microbial levels.

Results: Starting free chlorine levels ranged between 104.2–426.5 ppm, and by 80 minutes free chlorine levels were below 10 ppm in the float water. HPC and TC levels in the water were negatively correlated with free (-0.75 and -0.64 respectively) and total chlorine (-0.77 and -0.66 respectively). Below 200 and 50 ppm free chlorine, HPC and TC levels respectively started to increase in float water. There was an average increase of 0.92 ± 0.53 and 1.07 ± 0.54 log CFU/g in HPC and TC respectively on pecans after going through the float system. All water (<0 log MPN/g) and pecans (<0 log CFU/g) were below the limit of detection for *E. coli*.

Significance: The results of this study determine the need for identifying effective

sanitizer limits in pecan float systems that can remain effective at reducing the risk of microbial contamination. Maintaining free chlorine levels above 50 ppm will reduce the TC levels in recirculated float water.

P1-212: FREE CHLORINE AND PECAN SHELL INTERACTION INFLUENCE SALMONELLA CONTAMINATION ON PECAN NUT MEAT IN A SIMULATED FLOAT SYSTEM

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Pecan nutmeats typically go through a float system to remove shell pieces. Chlorine-based sanitizers are often used by pecan processors in these systems. An excess of pecan shells may lead to a potential reduction in chlorine effectiveness due to excess organic matter.

Purpose: Determine the efficacy of chlorine at varying concentrations at reducing *Salmonella* transfer between contaminated shells and pecan nut meats in a simulated float system.

Methods: A mix of pecan shells and/or pieces (100/0, 50/50, 25/75% shell/halves) were inoculated (~ 7 log CFU/mL) with rifampicin-resistant *Salmonella* cocktail (Typhimurium, Enteritidis PT 9c, Montevideo, Saintpaul, and Newport). The inoculated substrate (20 g) and 105 g of uninoculated nuts were submerged into a 0, 5, 25, or 200 ppm chlorine solution. Uninoculated nuts were removed at 0.5, 2, and 5 minutes, weighed (10 g), diluted with buffered peptone water, homogenized for 30 seconds, and enumerated for *Salmonella*. Free chlorine levels were measured at each time point. Trials were repeated in triplicate with three biological replications (n=9). Data were analyzed using a generalized mixed model and Tukey's HSD ($P \leq 0.05$).

Results: *Salmonella* transfer (log CFU/g) was significantly higher as time points increased from 0.5, 2, and 5 minutes (1.12 ± 0.57 , 1.92 ± 0.48 , and 2.30 ± 0.54 log CFU/g, respectively). Transfer significantly increased as chlorine concentration decreased (2.37 ± 0.48 , 2.04 ± 0.47 , 1.94 ± 0.68 , and 0.77 ± 0.50 log CFU/g at 0, 5, 25, and 200 ppm). The model determined that transfer was significantly higher for shell percentages of 100% and 25% compared to 50%. Free chlorine levels significantly reduced as time increased. Pecan shell percentage did not significantly influence free chlorine levels for the 200 and 5 ppm starting

concentrations, but significantly decreased free chlorine at the 25 ppm level.

Significance: Shell volume, chlorine concentration, and immersion time impact both the effectiveness of chlorine and the transfer of *Salmonella*. These parameters should be considered when implementing a float system.

P1-213: LYTIC BACTERIOPHAGE AND ZERO-VALENT IRON SAND FILTRATION INTERVENTIONS REDUCE *SALMONELLA* INFANTIS LEVELS IN SURFACE WATER

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: *Salmonella* enterica in irrigation water can contaminate fruits and vegetables, leading to potential outbreaks. Non-chemical interventions can reduce levels of *Salmonella* in surface water, but investigation into the effectiveness of single treatment or synergistic technologies is warranted.

Purpose: To compare the effectiveness of reducing *Salmonella* infantis levels in surface water by combinations of lytic bacteriophage application and sand or zero-valent-iron sand (ZVI-sand) filtration.

Methods: Pond water was collected and inoculated with 4 log CFU/mL nalidixic acid (NA)-resistant *Salmonella* infantis. inoculated water (500 mL) was treated with a commercial lytic bacteriophage (SalmoFresh, 9 log PFU/mL) at 20°C for 5 min with shaking (200 rpm). pHage-treated water (500 mL), or untreated water was then filtered through either 100% sand or 50% ZVI / 50% sand (ZVI-sand) filters at 1000 mL/min. Filters were constructed using 5 cm (w) x 10 cm (l) PVC pipe (4.25 cm diameter), wet-packed with sand or sand and ZVI (0.43–0.60mm, particle size). *S. infantis* levels in effluent were determined on XLD agar with 50 g/mL NA after incubation at 37°C for 48 hours. Pairwise t-test with Bonferroni correction was performed (level of

Significance=0.05). Each experiment was replicated three times.

Results: Sand filtration and ZVI-sand filtration significantly reduced *S. infantis* levels by ca. 0.20 log CFU/mL and ca. 1.54 log CFU/mL, respectively. Bacteriophage treatment + sand filtration reduced *S. infantis* levels by ca. 0.20 log CFU/mL; bacteriophage treatment + ZVI-sand filtration reduced *S. infantis* by ca. 1.74 log CFU/mL. Compared to the control treatment (no phage, no filtration), *S. infantis* levels were significantly reduced by sand filtration ($p=0.049$), ZVI-sand filtration ($p=0.001$), and bacteriophage + ZVI-sand filtration ($p<0.001$).

Significance: Lytic bacteriophage treatment combined with ZVI-sand filtration reduced *S. infantis* levels more than other treatments, and shows potential effectiveness of combining biological and filtration-based interventions to reduce bacterial pathogens in water.

P1-214: HIGH DIVERSITY AND WIDESPREAD DISTRIBUTION OF BACTERIAL PATHOGENS IN AN INTEGRATED LANDSCAPE WATERSHED

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Surface waters are diverse ecosystems that can harbor pathogens impacting public health and food safety through crop irrigation. Metagenomics helps to characterize organisms present in a sample without the need for culturing.

Purpose: To characterize the bacterial composition and pathogen distribution in the Mapocho River watershed in Santiago, Chile.

Methods: Twelve monthly samples were collected in 15 sites (river, canals, and pond), representing natural, urban, and agricultural zones of the Mapocho watershed. Using modified Moore Swabs, 10 L of water were filtered in situ. Shotgun metagenomic sequencing was performed for total microbiomes on Illumina Nextseq 2000. Reads were classified using Kraken2/Bracken. Shannon diversity indexes were calculated at the phylum, family, and species levels. The influences of land use, water type, seasonality, and site on the bacterial composition were analyzed using ANOSIM, Kruskal-Wallis, and PERMANOVA.

Results: Shannon index values, at the phylum level, ranged from 0.43 (April, Site 4) to 4.31 (January, Site 7), and at the species level, from 0.43 (June, Site 4) to 7.61 (November, Site 9). No significant temporal differences were observed within individual sites across 12 months ($p > 0.05$). Public health-relevant families (Enterobacteriaceae, Vibrionaceae, *Campylobacteraceae*, Mycobacteriaceae, and Listeriaceae) were present in all sites, showing significant variation according to land use, water type, and site ($p < 0.05$). Seasonality affected only Enterobacteriaceae and Vibrionaceae abundance ($p < 0.05$). At the species level, *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes*, *Vibrio cholerae*, *Campylobacter jejuni*, *Mycobacterium tuberculosis*, and *Clostridium perfringens* varied significantly by water type and site ($p < 0.05$), while land use influenced *E. coli*, *S. enterica*, *V. cholerae*, *M. tuberculosis*, and *L. monocytogenes*. Factors such

as temperature, pH, and conductivity only explained 6.64% of the total variation.

Significance: The widespread presence of pathogens and bacterial diversity variability in surface waters underscores the need for continuous monitoring and control measures to safeguard public health and food production.

P1-215: MICROBIAL SOURCE TRACKING AND PATHOGEN SURVEILLANCE OF AGRICULTURAL WATER

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: With recent revisions to the FSMA Produce Safety Rule, growers must conduct assessments on pre-harvest agricultural water for the purpose of hazard identification and risk management. Cultural and genetic detection methods paired with risk assessment can be utilized to aid growers in decision making.

Purpose: This study compares data on pathogens and indicator organisms in agricultural water along with climactic and observational data across two years.

Methods: Water samples (1L) were collected from farms in Delaware and Ohio in 2023 (n=36) and Delaware in 2024 (n=27) from July-October. Bacterial enumeration was performed on Delaware samples on Charm E.coli/coliforms and XLT-4 for *Salmonella*. Samples were ultrafiltered and ultracentrifuged for 45min at 3000xg. Concentrate was collected and extracted using Qiagen RNeasy PowerMicrobiome or Qiagen Dneasy Powersoil kit. dPCR was performed for viruses (hepatitis A virus, norovirus GI&GII) and indicator organisms (pepper mild mottle virus, human Bacteroides, ruminant Bacteroides, crAssphage, poultry parvovirus). Statistical analysis was performed using JMP.

Results: of the 2023 Delaware samples, 50%(9/18) were positive for at least one pathogen and 83.3%(15/18) were positive for an indicator. Out of the 2023 Ohio samples, 55.6%(10/18) were positive for at least one pathogen and 44.4%(8/18) were positive for an indicator. HAV was the most common pathogen detected in 2023 Delaware and Ohio samples, BacH was the most common indicator in 2023 Delaware, and crAssphage the most common indicator in Ohio. in 2024, 11.1% (3/27) samples were positive for a pathogen and 63%(17/27) were positive for an indicator. The most common pathogen and indicator were *Salmonella* and PaV, respectively. When comparing rainfall and pathogen/indicator presence, there was strong positive correlation between rainfall within 24 hours before sampling and indicator presence in 2023 ($r=0.6963$) and between rainfall within 24 hours and

pathogen presence in 2024 ($r=0.5434$).

Significance: Multi-year surveillance paired with observational data provides insights on risks growers may need to navigate.

Award Application: Developing Scientist Award

P1-216: SALMONELLA PERSISTENCE IN SURFACE WATER AND IMPACT OF RAINFALL ON SEROVAR COMPLEXITY

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Contaminated surface water is a risk factor *Salmonella* transmission, yet the persistence of *Salmonella* in surface water remains understudied.

Purpose: Determine the effect of significant precipitation on *Salmonella* prevalence and complexity and to use whole genome sequencing (WGS) to evaluate *Salmonella* persistence and transmission in water.

Methods: Modified Moore swabs were used to sample three sites along a creek. Water samples were taken twice monthly, once after significant rainfall (based on historical data; range: >0.16->1 inch/24 hour, depending on month) and once after at least three dry days, for 20 months. Weather and water variables (including conductivity, flow rate, pH, dissolved oxygen, total dissolved solids, water temperature) were recorded during each collection. *Salmonella* was isolated following standard enrichment and positive samples analyzed by deep serotyping (DST) and whole genome sequencing (WGS).

Results: *Salmonella* prevalence in water was 62.0% (80/129). Dissolved oxygen and total dissolved solids were negatively associated with *Salmonella* prevalence (t-test $p < 0.01$), however *Salmonella* was more likely to be isolated on days without precipitation (Chi2 $p < 0.05$). DST analysis on 68 samples showed 91% contained multiple serovars (range 1-10 serovars/sample). The average number of serovars/sample was 5.0 following significant rain, significantly higher than an average 3.6 serovars/sample following three dry days (Kruskal-Wallis, $p < 0.05$). WGS analysis revealed that 45.6% (47/103) of isolates are closely related (within five allelic differences) to at least one other isolate in the dataset. These related isolates were identified across several sites, and/or across several months, suggesting instances of *Salmonella* persistence or reintroduction, as well as examples of downstream transmission.

Significance: These observations highlight high *Salmonella* complexity in water, especially following significant precipitation, though this does not align with *Salmonella* prevalence after significant rain. This information may guide risk

analysis where surface water is used for produce irrigation.

Award Application: Developing Scientist Award

P2-01: BONE APPETIT: ASSESSING PET DIET CHOICE AND FOOD HANDLING PRACTICES OF DOG AND CAT OWNERS

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Presentation type/Format: Technical

Category: Animal and Pet Food Safety

Abstract:

Introduction: The availability of raw meat-based diets for pets, which have the potential to be contaminated with pathogens, has increased in recent years, raising concerns about risks to human and animal health.

Purpose: We assessed the prevalence of feeding various pet diets among dog and cat owners and pet food handling practices. **Methods:** We surveyed Minnesota dog and cat owners aged >18 years during the 2024 Minnesota State Fair. We asked about main meals, toppers (additions or enhancements to main meals), treats and feeding frequency along with food handling practices. Raw meat-based main meals included raw-coated kibble and commercially available and home-prepared meat or fish. We compared handling practices among those who reported feeding raw main meals or toppers in the previous year to those who did not using Chi-squared tests ($p < 0.05$).

Results: Among 717 respondents, 53.3% ($n=382$) owned dogs, 29.8% ($n=214$) owned cats, and 16.9% ($n=121$) owned both. Eighty dog owners (15.9%) and 42 cat owners (12.5%) reported feeding any raw meat-based main meals in the previous year. Overall, 11.1% ($n=56$) of dog owners and 6.9% ($n=23$) of cat owners reported feeding raw meat or fish toppers to their pets during the previous year. Among respondents who reported feeding raw meat-based main meals or toppers, 61.2% reported always washing their hands after compared to 38.3% of respondents who did not feed raw main meals or toppers ($p < 0.001$). Respondents who fed raw meat-based main meals or toppers were more likely to have tasted their pets' food or treats in the previous 3 months compared to those who did not feed raw main meals or toppers (9.7% vs. 3.8% respectively, $p=0.004$).

Significance: Nearly one-fifth of respondents fed their pets' raw meat-based main meals or toppers, and handling practices were suboptimal. These findings provide support for pet food as an emerging public health risk.

P2-02: IMPACT OF BRINING AND DRY SALTING ON THE SURVIVAL OF SALMONELLA SPP. AND LISTERIA MONOCYTOGENES ON INOCULATED SUGAR KELP (SACCHARINA LATISSIMA) DURING REFRIGERATED AND AMBIENT STORAGE

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: The short shelf life of seaweed and limited harvesting time have been challenging for the seaweed industry. Brining and dry salting can be used to improve the shelf life of seaweed, however, their impact on pathogen survival in seaweed is limited.

Purpose: The aim of this study was to investigate the survival of post-process pathogenic contaminants inoculated on brined and dry salted kelp subjected to different temperatures during storage.

Methods: Fresh kelp was first subjected to brining (40% w/v NaCl) or dry salting treatment (30% w/w Kosher salt) to achieve a final water activity level of ≤ 0.77 and then inoculated separately with a cocktail of two strains or serovars of *L. monocytogenes* and *Salmonella*, respectively (~ 4.0 log CFU/g). Samples were stored at refrigerated and ambient temperatures for up to 12 weeks. Microbiological analyses were performed weekly to evaluate the survival of pathogens. A multi-way ANOVA ($p < 0.05$) was used to assess the effects of treatment, storage temperature and time on pathogens population during storage.

Results: *Listeria monocytogenes* population decreased by 2.67 ± 0.56 and 3.00 ± 0.79 log CFU/g, while *Salmonella* decreased by 3.50 ± 0.31 and 3.73 ± 0.37 log CFU/g in brined and dry-salted kelp, respectively, during storage. Populations did not decrease significantly during the first week of storage regardless of storage temperature. Brining and dry salting had similar effects on the *Listeria* population during storage while treatment, storage duration and temperature had significant effects on *Salmonella* population. *Salmonella* and *Listeria* were detectable in all treatments other than brined kelp under ambient storage (*Salmonella* undetectable after 11 weeks) for 12 weeks.

Significance: Brining and dry salting contribute to pathogens reduction in sugar

kelp; however, additional processing steps are necessary to reduce microbial contamination levels to ensure safety.

P2-03: COMPANION ANIMALS AS RESERVOIRS OF MULTIDRUG-RESISTANT SALMONELLA AND THEIR IMPACT ON FOOD SAFETY AND PUBLIC HEALTH

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: *Salmonella* is a major foodborne pathogen, with companion animals shedding bacteria into households and contaminating pet food, increasing human exposure risks. Infected animals may show diarrhea, vomiting, fever, or remain asymptomatic while shedding the pathogen. Despite these risks, data on *Salmonella* prevalence, antimicrobial resistance, and virulence in Central Kentucky companion animals remain limited.

Purpose: This study aimed to assess the prevalence, AMR profiles, and virulence characteristics of *Salmonella* isolates from healthy dogs and cats to evaluate their potential food safety and public health risks.

Methods: Fecal samples (n=206) were collected from cats (n=66) and dogs (n=140) at pet clinics and animal shelters in Central Kentucky. Samples were enriched in tetrathionate broth and plated on XLT4 agar. DNA was extracted using the boiling method, and *Salmonella* presence was confirmed via polymerase chain reaction (PCR) targeting the *invA* gene. Swarming and swimming motility were evaluated, followed by biofilm formation screening using crystal violet assay. Genotypic virulence and AMR genes were detected via PCR, and antimicrobial susceptibility testing was conducted using broth microdilution assay.

Results: *Salmonella* was detected in 11.6% (24/206; 95% CI: 7.61–16.84) of fecal samples. Strong biofilm production was observed in 87.5% (21/24) of isolates. High swarming motility was detected in 45.8% of isolates, while 58.3% exhibited strong swimming motility. The most prevalent AMR genes were *bla*TEM (70.8%), *bla*CTX (79.1%), *strA* (66.7%), and *sul2* (70.8%). Key virulence genes included *invA* (100%), *hilA* (83.3%), and *siiA* (87.5%) that are associated with invasion, regulation, and survival. All isolates were multidrug-resistant (MDR), with the highest resistance to macrolides (azithromycin) and trimethoprim-

sulfamethoxazole, while carbapenems (meropenem) were most effective.

Significance: Contaminated pet food can infect companion animals with *Salmonella*, leading to pathogen shedding and household transmission risks. Proper pet food handling, hygiene practices, and enhanced veterinary surveillance are essential to reducing *Salmonella*-related food safety risks for both pets and humans.

Award Application: Developing Scientist Award

P2-04: AN EVALUATION OF VITAMIN D, COPPER, AND MICROBIAL CONTAMINANTS IN DOG FOOD AND TREATS

Primary Author:

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James Rogers, Consumer Reports

Sana Mujahid, Consumer Reports

Category: Animal and Pet Food Safety

Abstract:

Introduction: Dog food recalls and research data indicate recurrent contaminant and nutrient issues that can result in harm to not only pets but also pet owners.

Purpose: This study evaluated popular dog foods and treats for vitamin D, copper, and microbial contaminants that have previously been reported.

Methods: Fifty-eight products representing dry dog food, wet/semi-moist food, fresh food, and raw food and 13 products representing snacks/treats were selected based on market and recall data. Three samples of each of these 71 products (n=213) were purchased from stores in NY, NJ, CT, and online. Samples were tested for the presence of *Salmonella* spp. and *Listeria* spp. using AOAC methods RI 100701 and RI 021201, respectively. Cultural confirmation was performed following FDA BAM procedures and Whole Genome Sequencing. Copper was measured by ICP-MS using EPA 6020B. Vitamin D (D2 and D3) and Aflatoxins (B1, B2, G1, G2) were measured by LC-MS/MS for a subset of models (n=36 and n=101, respectively).

Results: Aflatoxin B1 was detected in 3 samples of one product and averaged 2.1 ppb. Two samples were confirmed positive for *L. monocytogenes*. *Salmonella* and Aflatoxin were not detected in any other samples. Samples from one product averaged below the Association of American Feed Control officials (AAFCO) minimum for Vitamin D of 500 IU per kg of dog food (dry matter basis) and one product averaged above the limit of 3000 IU per kg of dog food. Forty-five products averaged > 15 mg/kg of copper (dry matter basis), which is the AAFCO proposed Control Limit for Copper.

Significance: Popular dog foods can expose pets and their households to pathogenic microbial contaminants and not provide the recommended levels of vitamin D and copper.

P2-05: IMPACT OF VINEGAR-BASED ANTIMICROBIAL SOLUTION ON SPOILAGE MICROORGANISMS IN MECHANICALLY SEPARATED CHICKEN USED FOR PET FOOD

Primary Author:

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Mechanically Separated Chicken (MSC) is a cost-effective, protein-rich ingredient in pet food but is highly susceptible to spoilage due to its high water activity and nutrient content. Effective antimicrobial interventions are essential to enhance MSC's shelf life and quality.

Objective: This study evaluated the efficacy of dried vinegar (DV) system in controlling spoilage microorganisms in MSC over 10 days.

Methods: MSC was obtained from a commercial slaughterhouse and blended with 0.60% DV along with a no-antimicrobial control. Treatments were inoculated with 2–3 log CFU/mL of *Leuconostoc mesenteroides*. Samples were stored aerobically at 4°C for up to 10 days. At each sampling (day 0, 3, 5, 7, and 10), samples were plated in duplicates onto de Man, Rogosa, and Sharpe (MRS) and Tryptic Soy Agar (TSA) for the enumeration of lactic acid bacteria and aerobic plate counts (APC), respectively. Spoilage threshold was considered as 6 log CFU/g. One-way ANOVA was used to compare the treatment performance in JMP Pro version 15.1.0 with a significance set at ($p \leq 0.05$).

Results: Aerobic plate counts in the control sample exceeded the spoilage threshold (6 log CFU/g) by day 6, indicating rapid microbial growth without preservatives. In contrast, 0.60% DV significantly suppressed microbial growth, extending shelf life ($p \leq 0.05$). Lactic acid bacteria levels in the no-preservative treatment exceeded spoilage limits by day 2, while the DV treatment maintained significantly lower LAB levels (~ 4.00 log CFU/mL; $p \leq 0.05$), demonstrating its superior antimicrobial efficacy.

Significance: The findings underscore the potential of dried vinegar system as an effective antimicrobial solution for extending the shelf life and maintaining the

microbial quality of MSC in pet food applications.

P2-06: VALIDATION OF RAWHIDE AND PIG EAR PET TREATS FOR THE DETECTION OF SALMONELLA USING HYGIENA'S BAX® SYSTEM

Primary Author:

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Many pet owners give their animals chews and snacks as treats. These products often contain animal by-products, including ears, snouts or bones left over from slaughter. During processing, these types of products may be dried, frozen, cleaned and flavored but not always cooked, posing an inherent risk of pathogen contamination.

Purpose: The purpose of this study was to validate the performance of two PCR-based methods compared to the US FDA BAM reference method for the detection of *Salmonella* in rawhide and pig ear pet treats.

Methods: Two unpaired matrix studies were performed following the technical guidelines in AOAC INTERNATIONAL official

Methods of Analysis Appendix J. Pig ears were inoculated with *Salmonella* infantis and rawhide treats were inoculated with *Salmonella* Typhimurium to create a low fractional level and a high level. Additional samples were left uninoculated as negative controls. Samples were equilibrated at room temperature for two weeks prior to enrichment. Test method samples (n=30) were then enriched 1:10 with BPW and incubated at 35 °C for 18-24 hours. Reference method samples (n=30) were enriched following the FDA BAM Chapter 5. Test samples were assayed using real-time and standard PCR. All samples were confirmed by culture.

Results: For pig ears, 7/20 low inoculated and 5/5 high inoculated samples were detected by real-time and standard PCR. For rawhide, 7/20 low inoculated and 2/5 high inoculated samples were detected by real-time and standard PCR. All PCR results were identical to culture

Results, with 100% sensitivity and 100% specificity.

Significance: The results of these studies demonstrate that the BAX® System Real-Time and Standard PCR assays are sensitive and specific for the detection of *Salmonella* species in rawhide and pig ear pet treats. Furthermore, the test

Methods were equivalent or superior to the reference method.

P2-07: MATRIX VALIDATION OF REFRIGERATED, COOKED PET FOOD FOR THE DETECTION OF SALMONELLA AND LISTERIA USING HYGIENA'S BAX® SYSTEM REAL-TIME PCR ASSAY

Primary Author:

Julie Weller, Hygiena

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Deja Latney, Hygiena/Qualicon

Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Over the past few years, pet owners have sought higher quality food options that contain more natural ingredients for their animals. While these premium brands are more nutritious than the traditional dry kibble, the same microbiological risks remain.

Purpose: The purpose of this study was to evaluate the performance of the BAX® System method compared to the U.S. Food and Drug Administration Bacteriological Analytical Manual reference method for the detection of *Listeria* species and *Salmonella* species in cooked, refrigerated pet food.

Methods: in two separate validations, pet food was inoculated with either a heat-stressed culture of *Salmonella* or *Listeria monocytogenes* to create two levels of contamination: a low fractional level and a high positive level. Samples were equilibrated at 4 °C for 72 hours and then divided into 30 unpaired test portions for testing using both the alternative method and reference method. For *Salmonella*, 375 g alternative method samples were enriched in BPW, and 25 g reference method samples were enriched according to FDA BAM Chapter 5. For *Listeria*, 125 g alternative method samples were enriched in 24 LEB Complete, while 25 g reference method samples were enriched according to FDA BAM Chapter 10. Alternative method samples were analyzed using real-time PCR and all samples were confirmed by culture.

Results: in both validations, fractional results were obtained by real-time PCR in 10/20 low-level *Salmonella* samples and 11/20 *Listeria* samples. All 5/5 high-level samples were positive. These results were in complete method agreement with culture. Test and reference results were compared using the probability of detection (POD). No significant difference was determined, as the 95% confidence interval contained zero for all levels.

Significance: These studies provide a sensitive and accurate method to detect *Salmonella* and *Listeria* in cooked, refrigerated pet food that is fit for purpose.

P2-08: MATRIX VALIDATION OF 375 G OF FROZEN WAFFLES FOR THE DETECTION OF LISTERIA USING HYGIENA'S BAX® SYSTEM REAL-TIME PCR ASSAY

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Christine Chapman, Hygiena

Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: There are a wide variety of frozen bakery items that offer consumers the convenience of a “freshly made” product with a quick preparation time and extended shelf life. These products are fully cooked before being frozen. The heating process during baking will typically eliminate any microbial organisms, but contamination and recalls can still occur.

Purpose: The

Purpose of this study was to evaluate the performance of a rapid real-time PCR method compared to the U.S. Food and Drug Administration Bacteriological Analytical Manual reference method for the detection of *Listeria* species from 375 g samples of frozen waffles.

Methods: Frozen waffles were artificially inoculated with *L. monocytogenes* at two levels: a low level to obtain fractional recovery and a high level to obtain all positives. Samples were held at -20 °C for two weeks and then unpaired samples were divided into a 375 g test method and a 25 g FDA BAM reference method. Test method samples (n=30) were enriched with 3,375 mL pre-warmed (35 °C) Actero *Listeria* media and incubated at 35 °C for 22-24 hours. Reference method samples (n=30) were enriched following the FDA BAM Chapter 10.

Results: Test method samples were analyzed by real-time PCR and returned fractional positive results for 14/20 low-inoculated samples and all positive

Results for 5/5 high-inoculated samples. All samples were confirmed by culture with complete agreement. Test and reference results were compared using the probability of detection (POD). No significant difference was determined, as the 95% confidence interval contained zero for all levels.

Significance: Overall, the BAX System method is accurate and reliable for the detection of *Listeria* in 375 g samples of frozen waffles, equivalent to the FDA BAM reference method.

P2-09: VALIDATION OF THE GENE-UP® SALMONELLA METHOD FOR THE DETECTION OF SALMONELLA SPP. IN HUMAN GRADE PET FOOD

Primary Author:

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Amy Bosco, Cargill

Jessica Brown, Cargill Protein

Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: The GENE-UP® SLM (Performance Tested

MethodsM 121802 and OMA 2019.01) assay is a real-time PCR molecular detection method that utilizes Fluorescence Resonance Energy Transfer and proprietary hybridization probes for the rapid detection of *Salmonella* spp. in food and environmental samples.

Purpose: To conduct an AOAC style matrix extension of the candidate method for the detection of *Salmonella* spp. in human grade pet food at the 375 Gram sample size in comparison to the FDA BAM Chapter 5: *Salmonella*.

Methods: The validation method comparison consisted of evaluating 25 total sample replicates per method. Within the matrix sample set, there was a low level of 20 inoculated samples (0.2-2 CFU/test portion), and 5 high level inoculated samples (2-5 CFU/test portion) per method. All PCR method samples were analyzed by the GENE-UP SLM assay and the reference method samples tested per the FDA BAM Chapter 5. All enrichments, regardless of presumptive results, were culturally confirmed by both the GENE-UP alternative confirmation and traditional reference method plating. Test portions were analyzed per the instructions for use of the candidate method and the FDA BAM Chapter 5 method.

Results: Using an unpaired dPOD (difference in Probability of Detection) test at $p < 0.05$, for both levels, there were no statistically significant differences between the number of positive results obtained by the GENE-UP SLM method and the corresponding reference method for the validation study. The dPOD and corresponding 95% confidence intervals were 0.00 (-0.26, 0.26) for the low level and 0.00 (-0.43, 0.43) for the high level. No significant differences were observed between the number of positive samples detected by the GENE-UP method and

the reference method confirmations from the candidate enrichments.

Significance: The results of this study demonstrate that the GENE-UP SLM method is equivalent to the reference method for the detection of *Salmonella* spp. in samples of the human grade pet food evaluated during this study.

P2-10: COMPARISON OF UVM, MOPS-BLEB AND LPT ENRICHMENT BROTH IN TESTING *L. MONOCYTOGENES* IN READY-TO-EAT (RTE) FOODS AND LIQUID WHOLE EGGS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Consumption of ready-to-eat (RTE) foods and eggs remains a significant source of *Listeria monocytogenes* infections. As part of *L. monocytogenes*' routine testing, the USDA-FSIS recently replaced the UVM and MOPS-BLEB enrichment broths with the LPT broth (MLG 8.15). The change to LPT reduces the two-day process that utilizes a primary and secondary enrichment to a single step enrichment process.

Purpose: Utilizing verification studies, RTE foods and liquid whole eggs were tested using real-time PCR that compared the previous two-day USDA-FSIS enrichment process (MLG 8.14) to the LPT single-day process (MLG 8.15). Additional matrices were enriched in LPT to further confirm the broth's efficacy.

Methods: Two sets of 125g matrices were analyzed in each enrichment broth. Matrices included RTE hotdogs, RTE breaded chicken nuggets and liquid whole eggs each with 10 samples inoculated at ≤ 10 CFU and 5 uninoculated samples for both enrichment broths. For the MLG 8.14 set, samples were enriched at 1:10 in UVM at 30°C for 23h and transferred to MOPS-BLEB for secondary growth. Samples enriched in LPT were incubated 24h at $35 \pm 1^\circ\text{C}$ for the single-day enrichment process. Fifteen 125g samples of raw catfish, RTE breaded catfish and fermented sausage were also enriched in LPT alone. All enriched samples were evaluated on GENE-UP LIS and LMO. Traditional and alternative confirmation with direct plating on ALOA and MOX followed by biochemical identification was also performed.

Results: All inoculated samples correctly reported positive on LIS and LMO assays from UVM, MOPS-BLEB and from LPT. Interestingly, raw catfish and breaded RTE catfish tested as naturally contaminated with *L. monocytogenes* and *L. welshimeri*. Results from alternative and reference screening methods demonstrated 100% alignment with PCR results and biochemical identifications.

Significance: This data confirms the LPT single day enrichment process for MLG 8.15 in testing RTE products and liquid whole eggs for *L. monocytogenes*.

P2-11: NEEDS ASSESSMENT FOR A NEW 4-H FOOD SCIENCE AND SAFETY CURRICULUM

Primary Author:

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Despite an interest in food science and food safety from 4-H youth, there are limited curriculum and resources focused on this area to help meet that need.

Purpose: This study aims to identify educational competencies for a new food science and safety youth curriculum and describe the current challenges in this food project area.

Methods: A Delphi survey methodology was utilized to have food science and safety experts in Iowa and Florida come to a consensus around competencies and challenges within youth programming in this area. Participants across Iowa and Florida were asked what educational competencies should be included in a food science and safety 4-H program in order to develop adequate youth understanding within this area and what challenges currently exist within this area. There were 28 participants who completed all three rounds of the survey.

Results: Results from round one of the Delphi (n=40) compiled a list of answers to two open-ended questions focusing on competencies and challenges; 39 challenges, 62 competencies were reported. Round two (n=32) asked participants to rate their agreement for the items from round one for challenges (knowledge gap, resources, youth programming, youth interest, traditions), competencies (food safety, food science, foundational, nutrition, other). Round three (n=28) had participants confirm their agreement on items that achieved consensus with items focusing on challenges that include: knowledge gap, resources, youth interest, and tradition, competencies that include: food safety, resources, foundational, and nutrition.

Significance: This work helps to identify key learning outcomes and potential barriers necessary for curriculum development. Teaching youth about the principles of food science and safety within real world contexts will lead them to apply this knowledge it in their current and future adult lives which will assist in them creating safe products for their family, friends, and most important the food

project area.

P2-12: OUTBREAKS LINKED TO COUNTRY FOODS IN CANADA'S NORTHERN COMMUNITIES

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Country foods are foods which are trapped, fished, hunted, harvested, or grown that are sourced outside of commercial food systems. However, Canadians who reside in northern communities rely on locally sourced seafood and meat, opt for traditional foods for cultural reasons, and disproportionately experience greater risk of enteric illness compared to other Canadians.

Purpose: To identify and report on all known foodborne outbreaks linked to country foods in Canada's northern communities.

Methods: Outbreaks captured from peer-reviewed journal and news articles in the Publicly Available Foodborne Outbreak Database (PAIFOD) were descriptively analyzed. Northern communities were defined as Northern Canada (i.e., Yukon, Northwest Territories, Nunavut), and the provincial North as indicated by census divisions from Statistics Canada. All outbreak years between 1943-2024 contained within the database were considered.

Results: Overall, 15 outbreaks were identified from PAIFOD between 1987-2021 and the median number of cases across outbreaks was 4 (range: 1-34). Implicated foods included seal (n=6), walrus (n=3), whale (n=1), black bear (n=1), polar bear (n=1), clam (n=1), and unspecified meat (n=2). *Clostridium botulinum* (n=9) was the most commonly implicated pathogen, followed by *Trichinella* spp. (n=4), *Toxoplasma gondii* (n=1), and paralytic shellfish poisoning (n=1). For outbreaks with available information, four outbreaks reported at least one hospitalization (range: 1-9), and seven had at least one case of paralysis.

Significance: Despite a reduction in country food consumption between 2014-2024 reported in national surveys, outbreaks linked to these foods may be underreported in these regions due to surveillance limitations and access to healthcare. Culturally appropriate educational interventions aimed at improving

safe country food handling, particularly risks associated with fermentation, inadequate cooking, and hunting, may serve Canadians living in northern communities.

P2-13: UNPACKING POISONED: EVALUATING A FOOD SAFETY DOCUMENTARY ON YOUNG ADULTS' TRUST AND PERCEPTION

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Yaohua Betty Feng, Purdue University

Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Foodborne illnesses remain a significant public health issue, with 29,607 cases reported in the U.S. in 2023. Since its release, the documentary *Poisoned: The Dirty Truth About Your Food* has sparked widespread discussions about food safety and the responsibilities of stakeholders along the food supply chain.

Purpose: This study aimed to evaluate the impact of the documentary *Poisoned* on young adults' attitudes, perceptions, and trust in food safety and key food system stakeholders. It also explored the documentary's potential as a public health communication and education tool.

Methods: Undergraduate students at Purdue University (n = 59, 78% female) participated in the study. They completed a pre-survey before watching the documentary, a post-survey immediately after, and a follow-up survey nine days later to assess changes over time. Surveys measured confidence in various food safety stakeholders using a 7-point Likert scale. Additionally, participants joined 48-minute focus group discussions after viewing the documentary. Transcripts were analyzed using the Braun and Clarke thematic analysis approach. A paired t-test was used to assess changes in confidence levels between timepoints.

Results: Confidence levels in food supply chain stakeholders significantly decreased between pre and post survey ($p < 0.05$), particularly large framers (mean differences 2.18), grocery stores (2.13), large processors (2.00), and U.S. government (1.88). However, a rebound in confidence levels was observed for the restaurants (-0.46), grocery stores (-0.42), and self-cooking practices (-0.32) in the 9-day follow-up surveys. Focus group findings revealed that participants expressed high confidence that their own cooking won't make them sick. They viewed the documentary positively to enhance consumer awareness and food safety knowledge. Despite this, the documentary was observed to reinforce or introduce risky food-handling behaviors among some participants such as washing raw meat and pre-washed bagged lettuce.

Significance: These practices can lead to cross-contamination and increased risk of foodborne illness. This study contributes to the limited literature on the role of food safety documentaries in shaping consumer behavior and trust. Documentaries like *Poisoned* have the potential to raise food safety awareness while also influencing beliefs and behaviors that could increase or reduce foodborne illness risk.

Award Application: Developing Scientist Award

P2-14: SURVEY OF CONSUMERS' KNOWLEDGE OF MICROBIAL FOOD SAFETY REGARDING MEAT AND POULTRY PRODUCTS PURCHASED AT LOCAL WEST VIRGINIA FARMERS MARKET

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: Purchasing of meat and poultry products from local West Virginia farmers markets (WVFM) has become more popular and available although microbial safety concerns remain. Consumers' perceptions of food safety at WVFM, and their specific practices to ensure safety at both farmers' markets and households remain under-researched.

Purpose: This study aims to conduct a survey from consumers at FMs to determine their knowledge, attitude, and practices regarding microbial food safety of meat and poultry products purchased at FMs.

Methods: Face-to-face questionnaire with a total of 13 questions was interviewed to 116 consumers at 2 FMs in north central WV from June to September 2024. Questions include basic information (age, gender, and education level), purchasing frequency, handling including refrigerating of meat and poultry, concerns of microbial safety, and responsibility of food safety. Data from 116 completed surveys were analyzed using Chi-square tests (R-software) of independence to examine differences and bivariate relationships between categorical variables ($P = 0.05$).

Results:

Results showed that 36.2-62.1% of participants purchase meat products 1-3 times per season but less than half (25.9-45.7%, $P < 0.05$) purchase poultry. The majority of the participants (69.0%) thought "Eating local to assist our local farmers and economy" was the most important factor that encouraged them to

purchase meat and poultry products at farmers markets over supermarkets. only 4.3% ($P < 0.05$) of participants indicated food safety concerns as a reason that would prohibit their purchase of meat and poultry products, yet 71.6-81.9% ($P < 0.05$) could not identify a pathogenic bacteria associated with these products. Less than one-third of participants (28.4% and 27.7%, respectively) use disposable plastic bags to prohibit cross-contamination by leakage onto fresh produce, or pack a cooler, insulated bag, ice chest, or ice packs to keep meat and poultry products cool on the way home. An overwhelming 97.4% of participants, however, immediately place fresh meat and poultry products in a refrigerator that remains below 40 °F, while 94.8% always cook their meat products to a minimum internal temperature of 165 °F.

Significance: The results emphasized the need for continuing consumer education regarding safe food handling practices at WVFMs and at home.

P2-15: USING THE DELPHI METHOD TO DEVELOP AN INTRODUCTORY-LEVEL HIGHER EDUCATION FOOD SAFETY AUDITOR, INSPECTOR, AND ASSESSOR COURSE OUTLINE

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: High turnover and vacancy rates exist for food safety audit, inspection, and assessment (AIA) roles, and there is lack of programming within higher education exposing students to this career field.

Purpose: To solicit feedback from food safety AIA stakeholders on a higher education course curriculum outline that will expose students to career opportunities in AIA fields.

Methods: Food safety AIA stakeholders in industry, government, academia, extension, etc., were identified through professional networks. A Delphi survey was conducted to gather feedback on a proposed AIA curriculum outline, addressing the fundamentals of food safety, the methodology of conducting AIAs, and practical experience through scenario-based learning activities designed to engage students in immersive experiences. Stakeholders were selected from a list of contacts generated by the research team and recruited via email to participate. A survey was sent to participants between Feb. 16-17, 2023 and respondents were given until Feb. 24, 2023 to respond. Descriptive statistics were used to summarize

Results.

Results: Sixteen stakeholders were recruited with a 94% response rate; 47% of respondents had external or 3rd party audit experience, 33% had private contracting experience, and 27% had association or non-governmental organization experience. The average years of experience were 24 with state

agencies and 20 with non-governmental organizations. When asked about their reactions to the suggested learning objectives, 53% agreed with the flow and objectives of the curriculum as proposed, and 47% preferred to provide comments for topic and objective inclusion. In addition to the proposed module topics (e.g., personnel health and hygiene, pest control, equipment design), respondents also suggested prioritizing topics like allergen control and HACCP.

Significance: The results of the Delphi survey provided valuable information on the AIA curriculum by confirming that the outline and objectives are appropriate and suggesting additional, important topics for inclusion.

P2-16: WHAT DOES ‘READY-TO-EAT’ REALLY MEAN? EXPLORING DEFINITIONS, PRACTICES, AND FOOD SAFETY PERSPECTIVES ACROSS THE SUPPLY CHAIN

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Yaohua Betty Feng, Purdue University

Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Numerous recalls and outbreaks have been linked to ready-to-eat (RTE) foods, highlighting the need for clear regulatory guidance to control contamination. While the term “RTE” has a definition in guidance documents, its interpretation can vary among producers, processors, consumers, and regulators, influencing handling practices and potentially increasing foodborne illness risks.

Purpose: This study aimed to explore how three groups involved in the food chain interpret the definition of “ready-to-eat” foods.

Methods: An analysis of (1) four previous focus groups with small-scale growers, (2) 32 semi-structured interviews with food safety inspectors, (3) 47 interviews with consumers and (4) a survey of 916 consumers was conducted. All the studies explored the definition of “RTE foods” and participants’ interpretations of foods they considered RTE or non-RTE.

Results: Participants identified examples and food production processes as key elements to define RTE foods. The keywords mentioned by participants to describe RTE foods were “processed,” “raw,” “shelf-stable,” and “easy-to-eat.” A common theme across all groups was the definition of RTE as food that can be consumed without requiring further processing or kitchen preparation, including cooking and washing. Most consumers listed cookies and frozen foods as examples of RTE foods, while most produce growers listed salad kits and cut fruits as their examples. Participants expressed conflicting opinions about whether they regard mushrooms as RTE foods. Consumers who participated in the survey (59%) most commonly defined RTE food as “ready for consumption without further washing, heating, thawing, or other preparation,” which differs significantly from the definition in the regulatory guidelines.

Significance: This study provides valuable insights into varying interpretations of ready-to-eat foods, emphasizing the need for clearer communication and targeted guidance to reduce the risk of foodborne illnesses.

Award Application: Developing Scientist Award

P2-17: A SYSTEMATIC REVIEW OF HAND HYGIENE KNOWLEDGE, ATTITUDES, SELF-REPORTED PRACTICES, AND OBSERVED BEHAVIOUR IN FOOD MANUFACTURING AND FOOD SERVICE SETTINGS

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Hand-hygiene in food businesses is critical in controlling the transmission of pathogens. Despite its importance, inconsistent hand-hygiene practices in food service and food manufacturing can lead to contamination and public health risks. Understanding the cognitive and behavioural factors associated with hand-hygiene requires a comprehensive synthesis of existing research.

Purpose: To undertake a systematic review of primary research on food handlers' hand-hygiene knowledge, attitudes, self-reported practices, and observed behaviour in food manufacturing and food service settings. **Methods:** A comprehensive systematic search of peer-reviewed articles published in the past 25 years was conducted in Scopus, PubMed/MEDLINE, and ProQuest Central databases. The review included studies conducted in countries with developed economies that adopt CODEX General Principles of Food Hygiene. Screening and extraction was undertaken using a purpose-built tool in Covidence. Collated data from academic publications was synthesised according to hand hygiene cognitions and behaviours and analysed according to hand hygiene steps, occasions and compliance.

Results: A total of 23,076 studies were imported into Covidence, 2,338 duplicates were removed. After title/abstract screening, 283 studies were assessed for full-text eligibility, 41 studies met the inclusion criteria and were included for extraction in the review. Most studies (43%) were conducted in the United States, the remainder were from United Kingdom, Poland, Italy, France, Spain, and Belgium. The majority of studies (80%) were from food service sector, mainly restaurants. Although 45% of the studies were observational studies or included and observation, only 14 % were in the food manufacturing setting. Moreover,

about quarter of the studies assessed knowledge and attitudes of food handlers towards hand hygiene.

Significance: This review has provided an in-depth insight into food handlers' knowledge, attitudes, and practice regarding hand-hygiene. Findings indicate lack of observed data in food manufacturing settings and discrepancies between self-reported and observed behaviours have been determined.

P2-18: THE DEVELOPMENT AND EVALUATION OF A FOOD SAFETY TRAINING PROGRAM FOR FRONTLINE EMPLOYEES

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: Food safety training is required for all employees who manufacture, process, pack, or hold food. Small processors and farms have noted the need for training curricula designed for frontline workers that can be easily deployed and implemented for English and Spanish speaking employees.

Purpose: The purpose of this study was to 1) develop a food safety training curriculum for frontline employees, and 2) determine the effectiveness of the program in influencing knowledge and behavior change towards food safety practices in food processing facilities.

Methods: A curriculum framework was developed through 1) a review of regulatory requirements and third-party auditing standards; 2) an alignment with recognized learning objectives in the retail and restaurant workshops; and 3) a needs assessment among industry and regulatory contacts. Seven pilot trainings were conducted in both English and Spanish from October to December 2024 (N=61 participants). Pre- and post-test evaluations were used to measure knowledge change. ongoing interviews with managers and supervisors of the participants will be used to evaluate behavior change.

Results: of the 61 participants, 50 completed tests were included in the statistical analyses. Overall, paired t-tests conducted using JMP® indicated that the training was effective in meeting the program's learning objectives and increasing participant food safety knowledge ($p < 0.001$). However, in comparison to participants who completed the training in English ($n=37$), Spanish-speaking participants ($n=13$) did not have a statistically significant increase in knowledge scores. This highlights the challenge of creating a standardized curriculum for a diverse population across multiple food sectors in the industry. Data collection is ongoing which includes additional workshops and interviews with targeted

companies to evaluate behavior change in participants.

Significance: This study establishes the foundation for a standardized food safety educational program while highlighting the importance of supporting the diverse demographics of frontline employees given the significant impact of their role.

P2-19: A REVIEW OF FOOD SAFETY RISKS IN AQUAPONICS SYSTEMS: IDENTIFYING PATHOGEN SOURCES AND CONTAMINATION CHALLENGES

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Aquaponics is a food production system that associates aquaculture and hydroponics by using aquatic animal waste as a fertilizer for hydroponically grown plants. The practice has attracted attention from farmers and researchers around the world in recent years. However, an information gap exists regarding the potential food safety risks associated with aquaponics systems.

Purpose: This study aimed to identify the main food safety concerns and potential sources of risks involved in aquaponics systems.

Methods: We conducted a systematic literature review in October 2024 following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Through our search of Scopus, Purdue Libraries, Web of Science, and Agricola databases, we identified 3,016 papers. The first exclusion criteria were non-English studies, studies conducted before 2004, dissertations, abstracts, and non-peer-reviewed articles, which resulted in 1,722 studies. After screening, 46 peer-reviewed English-language studies from the past 20 years were included that focused on aquaponics systems, contaminants, pathogens, microbial quality, or food safety practices.

Results: The main insights from the analyzed studies indicate that common food safety risks in aquaponics systems include the presence of potential pathogens and spoilage bacteria, such as *Pseudomonas* spp., *Salmonella* spp., *Clostridium* spp., biofilms, mycotoxins, molds, and yeasts. Additionally, heavy metals, pesticides, contaminated water, and fish feed were identified as contamination sources. Inadequate personal hygiene practices and lack of periodic cleaning and sanitizing may also contribute to system contamination.

Significance: This study highlights the need to address food safety shortcomings in aquaponics systems by identifying key sources of contamination and potential pathogen risks, thereby contributing to safer and more sustainable food production methods.

Award Application: Developing Scientist Award

P2-20: MICRO AGRICULTURAL SYSTEMS: THE CHANGING LANDSCAPE OF SPECIALTY CROP PRODUCTION AND PRODUCE SAFETY

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: Small-scale, non-traditional food production, including specialty crop production in home gardens, schools, community gardens, and urban or rural municipalities, is a growing trend. As these producers transition from self-sufficiency to commercial sales, they face significant challenges in implementing good agricultural practices (GAPs). While new USDA initiatives, such as the Agricultural Marketing Service's Local Foods, Farmers Markets, and Resiliency grants, encourage food systems development, producers often lack understanding of potential food safety risks. Common issues include sanitation, cold-chain management, and post-harvest handling. Inadequate knowledge of agricultural water, temperature control and limited infrastructure increase the risk of foodborne pathogens.

Purpose: This program, informed by an Arkansas-based needs assessment, aims to address gaps in produce safety practices for small-scale producers from home to small-scale commercial and including those using USDA Resilient Food Systems infrastructure (RFSI)-funded facilities. The program provides targeted educational outreach to help producers navigate the complexities of the FSMA Produce Safety Rule (PSR) while effectively utilizing small scale or USDA-funded infrastructure.

Methods: The initiative supports in-person workshops, technical assistance, and tailored educational materials to guide producers on FSMA compliance. The program will provide guidance on proper sanitation, temperature control, allergen management, and record-keeping, with a particular focus on applying these practices within USDA-funded facilities. Hands-on demonstrations and customized support will help producers transition from small-scale or home-based systems to larger, compliant operations.

Results: An outreach program is being developed to help producers to gain practical knowledge on managing food safety risks, such as cold-chain management and post-harvest handling. Producers will also receive support in

maintaining FSMA compliance and optimizing the use of USDA-funded facilities for safe, high-quality produce.

Significance: This initiative enhances food safety practices among small-scale producers, reduces foodborne risks, and promotes sustainable agricultural practices. It supports resilient local food systems and strengthens food security in underserved communities.

P2-21: MICRO AGRICULTURAL SYSTEMS: THE CHANGING LANDSCAPE OF FOOD FREEDOM AND VALUE-ADDED FOOD PRODUCTION

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Presentation type/Format: Technical

Category: Communication, Outreach and Education*

Abstract:

Introduction: Legislative changes, such as the Arkansas Food Freedom Act and similar laws across the U.S., have opened new opportunities for home-based and small-scale producers to sell value-added foods. While these laws promote innovation and local food production, they also present significant food safety risks. Home producers, homesteaders, and small-scale farmers often operate with limited oversight, increasing the likelihood of unsafe practices. Risks include improper sanitation, lack of allergen control, inadequate labeling, and insufficient knowledge of retail and wholesale regulations. As producers scale from home kitchens or on-farm operations to larger models, they face challenges maintaining food safety practices and meeting complex regulations.

Purpose: This program, informed by an Arkansas-based needs assessment, aims to address the food safety challenges of non-traditional producers. The needs assessment highlights key issues in home kitchens, small farms, and institutional settings. The program provides educational tools, resources, and regulatory guidance to equip producers with the knowledge and skills to safely produce and sell value-added foods. Key areas of focus include sanitation, allergen control, labeling, and compliance with state and federal regulations, helping producers transition from small-scale to commercial food production.

Methods: The initiative supports in-person workshops, one-on-one technical assistance, and tailored educational resources for home producers, homesteaders, and small-scale entrepreneurs. The program will provide guidance on food safety practices, including sanitation, allergen management, labeling, and record-keeping. Workshops will also cover regulatory compliance and the requirements for scaling production.

Results: An outreach program is being developed to help producers manage food safety risks, ensure regulatory compliance, and transition to larger-scale operations. Ongoing support will guide producers through complex food safety challenges. Arkansas approaches to these emerging trends will be discussed to inform national practice.

Significance: This program fosters safer, more resilient local food systems, reduces foodborne risks, and supports regional food security, particularly in underserved communities.

P2-22: DEVELOPMENT OF PRODUCE SAFETY EDUCATIONAL MATERIAL FOR SMALL BEGINNING UNDERREPRESENTED AND UNDERSERVED FARMERS

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Although there is a wealth of produce safety educational material, resources specifically designed for underrepresented and underserved small beginning farmers are limited.

Purpose: The overall objective of this project was to identify the needs of small, underrepresented/underserved beginning farmers and develop produce safety educational material based on virtual focus group feedback.

Methods: The team created a questionnaire to recruit underrepresented/underserved small farmers for a virtual focus group, ensuring participants represented a variety of produce operations. Based on recommendations from the project's Advisory Board, the initial questions were revised and used in the recruitment process. A flyer was developed and circulated. From the review process, eight applicants from Alabama, North Carolina, and South Carolina were selected. As a training opportunity, graduate students enrolled in a Produce Safety class at Alabama A&M University participated in the virtual focus group.

Results: Focus group participants expressed the need for training materials focused on the use of sanitizers and record keeping, along with a manual tailored to small beginning farm operations. In response, the graduate students developed

produce safety materials covering proper sanitizer use and record keeping. Formats included short 3-minute videos and factsheets. All materials were reviewed and revised by the project's Advisory Board.

Significance: This innovative project addresses the educational needs of underserved beginning farmers while simultaneously training graduate students in produce safety. The resulting materials are both relevant and accessible, supporting knowledge growth among small beginning farmers in produce safety.

P2-23: FOOD SAFETY ATTRIBUTES ASSOCIATED WITH CEREALS, LEGUMES, AND CONDIMENTS IN URBAN AND PERI-URBAN HOUSEHOLDS IN BENIN, GHANA, AND NIGERIA

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Cereals, legumes, and fermented condiments are dietary staples in African households. In West African countries like Benin, Ghana, and Nigeria where informal systems are dominant, they are prone to contamination via handling practices.

Purpose: Sourcing, handling, and consumption patterns were assessed to estimate the exposure to multiple toxicants including mycotoxins, heavy metals, and pesticide residue in cereals, legumes, and condiments among urban and peri-urban households.

Methods: Data on the frequency of consumption and handling practices of the targeted food groups were obtained via questionnaires input on the KoboCollect application from adults in 300 households (100 households each in Benin, Ghana, and Nigeria).

Results: From 24-hour recall, cereal consumption was high across the three countries (91%, 88%, and 80%) in Ghana, Nigeria, and Benin respectively. In contrast, the consumption of legumes and condiments was lower in Benin (37% and 35% respectively), compared to Ghana (65% each for legumes and condiments) and Nigeria (67% and 72% for legumes and condiments respectively). Foods were sourced mostly from informal markets but also from home gardens, commercial farms, and received as gifts.

Significance: The data obtained provided insights into practices that contribute to food safety risks caused by multiple toxicants and entry points for addressing the risks.

P2-24: EVALUATING A HYBRID APPROACH TO PREVENTIVE CONTROLS FOR HUMAN FOOD EDUCATION FOR SMALL PROCESSORS IN VIRGINIA

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: introductory food safety education for small food processors across the Southeast primarily focus on state- or commodity-specific concepts, despite the science of food safety not significantly changing across state lines. Many of these food producers report a preference for hybrid courses, despite existing data showing this approach is not as effective with some content. To facilitate education in an increasingly virtual climate, it is necessary to understand which content can be effectively delivered in-person and virtually.

Purpose: Assess the knowledge gain, experience, and preferences of small food processors in Virginia via a novel hybrid course structure.

Methods: Extension educators from four states developed a two-part course consisting of 1) a virtual module on state-specific regulations and 2) four in-person modules about introductory food safety concepts. Content was independently reviewed by eight industry and government personnel in the respective states. Participants (n=41) in a pilot training in Virginia were asked to complete the virtual module prior to attending the in-person workshop. Pre-/post-tests were administered at the beginning and end of the in-person workshop to assess short-term knowledge gain using a paired t-test. Formative evaluations were conducted throughout the in-person workshop, followed by a summative evaluation at the workshop's end to assess participant experience and preferences for training using a standard qualitative approach. Statistical analysis was conducted in

RStudio version 4.3.3.

Results: of the 41 virtual module participants, 26 attended the in-person workshop (63.4%). of these, 12.2% (5/26) participants completed the virtual module prior to attending the in-person workshop as intended, with 53.8% (14/26) participants completing the module after the in-person event. Post-test scores (93.3%, 11.2/12) were significantly higher than pre-test scores (76.7%, 9.2/12, $p < 0.0001$, indicating an increase in knowledge of 21.7%. Qualitative analysis identified participant preference for the in-person format.

Significance: The hybrid course structure impacted course attendance and completion rates, indicating a need for optimization prior to multistate dissemination.

Award Application: Developing Scientist Award

P2-25: FLORIDA'S EXTENSION PROGRAMS PREPARE PRODUCE GROWERS FOR PRODUCE SAFETY RULE INSPECTION

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

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: The new regulation meant Florida produce growers would require education and technical assistance to meet the requirements of the rule.

Methods: The University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) and the Florida Department of Agriculture and Consumer Services (FDACS) have collaborated since 2017 to provide education and outreach through Produce Safety Alliance (PSA) Grower Training Courses and On-Farm Readiness Reviews (OFRR). Both programs assist growers in meeting the requirements of the PSR. Pre- and post-tests are administered at PSA Grower Trainings (n=1,724; 106 trainings since 2017) to measure knowledge increase during the class. A follow-up survey to determine practices changed or implemented is sent four months post-training. Following the completion of each OFRR (2018-2025), surveys (n=83) are completed to evaluate farm readiness for rule implementation, and highlighted areas where more education was needed to meet minimum requirements.

Results: Post-test score means (21.52/25) were significantly higher than pre-test score means (17.75/25), indicating an increase in knowledge after participation in the training ($t=-11.80$, $P<0.05$). Follow-up surveys indicated the creation or modification of recordkeeping systems was the most common action taken based on the knowledge gained in the training. OFRR surveys indicated sanitation (36%), pre-harvest water (13%), and worker training (27%) required the most

improvements, and that 49% of farms met the PSR requirements, 39% needed minor improvements, and 12% needed significant improvements to meet the FSMA PSR requirements.

Significance: Knowledge and practices of Florida farms regarding the FSMA PSR increased following training and site visits. As inspections continue throughout Florida, education and outreach programs are ongoing to ensure Florida farms are prepared for implementation of the FSMA PSR.

P2-26: ASSESSING THE SOUTHERN REGIONAL FOR FSMA FOOD SAFETY TRAINING AND OUTREACH IMPACTS

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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 requires training, outreach, and technical assistance for owners and operators of small and medium-sized farms, beginning and socially disadvantaged farmers, small food processors, and small fruit and vegetable wholesale merchants.

Purpose: The Southern Center for FSMA Training is a consortium of 26 institutions aimed at enhancing produce safety in 14 southern states and 2 territories through various educational means, including conducting and evaluating standardized courses (Produce Safety Alliance (PSA) and Food Safety Preventive Controls Alliance (FSPCA) Preventive Controls Qualified individual (PCQI)) targeting the PSR and PCHF.

Methods: Validated pre/post-tests were given at PSA and PCQI courses to assess short-term knowledge gains from September 09/2021- August 08/2024 across the region. A qualitative evaluation was conducted with participants of both courses 4no less than four months after training to evaluate medium-term outcomes of behavior changes related to food safety practices. PSA (103; 1,555 participants) and FSPCA (12; 169 participants) courses were held across 13 states and 1 states/territories.

Results: PSA post-test scores (20.63; $n = 1,132$) were significantly higher than pre-test scores (15.86; $t = 14.24$, $p < 0.01$), indicating a significant increase in knowledge. of 13 practices included in the 4- month follow up PSA surveys, the most adopted practices were Implement new or different trainings for employees on food safety/hygiene protocols (54.3%; 100/184) and Write or modify farm food safety plans (53.9%; 96/178). of seven practices included in the 4-month PCQI behavior change surveys ($n = 58$) the most frequent behavior change was they recommended the training to others ($n = 32$), followed by Trained employees on FSPCA rules ($n = 23$) and Fine-tuned existing food safety plans ($n = 22$).

Significance: Members of the SC continues to enhance food safety of growers and processors through short term knowledge gains immediately post training, and medium-term behavior changes of food safety practices.

P2-27: EFFECT OF SALT CONCENTRATION AND PEPPER VARIETY ON MICROBIAL POPULATION INCREASES AND PH REDUCTION DURING PEPPER MASH FERMENTATION

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Previous research shows home food preservers are experimenting with fermenting vegetables, but there is a lack of food safety research on lacto-fermentation methods for alternative vegetables like peppers.

Purpose: To develop and validate a pepper mash lacto-fermentation process by comparing the effects of salt concentration and pepper variety on microbial growth and pH reduction.

Methods: Two lacto-fermentation experiments were conducted with Red Bell and Serrano peppers at three NaCl concentrations (1.00%, 1.75%, and 2.50% w/w). Pepper mashes were prepared by rinsing, trimming, deseeding, chopping 500 g of peppers, and mixing with pickling salt in 500 g batches. Triplicate 1-pint jars were filled with 150 g mash, and water-filled bags were added to maintain anaerobic conditions. Fermentation was monitored at room temperature over 12 days, with samples taken on days 0, 2, 7, and 12 for pH and microbial analysis (lactic acid bacteria, aerobic plate count, and yeast & mold) using serial dilutions and Petrifilm plating. Statistical differences in microbial counts and pH values were analyzed using a mixed-effect model ANOVA.

Results: Lactic acid bacteria and aerobic plate counts significantly increased by Day 2 ($p < 0.05$) for all salt concentrations and pepper types, with no significant change after that. In Serrano peppers, LAB and APC increased by about 7 and 3 logs, respectively, while in Bell peppers, both increased by 7 logs. pH decreased similarly, from 4.50–5.48 on Day 0 to 4.76–3.82 for both pepper types. Yeast and mold counts were less consistent but significantly increased by Day 7, with Serrano peppers showing a 7-log increase and Bell peppers a 5-log increase for all salt concentrations.

Significance: Successful fermentation of peppers can occur over a wide range of NaCl concentrations, offering options for those wanting to reduce salt intake. Success with both mild (Red Bell) and hot (Serrano) peppers suggests microbial growth and pH reduction are not influenced by capsaicin concentration, as aerobic

plate count and LAB values support, though yeast and mold values show some variation.

P2-28: PACKED WITH PROTEIN OR PATHOGENS? ASSESSMENT OF PROTEIN COMPOSITION AND FOOD SAFETY COMMUNICATION IN COMMERCIAL MEAL-KITS

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Protein-rich foods require special handling and preparation to prevent foodborne illness. Meal-kits are boxes containing a variety of pre-measured ingredients, including raw proteins and fresh produce. Meal-kits are delivered directly to consumers and include step-by-step instructions for in-home preparation. While meal-kits have the potential to improve diet quality, their communication of safe protein handling is unknown.

Purpose: The objective of this study was to evaluate protein content and the presence and quality of protein-related food safety instructions in commercial meal-kits in the US.

Methods: Recipe cards (n=126) were collected from meal-kit suppliers (n=11). A data extraction tool was designed in Qualtrics to capture protein and food safety content of recipes. The type and quantity of protein foods in meal-kits were analyzed and compared to the Dietary Guidelines for Americans. Food safety practices, including proper storage, hand hygiene, cross-contamination prevention, and cooking adequacy were captured and evaluated using the Partnership for Food Safety Education 'Safe Recipe Style Guide.'

Results: On average, analyzed recipes provided 36 grams of protein per serving (median: 37 grams). Over three-fourths (n=96) of recipes included meat, poultry, or fish; poultry was most frequently featured (n=49). No recipes instructed handwashing before preparation, and only seven recommended handwashing after handling raw protein. Approximately one-half of recipes containing raw protein (51%, n=49) included instruction to dry raw proteins with paper towels without additional guidance to prevent cross-contamination. Less than half of all recipes (n=57) included minimum internal temperatures, and 3% stated an incorrect temperature.

Significance: Meal-kits may provide a significant portion of the average American's protein requirement, presenting the possibility of protein

overconsumption if consumed frequently. Communication regarding safe protein handling is insufficient to prevent foodborne illness, especially among consumers susceptible to foodborne disease. Further research is needed to guide future meal-kit development to optimize nutrient composition and food safety communication.

P2-29: "I'VE NEVER CONSIDERED MYSELF HIGH RISK": DEFINING AND COMMUNICATING SUSCEPTIBILITY TO FOODBORNE ILLNESS

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Food safety communication is essential to prevent foodborne disease among consumers with increased susceptibility to foodborne illness. However, current definitions of susceptibility or "clinical vulnerability" are vague and may omit at-risk groups. Similarly, the preferred communication methods for consumers in higher risk groups are unknown.

Purpose: The objective of this study was to identify the current definitions of susceptibility or "clinical vulnerability" used by food safety organizations worldwide and to evaluate consumer communication preferences surrounding food safety risk communication.

Methods: A grey literature review of definitions of susceptibility utilized by global food safety agencies was conducted. in-person discussion groups (n=6) were conducted with members of susceptible groups (adults aged 65 years and older) to assess communication preferences of foodborne illness risk. Discussion topics included awareness of susceptibility, appropriate terminology, and the level of detail preferred in communication of susceptibility.

Results: A total of 515 definitions were identified from 14 global agencies, 33% (n=217) originating from American organizations. Groups identified in definitions of clinical vulnerability most frequently included older adults (96%), people with compromised immune systems (72%), pregnant people (64%), and children (52%). Pregnant people and older adults were most frequently referred to in relation to listeriosis (90% and 83% respectively). Other at-risk groups (people with cancer or HIV, people prescribed proton-pump inhibitors, etc.) were less frequently referred to (11%, 6%, 1% respectively). Discussion groups revealed lack of prior knowledge surrounding increased foodborne illness susceptibility, despite belonging to a "clinically vulnerable" group. Participants indicated that the provision of a rationale enhanced understanding of their increased susceptibility.

Significance: Findings indicate that adjustments to existing definitions of susceptibility and risk communication practices are required. Definitions should be

based on incidence data and be inclusive of all individuals with increased susceptibility. Consideration for the communication preferences of consumers within vulnerable groups should be considered to enhance consumer understanding.

P2-30: CULTURAL INFLUENCES AND BARRIERS IN ADOPTING FOOD SAFETY PRACTICES: A CASE STUDY OF HMONG FARMERS

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Current Food Safety Modernization Act (FSMA) training programs often overlook the cultural values and challenges faced by minority farming communities, including Hmong farmers. These farmers rely on traditional practices, family labor, and strong community ties, which can create difficulties in integrating FSMA protocols.

Purpose: This study explores how cultural values influence food safety practices among Hmong farmers and identifies barriers to adopting FSMA training.

Methods: Semi-structured interviews were conducted with 12 Hmong farmers across Missouri (n=6) and Minnesota (n=6). On-farm observations (n=12) were also conducted to evaluate food safety practices. A bilingual translator facilitated farmer interviews. All interviews were recorded, transcribed verbatim, and used to initially identify key concepts and themes.

Results: Hmong farmers' food safety practices reflect cultural values such as collectivism, reliance on family-centered labor, and adherence to traditional farming methods passed down through generations. Their cultural heritage influences their preference for growing culturally significant crops and using traditional irrigation and composting techniques. However, language barriers, financial constraints, and limited access to resources such as coolers, washing stations, and adequate storage facilities present significant challenges. Farmers expressed a need for simplified, culturally tailored guidance to bridge the gap between traditional practices and FSMA compliance. Examples of potential strategies include picture-based SOPs, multilingual training materials, tabletop demonstrations for proper cleaning and sanitizer preparation, and community-focused skits or plays to reinforce key food safety concepts. Additionally, hands-on workshops and resource-sharing initiatives were identified as particularly effective tools.

Significance: This study highlights the need for culturally sensitive FSMA training programs that respect Hmong farmers' cultural values while offering tangible and

practical tools. By addressing their unique challenges, such programs can empower Hmong farmers to adopt food safety practices, preserve their heritage, and enhance sustainability and equity in minority farming communities.

P2-31: EXAMINING ILLNESS REPORTING IN FOODSERVICE EMPLOYEES

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Illness reporting in the restaurant and foodservice industry is critical for ensuring food safety and protecting public health. However, personal, organizational, and other behavioral factors (e.g., injunctive norms) often influence employees' decisions to report illnesses. The complex interplay of these factors remains underexplored, necessitating a deeper investigation into the motivations and barriers associated with illness reporting.

Purpose: Drawing from Focus Theory of Normative Conduct, this study aimed to investigate the determinants of illness reporting behavior among foodservice and restaurant employees.

Methods: A total of 350 employees, recruited from a crowdsourcing platform (Prolific), completed an online survey. Exploratory Factor Analysis (EFA) was conducted to identify latent constructs, followed by Confirmatory Factor Analysis (CFA) to validate the measurement model. Multiple regression analysis was employed to analyze the relationships between personal factors (e.g., team loyalty, presenteeism drivers), organizational factors (e.g., group identification, managerial and peer influences), and behavioral intentions to report illness.

Results: More than 50% of the participants were aged 19–29 years and were predominantly female respondents (60.6%). Managerial-level behaviors ($\beta = .264$, $p < .001$) and peer-level behaviors ($\beta = .145$, $p < .001$) were the strongest predictors of illness reporting intentions. Injunctive norms ($\beta = -.129$, $p < .001$) and team loyalty ($\beta = -.092$, $p = .016$) had significant negative impacts on behavioral intention, highlighting potential barriers to illness reporting. Presenteeism drivers, including motivation and commitment to work ($\beta = .109$, $p = .002$), also significantly influenced behavioral intentions, implying the need to address workplace culture and individual perceptions to promote better illness reporting practices.

Significance: This study highlights the need for managerial support, team loyalty, and better communication to foster illness reporting in foodservice settings.

P2-32: ASSESSING FOOD SAFETY KNOWLEDGE GAPS AMONG UNITED STATES INDOOR GROWERS

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: The rising demand for locally sourced produce has driven interest in Controlled Environment Agriculture (CEA) for its ability to grow food locally. While CEA is often considered safer than traditional field farming, recent recalls and outbreaks have raised food safety concerns.

Purpose: This study explored knowledge gaps and assessed food safety and hygiene practices among indoor growers through a nationwide survey of 64 indoor farming operations across the United States.

Methods: The survey addressed facility types, farm sizes, growing systems, and food safety practices aligned with regulatory standards and industry guidelines. Respondents represented diverse regions, with the Southern region contributing the most participants, followed by the North Central, Northeast, and Western regions.

Results: Significant variability in indoor farming characteristics was observed. Greenhouses were the predominant facility type, especially in the Northeast (83%), while hydroponics was most common in the South (87%). Smaller farms (<10,000 sq. ft.) dominated the Western and Southern regions, while larger farms (>100,000 sq. ft.) were concentrated in the Northeast and North Central regions. Urban farming was most prevalent in the West (75%), while the Northeast had a rural majority (67%). Knowledge gaps were identified in areas such as soil amendments and managing animal access to production areas. Only 50% of farms with animal presence implemented pest control programs. A lack of understanding was also noted regarding food safety during harvest and postharvest practices.

Significance: These findings highlight the diversity of indoor farming systems and practices and underscore critical areas for improvement in food safety and hygiene knowledge among CEA producers.

P2-33: EVALUATION OF AN AUTOMATED DNA EXTRACTION PROTOCOL AND REDUCED ENRICHMENT TIMES FOR THE DETECTION OF SALMONELLA OR STEC IN A VARIETY OF MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Advances in molecular methods have allowed industry to rapidly release product. However, for certain industries these advancements have not provided this benefit due to the complexity or perishability of the product. There continues to remain a need for accurate methods with reduced incubation periods. The objective of this project was to evaluate an automated process using NucliSENS® reagents to evaluate shortened enrichment protocols for the detection of *Salmonella* in four complex matrices (dry dog food, milk and white chocolate paste, and peanut butter) and for the detection of pathogenic *Escherichia coli* in carcass sampling cloths.

Purpose: To evaluate an automated DNA extraction protocol to reduce incubation time requirements for the detection of select pathogens by real-time PCR in chocolate products, peanut butter, dry dog food, and carcass sampling cloths.

Methods: Validation studies (dry dog food and carcass sampling cloth) and verification studies (raw materials for the chocolate industry) were performed. Validation studies included the evaluation of 30 test replicates (5 control, 20 low inoculum, and 5 high inoculum) after a 10 h enrichment (dry pet food) or 4 h enrichment (carcass sampling cloth). For the verification study (chocolate products), a 10 h enrichment protocol was evaluated with 10 replicates at a low level. All food matrices were analyzed using 375 g test portions and all matrices were enriched in BPW plus BACTBOOST™ supplement followed by DNA extraction using the automated protocol. Testing was performed using AOAC certified real-time PCR methods.

Results: No false positive or negative results were observed with the new

automated DNA extraction method for any of the matrices evaluated. When results of the new method were compared to the reference method, no statistically significant differences were observed.

Significance: The new automated preparation can reduce the enrichment time needed for *Salmonella* detection to 10 h and pathogenic *Escherichia coli* to 4 h for select matrices.

P2-34: A STUDY TO DETERMINE THE BARRIERS AND IMPACT OF OPERATING EFFECTIVE INTERNAL SYSTEMS AUDITS OF FOOD SAFETY CULTURE WITHIN WALES'S FOOD AND DRINK MANUFACTURING SECTOR

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: The Welsh Government has identified that to enable growth of the food and drink sector in Wales there is a need to support food and drink manufacturing businesses to proactively maintain strong food safety culture (FSC) on site. In businesses, Food Safety Culture is variable, leading to food safety incidents and Non-conformances which could be prevented.

Purpose: To determine the operational profile of FSC in manufacturing sites to identify barriers and appropriate interventions to enable the staff to comply with established food safety systems.

Methods: A multiple-method approach included: desk-based research to determine Food Safety Scheme standard requirements and the completion of independent systems audit on-site with food manufacturing businesses (n=9) of varying size and discipline. It included the design and implementation of a systems audit checklist to assess the climate of food safety culture on site.

Results: The study determined 90% of the businesses were certificated to a third-party food safety standard with 50% manufacturing ready to eat(RTE) or heat(RTH) products. Sixty-five requirements were assessed across nineteen food safety themes to determine the levels of compliance through observation, interviews, and documentation on site. Compliance to the requirements ranged from 30% to 90%. Only seven of the themes achieved more than 70% compliance.

Significance: The study highlighted a variation in systems compliance indicating a negative impact on food safety culture. Food safety systems compliance is dependent upon multiple factors such as staff skills, capability, and resource. Findings have informed the development of support mechanisms to improve CAPA, Hygiene and Housekeeping, Environmental monitoring. Delivering multiple bespoke interventions to improve teamwork, applied learning and understanding of FSC communication and policies. A series of bespoke interventions have been developed to improve the effectiveness of operational systems and subsequently

food safety culture.

P2-35: UK MEAL-KITS AND FOOD SAFETY: INVESTIGATING TEMPERATURE CONTROL FROM DELIVERY TO PLATE

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Meal-kits are boxes of fresh, measured ingredients delivered to the consumers' home for subsequent storage, handling, and meal preparation by the consumer. To maintain safe temperatures, food is delivered over extended distances in a box with insulation and ice packs. Consumers should ensure food safety by following recommended refrigeration practices and checking the internal cooking temperatures of cooked meat using recommended techniques.

Purpose: The aim was to focus on three aspects of temperature control: measure the surface temperatures of raw meat and poultry delivered in meal-kits, assess consumer practices by determining the temperatures of domestic refrigerators, and evaluate the temperatures of meat cooked by consumers at home.

Method: Using an infrared thermometer, the front and back surface temperatures of raw meat and poultry samples (n=76) delivered in UK meal-kits (n=17) were measured, in triplicate, within four hours of delivery. One hundred consumers were provided with refrigerator and meat thermometers to record in-home refrigeration temperatures (n=48) and cooking temperatures (n=36). Respondents (n=60) were also asked to submit photos of their recordings.

Results: Average surface temperatures recorded for raw meat (n=23) and raw poultry (n=53) were 5.3°C and 4.1°C. When cooking poultry (n=20), 75% of consumers were compliant with recommended internal cooking temperatures. For refrigeration (n=48), 35% adhered to recommended temperatures ($\leq 5^{\circ}\text{C}$).

Significance: The study highlights that while consumers were occasionally compliant with recommended food safety practices, there is room for improvement in temperature control when cooking poultry. Average surface temperatures of raw meat and poultry were determined to be delivered within the

legislative standards of $\leq 8^{\circ}\text{C}$. However, surface temperatures were recorded within four hours, but meal-kit providers validate ice packs to maintain safe temperatures for over eight hours. It remains uncertain whether surface temperatures would stay within 8°C over an extended period. Results could inform research designing interventions to enhance consumers' domestic food safety practices when using meal-kits.

P2-36: EVALUATING THE ACCURACY OF CHATGPT 4.0 IN ANSWERING CONSUMER FOOD SAFETY QUESTIONS: THE IMPACT OF PROMPT DESIGN ON RELIABILITY

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Chatbots have become popular in recent years for their capability to analyze substantial amounts of data in a brief time, allowing them to answer questions in real-time. Based on a previous study that analyzed different chatbots available for consumers in answering the food safety risks associated with 20 food safety practices discussed on the podcast **Risky or Not**, hosted by food safety experts Dr. Ben Chapman and Dr. Don Schaffner, this study investigated the effectiveness of ChatGPT-4o mini in answering if consumers' food safety practices are risky and the impact of prompt variations in the chatbot response.

Purpose: We asked if food safety practices were risky, and, additionally, we inserted the prompts "Answer Yes or No" and "Answer Yes, No, or Unsure" after the same food safety practice was tested previously. The goal was to assess ChatGPT-4o mini's ability to align with expert opinion and maintain response consistency.

Methods: Twenty food safety scenarios were selected from the **Risky or Not** podcast. Each scenario was input into ChatGPT-4o mini three times using three different prompt styles: no prompt, "Answer Yes or No," and "Answer Yes, No, or Unsure." The chatbot's responses were recorded and compared to the expert opinions expressed on the podcast. Agreement with expert opinion, response consistency across repetitions, and impact of prompt type were evaluated. Percentage agreement and variation in repeated responses were calculated.

Results: In comparison with the positions discussed by the experts on the podcast, the overall agreement of ChatGPT-4o mini's responses was 35%. The prompts "Answer Yes or No" and "Answer Yes, No, or Unsure" resulted in 50% and 40% agreement, respectively, indicating that ChatGPT-4o mini did not consistently align with expert opinion. Most errors occurred when the chatbot characterized actions considered safe by the experts as risky. ChatGPT-4o mini demonstrated moderate consistency, with 15% and 5% variation in repeated answers for the direct and prompted versions, respectively.

Significance: This study highlights the need for food safety content validation on ChatGPT-4o mini to optimize chatbot effectiveness and transparency. Enhancing

alignment with expert consensus will enable consumers to make more informed decisions about the risks associated with food practices and support the safe integration of chatbots in real-world applications.

Award Application: Developing Scientist Award

P2-37: SINGLE-LABORATORY VALIDATION (SLV) STUDY OF A REAL-TIME PCR METHOD FOR THE DETECTION OF SALMONELLA IN SOIL WITH IMPROVED AUTOMATED DNA EXTRACTION METHODS ON THREE REAL-TIME PCR SYSTEMS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The FDA qPCR method has been validated for the detection of *Salmonella* in a wide range of food samples and environmental swabs. A SLV study of this qPCR method for the detection of *Salmonella* in complex environmental samples is needed for outbreak investigations.

Purpose: To conduct a SLV study of qPCR for the detection of *Salmonella* in soil on three real-time PCR systems, using improved automated DNA extraction

Methods to effectively remove PCR inhibitors expected in soil.

Methods: Three types of soil (Silt, Sand, and Sediment) were used in the study. Each type of soil, 30 replicates of 25 g test portion size, were inoculated at three levels (5 uninoculated controls, 20 replicates of fractional level, and 5 replicates of high level). All 30 test portions were analyzed by BAM culture method and qPCR method in 24 h pre-enriched cultures. Automated DNA extraction kits (Promega Maxwell culture cell kit and indical Bioscience BioSprint 96 one-for-all vet kit) were used for the DNA extraction of 24 h pre-enriched cultures with and without using a pre-clarification step on a 96 deep-well filter plate. The cultural results were compared to the qPCR results from Applied Biosystems 7500 Fast, QuantStudio 5, and Bio-Rad CFX Opus Deep-Well systems.

Results: Ct (Cycle threshold) values of internal Amplification Control in the qPCR analysis were only in the expected range of 28 ± 1 when DNA templates were prepared from pre-clarified cultures showing that pre-clarification steps effectively removed PCR inhibitors. Results obtained for the three real-time PCR systems had 100% agreement with culture Results for all three types of soil.

Significance: This study provided a validated qPCR method for the detection of *Salmonella* in complex environmental samples in multiple Real-time PCR platforms, promoting a rapid screening method application to accelerate the testing during outbreak investigation.

P2-38: PERSISTENCE OF HUMAN NOROVIRUS AND ITS SURROGATES IN VOMITUS

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human norovirus (HuNoV) is a major foodborne virus that causes gastroenteritis which is transmitted mostly by the fecal-oral routes but also by vomitus episodes.

Purpose: The aim of this project was to determine the persistence of HuNoV and its surrogates (MNV-1, MS2 phage and Tulane) in artificial vomitus deposited on different surfaces (stainless steel coupons).

Methods: Artificial vomitus was obtained using the standard INFOGEST 2.0 protocol via static in vitro digestion. Viruses were individually inoculated into the vomitus with a final concentration of 10^7 PFU/mL and a volume of 1.0 mL. Samples were then spotted onto three stainless steel coupons (1 cm^2) each containing 30 μL and dried for 1 hour. Samples were then incubated for 0, 2, 4 or 6 days at room temperature and recovered in 900 μL of EBSS 1X buffer. Controls with PBS 1X and samples in solution were used to determine loss during recovery. Finally, viral titer was determined by RT-qPCR and plaque assays. All experiments were performed in triplicate.

Results: For HuNoV and MS2 RNA detected by RT-qPCR, both viruses persisted in vomitus, in PBS solutions and on stainless steel coupons. For MS2 in solution using plaque assays for detection, the virus persisted in both vomitus and PBS similarly. However, on stainless steel coupons, there was no detection at day 2 in vomitus (2-way ANOVA, $p = 0.0004$), but MS2 was still detected in PBS 1X after 6 days. For MNV-1 in solution, the virus persisted both in vomitus and in PBS similarly. However, on stainless steel coupons, MNV 1 persisted longer in vomitus with no detection at day 2 in PBS 1X ($p < 0.0001$) and at day 4 in vomitus ($p = 0.0007$).

Significance: This project enhances understanding the impact of vomitus in the persistence of HuNoV and the importance of proper disinfection following vomiting episodes.

Award Application: Developing Scientist Award

P2-39: UTILISING THE CAPABILITY, OPPORTUNITY, AND MOTIVATION (COM-B) MODEL OF BEHAVIOUR TO IDENTIFY BARRIERS AND FACILITATORS FOR A ROBUST FOOD SAFETY CULTURE

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Food Safety Culture is pivotal to the success of organizations within the food industry, ensuring compliance to safety standards, efficiency and reputation. Despite its importance, achieving a strong food safety culture remains challenging due to various organizational and individual factors, including limited resources and varying levels of commitment across teams.

Purpose: This research explored industry and academic experts' perceptions of what contributes to the success or failure of food safety culture in food businesses. The study also investigated barriers and facilitators to achieving a robust food safety culture and provided insights into how organizations can better support food safety practices.

Methods: Twenty-three qualitative, semi-structured interviews were conducted with experts in the field. Data analysis was guided by the Capability, Opportunity, and Motivation (COM-B) model of behaviour. This provided a structured approach to identify critical factors affecting food safety culture.

Results: 'Capability' was a significant barrier, with participants citing a lack of knowledge, understanding, and the perception that food safety culture is intangible and unachievable. Training, education, and clear communication emerged as critical facilitators to enhance capability. 'Social opportunities' and 'physical opportunities' presented both barriers and facilitators. Language barriers, cultural differences, and resource constraints such as time, staffing, and finances hindered progress. Conversely, fostering inclusive communication, providing resources, and adapting strategies to organizational needs created

opportunities for food safety culture to thrive. Both automatic reflective motivations influenced food safety practices. 'Automatic motivation', such as ingrained safety habits, and 'reflective motivation', such as continuous evaluation and improvement, were highlighted as key facilitators. Barriers included challenges in maintaining focus on food safety goals and aligning them with organizational values.

Significance: This study highlights the complexity of fostering food safety culture, emphasizing individual, social, and organizational factors. Findings offer recommendations for addressing gaps in motivation, resources, and leadership to improve performance and compliance.

P2-40: CONSUMER FOOD-SAFETY AND NEURODIVERSITY: IDENTIFICATION OF BEHAVIOURAL CHALLENGES

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Presentation type/Format: Poster

Category: Communication, Outreach and Education*

Abstract:

Introduction: Neurodiversity is defined as the range of differences in individual brain function/behavioural traits, regarded as part of normal variation among consumers. Neurodiverse tendencies have been associated with food-related challenges; however, little consideration has been given to food-preparation behaviours associated with home food safety.

Purpose: To align and consider domestic food-safety behaviours with neurodiverse conditions to understand consumer challenges during home food preparation and inform targeted food-safety education.

Methods: A review of neurodiverse health-related literature and consultation with neurodiverse professional experts facilitated an in-depth understanding of key neurodiverse conditions, traits, and strengths. Such tendencies were aligned with UK/US-recommended food-safety practices and considered in the context of current/key consumer food-safety education resources.

Results: Findings have highlighted that neurodiverse people may experience challenges with “communication, coordination and learning” that are directly applicable to implementation of food-safety behaviours required for reducing the risk of foodborne illness during food preparation in the home. Identified tendencies of common neurodiverse conditions (autism, ADHD, dyslexia, dyscalculia and dyspraxia) that may impact domestic food-safety behaviours included ‘sensory sensitivities’, ‘executive functioning’, ‘communication challenges’, ‘preference for routine’, ‘hyperfocus’ and ‘cognitive rigidity’. For example, ‘sensory challenges’ (smell, noise and texture of different foods) may affect hand contact with foods/glove wearing and avoidance of handwashing and resultant risks of microbial cross-contamination. Examples of ‘cognitive rigidity’ and ‘communication’ may align with taking on board others’ thoughts/opinions

and understanding written instructions, thus may impact adherence with recipe or manufacturers' cooking (time/temperature) instructions intended for cooking efficacy and thermal inactivation of pathogens.

Significance: Aligning risk-reducing domestic food-safety behaviours with neurodiverse conditions/tendencies will be valuable to educators, caregivers and health professionals and inform development of practically applicable, targeted education approaches, tailored to the unique needs of neurodiverse consumers.

P2-41: NOURISHING WITH CARE: SAFE FOOD HANDLING KNOWLEDGE AND BEHAVIORS OF VIRGINIAN CAREGIVERS OF CHILDREN UNDER FIVE

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Category: Communication, Outreach and Education*

Abstract:

Introduction: The CDC estimates that roughly 48 million people in the US will experience foodborne illness annually. Children are particularly at risk due to their underdeveloped immune system, inability to prepare their own food, and smaller size thereby requiring a lower infectious dose. Primary caregivers have an important role in helping protect children from foodborne illness.

Purpose: We explored the food safety knowledge, perceptions, and behaviors of caregivers of children under age 5. The information will be used to develop targeted educational programs and inform evaluation to measure changes in knowledge, perceptions, and behaviors.

Methods: Rooted in the Health Belief Model (HBM), we conducted a survey of 318 Virginians who self-identified as a primary caregiver for a child under 5 (VT IRB 23-541). The Survey collected qualitative data, analyzed using open coding, and quantitative data, analyzed using Chi square tests. Type II error was accounted for by using the Benjamin Hochberg correction formula.

Significance was determined by $p < 0.05$ after adjustments.

Results: The HBM-based questions showed inconsistencies in how respondents correctly identify a child at high risk for foodborne illness or know all the symptoms of foodborne illness. They also were not completely confident in safely preparing food; only half of respondents reported always washing their hands and cleaning surfaces. Having a lack of information was cited as the most common barrier to safe food handling, and most people reported trusting government sources. More than half of respondents received a failing score ($< 70\%$) on a food safety questionnaire, indicating a need for better education.

Significance: Results document educational needs and provide vital information that can inform new programs delivered through Cooperative Extension. programs can utilize constructs of the HBM to more effectively influence knowledge, perceptions, and behavior change.

P2-42: SURVIVAL OF NOROVIRUS AND ESCHERICHIA COLI IN PREHARVEST AGRICULTURAL WATER

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Presentation type/Format: Poster

Category: Food Defense

Abstract:

Introduction: Human norovirus (HuNoV) is the leading cause of foodborne illnesses in the United States and is characterized by its high infectivity and environmental persistence. Sewage-contaminated water can increase the chance of microbial transfer to fresh produce during production.

Purpose: To evaluate the survival of norovirus and *Escherichia coli* (as an enteric bacterial model) in agricultural water.

Methods: Preharvest agricultural water samples collected from a Florida farm (treatment) and phosphate-buffered dilution water (PBDW, control) were inoculated with HuNoV GII, its surrogate Tulane virus, and *Escherichia coli* TVS 353 at $5.9 \log_{10}$ Genome copies (GC)/mL, $6.0 \log_{10}$ TCID₅₀/mL, and $7.0 \log_{10}$ CFU/mL, respectively. Samples were stored at 12°C for 21 weeks and tested at various time points with three biological and two technical replicates. Viral quantification was performed with RNase RT-qPCR. Infectious Tulane virus particles were estimated via TCID₅₀ assay, and *E. coli* survival was quantified using plate counts on rifampicin BHI agar.

Results: HuNoV and Tulane virus $\log_{10}$ GC/mL declined at similar rates within each matrix ($p > 0.01$) in agricultural water (first *D*-value of 2.35 ± 0.46 weeks) and control group (first *D*-value of 9.74 ± 0.63 weeks). Pearson's product-moment correlation analysis showed a strong relationship between HuNoV and Tulane virus inactivation ($r = 0.87$, $p < 0.01$). Using TCID₅₀, the Tulane virus demonstrated the first *D*-value of 0.37 ± 0.37 weeks in agricultural water and 2.62 ± 1.70 weeks in the control group. Both inactivation kinetics for infectious Tulane virus showed a tailing at $1.42 \log_{10}$ TCID₅₀/mL weeks 11 to 21. In agricultural water, *E. coli* showed an initial 1-log reduction in 8.7 ± 1.37 weeks.

Significance: This study provides laboratory-based data on the persistence of norovirus and *E. coli* in agricultural water, aiding growers, regulators, and public health officials in improving water management, setting standards, and mitigating risks.

Award Application: Developing Scientist Award

P2-43: ENSURING SEAFOOD SAFETY: A NOVEL RECOMBINASE AIDED AMPLIFICATION (RAA) COUPLED WITH CRISPR/CAS12A FOR AUTHENTICATION OF COMMERCIALY IMPORTANT PACIFIC OYSTER

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Presentation type/Format: Technical

Category: Food Fraud

Abstract:

Introduction: Seafood fraud, including species substitution and mislabeling, has become a widespread problem in the global seafood market. This practice not only undermines consumer trust but also has severe economic and ecological implications, which ultimately compromise seafood safety and traceability.

Purpose: With half of global oyster consumption, Pacific oyster (*Crassostrea gigas*) of its native range from Japan and northeast Asia holds considerable and commercial importance in the seafood industry. However, they are susceptible to fraudulent practices by substitution with other lower-valued *Crassostrea* species due to relatively similar appearances. Therefore, efficient and accurate identification techniques for oyster species must be developed to ensure precise labeling and prevent fraudulent activities.

Methods: In this study, a species-specific RAA (recombinase-aided amplification) assay targeting the COI gene of Pacific oyster was developed and coupled with the CRISPR/Cas12a system to generate a fluorescent signal detectable by handheld blue LED light.

Results: The developed RAA assay provided accurate results at 40°C for 25 min, followed by CRISPR/Cas12a digestion at 37°C for 40 min. The combination of RAA-CRISPR/Cas12a showed high specificity with no cross-reactivity against two non-targeted *Crassostrea* oysters and demonstrated greater sensitivity up to DNA concentration 10^{-5} ng/reaction. The developed assay also showed greater sensitivity in cooked Pacific oysters for in-house validation using boiling, steaming, frying, and canning at different time intervals. Further validation with commercial oyster products revealed 6.7% mislabeling.

Significance: In conclusion, the RAA coupled with CRISPR/Cas12a presents a promising solution to the issue of seafood fraud. Their speed, precision, user-friendliness, and cost-effectiveness make them valuable tools for ensuring the

integrity and safety of the seafood supply chain.

Award Application: Developing Scientist Award

P2-44: PREVALENCE OF ADULTERATION OF GROUNDNUT PASTE AND POWDERED PEPPER SOLD AT MARKETS IN THE GREATER ACCRA REGION OF GHANA

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Presentation type/Format: Poster

Category: Food Fraud

Abstract:

Introduction: Food is a basic need. However, food products are prone to adulteration with cheaper ingredients or materials. Food adulteration is done intentionally or unintentionally.

Purpose: This research investigated consumer knowledge, perception, and prevalence of food adulteration (in groundnut paste and powdered pepper) and identified the impact of adulterants on the quality of these food products.

Methods: A cross-sectional survey involving 398 participants was conducted using a structured questionnaire to collect information on consumer knowledge and perceptions of food adulteration. A total of 30 samples of groundnut paste and 30 samples of ground pepper were collected from six (6) markets in the Greater Accra region of Ghana. Various standard techniques including FT-IR were used to investigate adulteration levels in these food products. Different analytical techniques were also used to determine the effect of adulteration on the physico-chemical properties of the products (groundnut paste and powdered pepper).

Results: The survey found that consumer awareness and knowledge of food adulteration was very low (11%). In general, the prevalence of food adulteration with groundnut paste and powdered pepper was about 70%, with most market samples showing high evidence of additive substitution (from the FTIR spectral analysis). Also, adulteration affected the physicochemical properties of the groundnut paste and powdered pepper.

Significance: Intensive public education about food adulteration and its effects on health is recommended so consumers become aware of these fraudulent activities and are more cautious when purchasing certain food products. Market surveillance of food products and enforcement of food regulation laws should be carried out regularly so that fake food products can be removed from the market and the culprits punished. Ultimately, this will protect the public from the adverse consequences of consuming adulterated food and protect their health.<

P2-45: ASSESSING HYGIENE PRACTICES OF KOREAN FRESH PRODUCE FARMS FOR THE IMPLEMENTATION OF FSMA PRODUCE SAFETY RULE

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Presentation type/Format: Poster

Category: Food Law and Regulation

Abstract:

Introduction: The recent recall of enoki mushrooms exported from Korea due to contamination with *Listeria monocytogenes* underscores the critical importance of hygiene management in fresh produce production and emphasizes the necessity of implementing the FSMA Produce Safety Rule.

Purpose: This study aims to evaluate the hygienic management practices of Korean fresh produce farms and identify the differences between the Korean GAP certification and FSMA regulations.

Methods: A comparative analysis was conducted between the Korean GAP and the U.S. FSMA to identify regulatory differences. A survey using a 5-point Likert scale was developed, where a score of 5 indicated excellent management and 1 indicated inadequate management. The survey included 99 items across 7 fields and was administered to fresh produce farms in Korea (n = 196). The collected data were analyzed to assess the necessity of implementing the FSMA Produce Safety Rule.

Results: The survey showed that farms with GAP certification (n=130) had an average score of 3.38, which was higher than those of non-certified farms (n=66, 2.79). When considering all farms, including GAP-certified farms, the overall average score was 2.91. Notably, the scores for agricultural water management (2.55) and record-keeping (2.51) were below this overall average, indicating that these areas require significant improvement, even among GAP-certified farms. These deficiencies among certified farms highlight critical areas for improvement to align with FSMA regulations.

Significance: This study compared Korean GAP and FSMA regulations to investigate hygiene practices in Korean fresh produce farms. The findings emphasize the need to implement the FSMA Produce Safety Rule and offer foundational data for improving Korean farm hygiene management practices.

P2-46: THE EFFECT OF WATER ACTIVITY AND FAT CONTENT ON THE INACTIVATION AND RECOVERY OF LISTERIA SPP. IN DRY-CURED AND DRY-FERMENTED READY-TO-EAT (RTE) MEAT PRODUCTS AFTER HIGH PRESSURE PROCESSING (HPP): A REVIEW

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: High pressure processing (HPP) is an intervention approved by the USDA FSIS for controlling *Listeria monocytogenes* (Lm) in post-lethality exposed ready-to-eat (RTE) meat and poultry products. Its effectiveness at controlling Lm is affected by intrinsic factors of the food matrix, e.g., fat content and water activity (a_w).

Purpose: Assess the impact of fat content and a_w on the effectiveness of inactivating and preventing the recovery of *Listeria* spp. in dry-cured and dry-fermented meats by HPP.

Methods: The PRISMA-ScR reporting guidelines and the Arksey and O'Malley framework were used to conduct a scoping review. Initially, 986 articles focusing on HPP treatment of RTE meats for the control of Lm and *Listeria innocua* were screened, with 27 studies related to dry-cured and dry-fermented products reporting a_w and/or fat levels selected for detailed analysis. From these studies, data on microbial inactivation, recovery, and associated HPP and storage conditions were extracted and evaluated.

Results: Water activity values of fermented and dry-cured meats ranged from 0.784 to 0.983. As a_w increased, microbial inactivation also increased with rising pressure. Fat levels ranged from 10% to 55.1%. The data suggested that fat levels > 25% may exert a baroprotective effect, reducing the effectiveness of HPP treatment. In terms of recovery, findings indicated that $a_w > 0.9$ is associated with increased microbial growth during storage. Likewise, in dry-cured products, fat content > 25% promoted greater microbial recovery and growth post-HPP.

Notably, it was observed that dry-fermented products generally do not support *Listeria* growth over time, likely limiting recovery due to their intrinsic characteristics.

Significance: This review emphasizes the pivotal role of a_w and fat content in these products, as they greatly influence the effectiveness of HPP. Additionally, it highlights the importance of accounting for food matrix characteristics when selecting a post-lethality processing technology.

Award Application: Developing Scientist Award

P2-47: USE OF NATURAL ANTIMICROBIALS TO INACTIVATE SALMONELLA ON IN-SHELL PECANS

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: There are potential points of microbial contamination along the pecan production and shelling supply chain. Thus, effective treatment to inactivate pathogens such as *Salmonella* is crucial.

Purpose: This study evaluated the efficacy of clove bud essential oil (EO) and acids (ascorbic, citric, and lactic) as natural antimicrobial solutions for inactivating *Salmonella* on inshell pecans.

Methods: Antimicrobial solutions of EO (0.05%, 0.1%, and 0.2% v/v), and acids (Lactic, citric, and ascorbic at 0.2% and 0.3% v/v) alone or in combination were prepared in distilled water. inshell pecans inoculated with a cocktail of four nalidixic acid resistant *Salmonella* (~ 6.5 log₁₀ CFU/pecan) were treated with each solution for 1, 5, and 10 min. Treated, and control pecans were placed in bags containing 10 mL of 0.1% sterile peptone water and massaged for 1 min. Samples were plated on Tryptic Soy Agar containing 50 µg/mL nalidixic acid and enumerated. Experiments were conducted in triplicate with three replications (n = 9). Significance was determined by ANOVA and Tukey's test (P≤0.05).

Results: *Salmonella* reductions ranged from 0.39–0.90 log₁₀ CFU/pecan (ascorbic acid), 0.70–1.40 log₁₀ CFU/pecan (citric acid), and 0.48–2.18 log₁₀ CFU/pecan (lactic acid). Clove bud EO at 0.2% for 10 min resulted in a reduction of 2.68 log₁₀ CFU/pecan. Maximum reduction (3.94 log₁₀ CFU/pecan) was achieved with a combination of lactic acid (0.3%) and EO (0.2%) for 10 min of treatment. The results of this study indicated that both the concentration and treatment time significantly influenced the reduction of *Salmonella* (P ≤ 0.05) on inshell pecans. The antimicrobial solution remained effective for 30 days at ambient and refrigerated temperatures, maintaining a reduction of approximately 3.0 log₁₀ CFU/pecan.

Significance: Clove bud EO and lactic acid can serve as safer alternatives to

conventional chemical treatments effectively enhancing the microbial safety of inshell pecans.

P2-48: IONIZING RADIATION ENERGY INFLUENCES INACTIVATION OF *LISTERIA MONOCYTOGENES*

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Ionizing radiation is used in food and other industries for pasteurization or sterilization

Purposes, and the energy measured in million electron volts (MeV) could potentially impact the inactivation kinetics of pathogens.

Purpose: This study compared the inactivation kinetics of three strains of *L. monocytogenes* using Cobalt 60 (1.17 and 1.33 MeV), 1.4 MeV eBeam treatment, and 3 MeV eBeam treatment.

Methods: Three strains of *L. monocytogenes* (~ 8 log CFU/mL) suspended in PBS buffer were exposed to specific doses between 0.2 kGy and 2 kGy at commercial scale eBeam and gamma treatment facilities. The samples were triple-packaged in leak proof containers to meet biosafety protocols. Alanine dosimeters were used to record the actual measured doses. After exposure to the specific doses, the samples were serially diluted and enumerated on TSA agar in triplicates. The viable colonies were enumerated after 48 hours of incubation at 37°C.

Results: The D10 values were calculated for each strain of *Listeria monocytogenes* using three different ionizing radiation treatments. For Cobalt 60 gamma treatment, the D10 values for the three strains were 60 ± 5 Gy (ATCC 19115), 89 ± 2 Gy (ATCC BAA-839), and 94 ± 14 Gy (ATCC BAA-679). With 1.4 MeV eBeam treatment, the values were 400 ± 9 Gy (ATCC 19115), 300 ± 6 Gy (ATCC BAA-839), and 510 ± 2 Gy (ATCC BAA-679). The D10 values using 3 MeV eBeam treatment were 340 ± 15 MeV (ATCC 19115), 370 ± 35 Gy (ATCC BAA-839), and 680 ± 90 Gy (ATCC BAA-679).

Significance: Understanding the inactivation kinetics of foodborne pathogens under varying ionizing radiation energies has significant importance- therefore, an understanding of the energy used in commercial facilities and how it could influence the inactivation kinetics of the target pathogens is needed.

Award Application: Developing Scientist Award

P2-49: INACTIVATION OF LISTERIA MONOCYTOGENES ON ENOKI MUSHROOMS USING A SEQUENTIAL PEROXONE AND GAS PHASE HYDROXYL RADICAL PROCESS

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: There have been several high profile recalls/outbreaks linked enoki mushrooms contaminated with *Listeria monocytogenes*. Enoki mushrooms are especially challenging to decontaminate due to the long stalks arranged in clusters along with sensitivity to spoilage when wet.

Purpose: To optimize and validate a sequential decontamination method based on peroxone (hydrogen peroxide and ozone gas) and gas phase hydroxyl-radical process (UV-C, hydrogen peroxide mist, and ozone gas) to inactivate enoki mushrooms inoculated with *Listeria monocytogenes*.

Methods: Bunches of enoki mushrooms (125 – 250 g) were spray-inoculated with a five-strain cocktail of *Listeria monocytogenes*, ensuring the bacteria reached the inner stalks. The peroxone treatment was applied by electrospraying the hydrogen peroxide (1 – 3%) onto the mushrooms followed by a 1-10 min treatment with ozone gas (400 – 1000 ppm). The gas phase hydroxyl-radical treatment was applied within a continuous reactor that introduced hydrogen peroxide mist (1-3%), ozone gas (20 ppm) and UV-C (114 mJ/cm²). The *L. monocytogenes* was recovered from control and treated samples by slicing then homogenizing 25 g lots in saline before plating onto selective agar.

Results: The average *L. monocytogenes* loading was 5.43±0.53 log CFU/g mushrooms. The log reduction of *L. monocytogenes* by peroxone depended on the concentration of hydrogen peroxide and duration of ozone treatment with 3% hydrogen peroxide and 10 min ozone treatment supporting 1.15 log CFU/g reduction of *Listeria*. The hydroxyl-radical treatment alone supported a 1.62 log CFU/g reduction of *L. monocytogenes* with the combined treatment, resulting in a 3.85 log CFU/g that was significantly ($p<0.05$) higher than the individual

treatments alone. None of the applied treatments did not negatively affect the sensory or shelf-life of mushrooms.

Significance: A combination of peroxone and gas phase hydroxyl treatment can form part of a *L. monocytogenes* control proGram in enoki mushroom production.

Award Application: Developing Scientist Award

P2-50: ENSURING SAFE AND NUTRITIOUS BEETROOT BLENDED JUICE: EXPLORING THERMAL AND NON-THERMAL TECHNOLOGIES FOR LONGER SHELF LIFE

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Preserving the safety, quality, and nutritional value of beetroot blended juice is essential to meet consumer demand, and this study explores the potential of thermal and non-thermal technologies to extend its shelf life effectively.

Purpose: This study investigates the effectiveness of thermal and non-thermal technologies in extending the shelf life of beetroot-based blended juice.

Methods: Formulated and prepared samples were analyzed in response to the averaged value of OAA, pH, TSS and ascorbic acid contents of blended juices. Optimized formulated blended juice was heated up to 85°C, hot filled in pre sterilized 200 ml glass bottles and crown corked. Thermally and non-thermally processed blended juice was analyzed in response to detailed chemical and microbiological quality characteristics. The samples were stored for 60 days.

Results: The thermal and non-thermally treated juice characteristics were found in the range to consume even after storage. The optimal blended juice had a desirability value of 0.921, with 0.60% acidity, 14.17 mg/100 ml ascorbic acid, 93.94% antioxidant activity, and 8.07 OAA. It included 53.12 ml beetroot juice, 40 ml grape juice, 1.88 ml turmeric juice, and 5 ml lemon juice. Beetroot had 87.10% moisture, 0.43% fat, 1.37% ash, 1.78% protein, 1.47% fiber, and 7.51% carbohydrates, with 0.44% acidity, 4 °Brix, and pH 6.1.

Significance: This study optimized a safe, nutritious beetroot-based blended juice with high antioxidant activity and extended shelf life using thermal and non-thermal preservation technologies which can be helpful to the beverage industry to support sustainable goals.

Award Application: Developing Scientist Award

P2-51: EVALUATION OF A PILOT SCALE UV-C SYSTEM FOR THE INACTIVATION OF PERTINENT MICROORGANISMS IN PLANT-BASED BEVERAGES

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: The growing demand for safe, minimally processed plant-based beverages (PBBs) necessitates effective microbial control methods without compromising nutritional quality. UV-C technology has emerged as a promising non-thermal approach for microbial inactivation while preserving quality.

Purpose: To evaluate the efficacy of a pilot-scale UV-C system for inactivating pertinent micro-organisms in almond milk (AM).

Methods: A continuous-flow UV-C system was used to treat AM inoculated with *Escherichia coli* O157:H7 and *Salmonella* Enteritidis at concentrations $> 10^6$ CFU/mL. The absorbance of the test fluid was assessed using a double beam spectrophotometer. D10 value of microbial strains was quantified using bench scale collimated beam apparatus. The data was used to calculate the Reduction Equivalence Fluence (REF) delivered by the system. The system was operated at a 200 Liter/hr. (Dean Number > 950) flow rate, delivering a REF of ~ 20 mJ/cm². Microbial log₁₀ reductions were enumerated using selective media plating.

Results: AM exhibited high absorbance $> 12.76 \pm 1.16$ cm⁻¹, with diffuse reflectance $< 5\%$, signifying low scattering. The calculated D10 values, indicating the UV-C dose required for a 1-log reduction, were 2.97 mJ/cm² for *E. coli* O157:H7 and 3.8 mJ/cm² for *Salmonella* Enteritidis. The UV-C system achieved a 6.14 log₁₀ reduction for *E. coli* O157:H7 and a 5.06 log₁₀ reduction for *Salmonella* Enteritidis at a REF of ~ 20 mJ/cm². The system demonstrated robust efficacy in inactivating pathogens within an opaque fluid matrix, such as almond milk.

Significance: The pilot-scale UV-C system achieved over 5-log₁₀ reductions of significant pathogens in almond milk while maintaining product safety. These results validate UV-C technology as a promising non-thermal intervention for enhancing microbial safety in plant-based beverages (PBBs). Further optimization and scalability could broaden its applicability to other beverage matrices.

Award Application: Developing Scientist Award

P2-52: EFFECTS OF PLASMA-ACTIVATED MICROBUBBLE WATER (PAMW) TREATMENTS ON THE INACTIVATION OF ESCHERICHIA COLI ON FRESH PRODUCE

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Plasma-activated microbubble water (PAMW) combines the antimicrobial properties of plasma-activated water with enhanced surface interaction of microbubbles, offering an efficient, sustainable alternative to chemical sanitizers for mitigating cross-contamination in postharvest washing of fresh produce.

Purpose: To evaluate the application of a continuous PAMW system for the inactivation of *E. coli* on strawberries, blueberries, and wash water.

Method: A rod-to-mesh dielectric barrier discharge (DBD) reactor was used to ionize compressed air into gas plasma, which was then injected into a 2 L water container via a microbubble diffuser to generate PAMW. The flow rate was 2 SCFH, with the power supply operating at 36.4 kVpp and a 500 Hz discharge frequency. Strawberries (n = 36) and blueberries (n = 36) were spot inoculated with *E. coli* TVS355 and then suspended in PAMW, microbubble water (MBW), and distilled water (DW) for 10, 15, and 20 minutes. *E. coli* recovery from berry surfaces and wash water was enumerated on MacConkey agar plates with rifampicin (50 mg/L). Statistical analysis was performed using one-way ANOVA (p = 0.05).

Results: The average initial *E. coli* populations were 6.93 ± 0.33 log CFU/mL on strawberries and 6.49 ± 0.37 log CFU/mL on blueberries. PAMW treatment achieved a significantly higher reduction of *E. coli* by 1.47 ± 0.35 log CFU/mL on strawberries after 15 minutes, compared to 0.79 ± 0.04 log CFU/mL for MBW and 0.69 ± 0.01 log CFU/mL for DW. PAMW treatment resulted in a 1.87 ± 0.18 log CFU/mL reduction on blueberries after 10 minutes. PAMW reduced *E. coli* to undetectable levels (<1.0 log CFU/mL) in wash water, while MBW and DW had *E. coli* populations ranging from 3.39 to 5.26 log CFU/mL.

Significance: This research indicates that PAMW can effectively inactivate *E. coli* on fresh produce and prevent cross-contamination in wash water.

Award Application: Developing Scientist Award

P2-53: REDUCTION OF FERMENTATION DURATION OF CASSAVA TO REMOVE HYDROGEN CYANIDE

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Cassava is a widely consumed root crop that contains high levels of cyanide, which can be toxic if not properly processed, and inappropriate processing can lead to disease and even death. The long soaking period for cyanide removal in cassava also delays the farmers' ability to earn their income.

Purpose: This study aims to address the issue of high cyanide levels in cassava consumed in Rwanda. This was done by determining the effect of slicing, blanching, and soaking on the reduction of fermentation duration for hydrogen cyanide removal in cassava.

Methods: Fresh samples from five of the most produced and consumed cassava varieties in Rwanda were collected and subjected to various processing methods. Chemical analysis was conducted to measure cyanide and starch content in sliced (1, 2, and 5 cm size) samples treated in the following ways: (a) fermentation (soaked water) up to 7 days; (b) blanching (2, 5, and 10 min); and (c) blanching combined with fermentation. All samples were then dried and milled into cassava flour. The difference in cyanide levels was determined by comparison of means using LSD at the 5% level of significance.

Results: The study found that usual fermentation can reduce cyanide levels in cassava, but size reduction is recommended to facilitate the diffusion of hydrogen cyanide, achieving a reduction of 94.44% with significantly different ($p < 0.05$) total hydrogen cyanide to the safe level of consumption in four days. Blanching combined with fermentation was the most effective method, reducing cyanide levels by 95.56% with significantly different ($p < 0.05$) results in three days.

Significance: This research adds to the subject area by providing a cost-effective method for reducing cyanide levels in cassava. It highlights the importance of proper processing techniques to ensure the safety of cassava-based dishes.

P2-54: SUGAR ESTIMATION USING ABSORBANCE AS AN ALTERNATIVE AND RAPID APPROACH TO PREDICTING CHEMICAL OXYGEN DEMAND (COD) IN PRODUCE WASH WATER

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction :Monitoring chemical oxygen demand (COD) levels present in post-harvest wash water is critical for maintaining the safety of fresh produce, however the time required (~2-3 h) for traditional COD measurements can create a burden for wash operations.

Purpose:Since sugars can be released into the wash water because of tissue damage, this study investigated whether sugar estimation using the colorimetric Sulfuric Acid-UV spectrometry method (SA-UV method) can be a predictor of COD.

Methods:The SA-UV method relies on the reaction of concentrated sulfuric acid with the sugar standards (glucose, fructose, and sucrose) or produce wash samples (grape, cantaloupe, and pineapple) to produce their furfural derivatives followed by UV light absorbance read at 315 nm. Then the correlations between the absorbance and actual measured COD of sugar standards and produce wash samples were assessed using a linear regression model to define the coefficient of determination (R^2). A prediction model was then developed to predict COD from absorbance measurement. Accuracy of the method was assessed in terms of percentage recovery (r).

Results: Strong positive correlations between absorbance and measured COD were observed in the sugar standards (2-100 mg/L) with R^2 values of 0.97, 0.91, and 0.98 for glucose, fructose, and sucrose, respectively. For produce wash samples, positive correlations were observed with R^2 values of 0.82, 0.85, and 0.62, for grape, cantaloupe, and pineapple wash samples, respectively. Overall, we saw a high percentage recovery for the sugar standards at concentration ranges between 20-100 mg/L while lower accuracy was observed at lower concentrations (2-20 mg/L). High accuracy was observed at a COD range between 30-400 mg/L for all produce wash samples.

Significance: This alternative approach for estimating COD can be useful for produce operations for its speed, low-cost of reagents and supplies, and minimal exposure to toxic chemicals.

Award Application: Developing Scientist Award

P2-55: IDENTIFICATION OF PEPTIDE BIOMARKERS FOR SALTINESS AND UMAMI TASTE ENHANCEMENT VIA BACILLUS SPP. FERMENTATION OF SOY FLOUR AND OYSTER EXTRACT

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: The growing demand for natural taste enhancers in the food industry has prompted research into fermentation-derived bioactive compounds. This study explores the potential of *Bacillus* spp. to produce peptide metabolites that enhance saltiness and umami flavors.

Purpose: The objective of this study was to identify key peptides generated during the fermentation of soy flour and oyster extract by *Bacillus* spp. that contribute to saltiness and umami taste enhancement.

Methods: Seven *Bacillus* strains, isolated from traditional fermented foods, were screened based on proteolytic activity and confirmed safe for use as food ingredients. Fermentation experiments were conducted using soy flour and oyster extract under controlled conditions. Taste profiles of fermentation metabolites were analyzed using an electronic tongue, and peptides were identified and quantified through LC/MS and UPLC-Q-TOF-MS. Statistical correlations between peptide concentrations and taste attributes were assessed.

Results: Key peptides, anonymized as Peptide-A and Peptide-B, demonstrated strong positive correlations with saltiness ($r = 0.96$ and 0.92) and umami ($r = 0.74$ and 0.92), respectively. In contrast, inhibitory peptides such as Peptide-C and Peptide-D showed negative correlations with these taste attributes. Substrate-dependent variations were observed, with soy flour yielding a broader diversity of peptides, while oyster extract fermentation produced distinct umami-enhancing compounds. Additionally, γ -aminobutyric acid (GABA), a known umami enhancer, was significantly elevated under select fermentation conditions.

Significance: This study highlights the potential of *Bacillus* spp. fermentation as a sustainable method for developing natural flavor enhancers, providing an innovative approach to reducing reliance on conventional salt and umami sources in food applications.

P2-56: OHMIC HEATING ON THE INACTIVATION OF CLOSTRIDIUM SPOROGENES IN GREEN BEAN PUREE AND ITS EFFECT ON THE SENSORY ATTRIBUTES OF THE PUREE

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Conventional retort processing (CH) ensures food safety but often compromises quality. Ohmic heating (OH), which uses electric currents to generate heat, offers a promising alternative by providing rapid, uniform heating while retaining food quality.

Purpose: This study evaluated the efficacy of OH for inactivating *C. sporogenes* spores in green bean puree, comparing it with CH. Additionally, the sensory quality of OH-treated puree was analyzed to assess its potential for better retention of desirable attributes such as color and aroma.

Methods: Green bean puree was inoculated with *C. sporogenes* spores, and initial inactivation experiments were conducted at various electric field frequencies (60–5000 Hz) and temperatures (110–130°C) without a holding time using capillary tubes in a specialized ohmic cell. Optimized conditions were used to process puree in custom-designed laminated pouches. CH pouch studies were conducted in a retort (holding at 121.1°C for 3.7 minutes). Sensory evaluation involved descriptive analysis by a trained panel assessing color and aroma intensity on a 15-point scale across raw untreated, OH-treated, and CH-treated samples.

Results: OH consistently achieved significantly greater ($P < 0.05$) spore inactivation compared to CH at similar temperatures. For example, at 130°C in capillary experiments, OH reduced spore counts by 4.04 ± 0.0 log CFU/ml, whereas CH achieved only 0.88 ± 0.16 log CFU/ml. OH also demonstrated enhanced efficiency, reaching the target temperature in 190 seconds in pouch experiments compared to 23 minutes for retort processing. Sensory results indicated OH-treated samples retained significantly more green color and aroma

compared to CH-treated samples, though slight deviations from raw untreated samples were observed.

Significance: OH represents an effective and efficient alternative to conventional thermal processing for spore inactivation in low-acid foods. Its ability to achieve superior microbial safety with reduced sensory degradation makes it ideal for minimally processed, high-quality foods.

P2-57: DEVELOPMENT OF A SELF-SANITIZING SURFACE COATING WITH OLIVE POMACE EXTRACT TO REDUCE SURFACE CONTAMINATION IN FOOD PROCESSING ENVIRONMENT

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: The current sanitizing methods for food processing environments are resource-intensive and unsuitable for continuous sanitation of food processing surfaces. There is a high demand for effective and sustainable surface-sanitizing approaches, especially those using food-grade materials like olive pomace, which is rich in antimicrobial phenolics.

Purpose: To develop a self-sanitizing surface coating with crude extracts from olive pomace to reduce contamination on food processing surfaces.

Methods: Olive pomace extract (OPE) was prepared using sonication, mixed into gelatin (2%), and applied onto polypropylene (PP) or stainless steel (SS) coupons. The coating properties were characterized by various chemical, mechanical, and microscopical analyses. *Escherichia coli* O157:H7 survival on inoculated coupons was measured after dark incubation at 20 °C. Coating effectiveness was further assessed through a cross-contamination experiment using spinach leaves.

Results: Contact angle measurements showed that the OPE-gelatin coating increased hydrophobicity on SS compared to bare coupons ($P < 0.05$), suggesting reduced bacterial colonization. Scanning electron microscopy and energy dispersive spectroscopy revealed micron-sized salt crystals on the coated surface. The phenolic levels (225–260 μg gallic acid equivalent/ cm^2) remained unchanged during 1-month storage at 20 °C ($P > 0.05$). Peeling strength ranged from 13.89–24.4 N/cm^2 (25–50% of double-sided tape strength). The coating inactivated ~ 5 log *E. coli* O157:H7 within 10 minutes and remained effective (> 3 -log reduction) after 20 inoculation-treatment cycles. Compared to uncoated surfaces, OPE-gelatin significantly reduced cross-contamination from spinach to SS surfaces (> 4 log, $P < 0.05$) and from SS to spinach leaves (> 3 log, $P < 0.05$).

Significance: The significant antimicrobial effect suggests that the OPE-gelatin coating can reduce contamination on food processing surfaces and maintain its

efficacy over time.

P2-58: INVESTIGATING THE IMPACT OF SUBLETHAL ORGANIC PEROXIDE OR ALKALINE STRESS ON *SALMONELLA* THERMOTOLERANCE IN RAW, GROUND TURKEY

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: USDA-FSIS is evaluating enhanced regulation of *Salmonella* in poultry products, and the thermal resistance (and impact of stress thereon) of specified *Salmonella* strains may inform processing parameters.

Purpose: We evaluate the thermal resistance of *Salmonella* strains in raw ground turkey and the impact of prior organic peroxide or alkaline stress (3 or 24 h exposure) on thermal resistance.

Methods: *Salmonella enterica* strains (Hadar, Typhimurium, and Muenchen; identified as significant in raw turkey by USDA-FSIS) were grown in tryptic soy broth (TSB; 24 h at 37°C; repeated). Cells were harvested and resuspended in 0.1% peptone water (PW; non-stressed) or sterile DI water containing 30 ppm peroxyacetic acid (PAA stress) or 1.0 mM NaOH (alkaline stress), and permitted to rest (3 h or 24 h; 20°C). Suspensions were centrifuged again, the pellet resuspended in 10 mL PW, and cells were inoculated into raw, ground turkey (93% lean). Samples were vacuum sealed and stored for equilibration (24 h; 4.4°C) and then subjected to heat by submerging in a circulating water bath (60°C) for prescribed time periods. Following heat exposure, samples were immediately transferred to an ice water bath and cells were enumerated on xylose-lysine-deoxycholate with a thin tryptic soy agar overlay (XLD-TAL; 24 h at 37°C). Thermal death time curves and D-values were obtained and compared.

Results: Presence of stress was significant ($p=0.0300$) per ANOVA; however, non-stressed *Salmonella* had a D-value (0.38 min) similar ($p>0.05$) to all stress conditions. Difference was observed between 3 h NaOH stress (the largest overall D-value of 0.43 min) and 3 h PAA stress (the lowest overall D-value at 0.27 min; $p<0.05$; Tukey-Kramer HSD).

Significance: Exposure to organic peroxide or alkaline stress under the conditions here did not confer a change in thermal tolerance; however, the significance of stress in the overall analysis warrants further genetic, proteomic, and phenotypic analysis.

Award Application: Developing Scientist Award

P2-59: EVALUATION OF A LABORATORY-DESIGNED DIELECTRIC BARRIER DISCHARGE PLASMA SYSTEM FOR LISTERIA MONOCYTOGENES INACTIVATION AND QUALITY PRESERVATION IN READY-TO-EAT COLD-SMOKED SALMON DURING 30-DAY REFRIGERATED STORAGE

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Cold-smoked salmon (CSS) is frequently linked to listeriosis outbreaks, with *Listeria monocytogenes* posing serious food safety risks. Non-thermal technologies offer promising solutions to enhance microbial safety while preserving product quality.

Purpose: This study investigates atmospheric cold plasma (ACP) for the inactivation of *L. monocytogenes* in ready-to-eat (RTE) CSS, aiming for effective listericidal outcomes without compromising quality.

Methods: The CSS samples were inoculated with a *L. monocytogenes* cocktail (serotypes 1/2a, 1/2b, and 4b) and treated with ACP using helium gas at 4 SLPM flow rate and 7.80 kV for 0, 4, and 8 minutes. Treated and control samples were vacuum-sealed and stored at 4°C, with microbial enumeration on days 0, 15, and 30. Quality attributes, including pH, water activity, texture, color, water-holding capacity, and lipid oxidation, were analyzed using non-inoculated samples. All experiments were conducted in triplicate, with

Significance evaluated at $p \leq 0.05$.

Results: The ACP significantly reduced *L. monocytogenes* counts, with reductions increasing with longer treatment times (8 min > 4 min > 0 min). Despite microbial growth during storage, treated samples consistently maintained lower counts than controls, demonstrating ACP's potential for microbial inhibition and shelf-life extension. Quality attributes remained unaffected, as lipid oxidation levels showed

no significant difference between treated and untreated samples.

Significance: These findings highlight ACP as a promising non-thermal technology for enhancing the safety and extending the shelf life of RTE CSS. Future research should investigate alternative gases, various gas compositions, and the role of relative humidity in optimizing germicidal efficacy and maintaining product quality.

P2-60: UV-C AS A SUSTAINABLE AND EFFECTIVE SOLUTION FOR MICROBIAL INACTIVATION OF PLANT-BASED BEVERAGES

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Foodborne pathogens and spoilage microbes pose challenges for the beverage industry, particularly for low acid beverages such as plant-based beverages (PBBs). UV-C technology offers a non-thermal alternative to treat beverages, ensuring microbial safety (pasteurized equivalent products) with minimal impact on nutritional and sensory qualities.

Purpose: To assess the effectiveness of UV-C technology for microbial inactivation in almond milk (AM), oat milk (OM), and Next Milk (NM).

Methods: Optical properties of the test fluid and the microbial inactivation sensitivity (D10 value) were experimentally and mathematically determined to calculate the Reduction Equivalence Fluence (REF) delivered by the system. A custom-designed continuous flow UV system with a 40 W low-pressure mercury lamp (253.7 nm) was used for the experiments with a fixed flow rate of 515 mL·min⁻¹ (Dean number >100). Fluids inoculated with *E. coli* O157:H7, *Salmonella* Enteritidis, and *Saccharomyces cerevisiae* (~10⁷ CFU·mL⁻¹) were treated, and reductions were assessed via standard plate count. Data was analyzed against log-linear regression models.

Results: AM, with reduced scattering (6.08 cm⁻¹) and absorption (11.89 cm⁻¹), achieved 6.83 log₁₀, 7.06-log₁₀, and 4.89-log₁₀ reductions of *E. coli* O157:H7, *Salmonella* Enteritidis, and *S. cerevisiae* at a REF of 22.02, 21.65, and 48.45 mJ·cm⁻², respectively. OM (absorption 50.43 cm⁻¹) achieved a 4.83 log reduction of *E. coli* O157:H7 and, a 7.17 log₁₀ reduction for *Salmonella* Enteritidis, 1.69 log₁₀ for *S. cerevisiae* at a REF of 15.94, 25.49 and 13.43 mJ·cm⁻². NM (absorption 81.05 cm⁻¹) required 15.85 and 16.18 mJ·cm⁻² for 5-log₁₀ reductions for *E. coli* and *Salmonella* Enteritidis respectively. All models showed high linearity (R² > 0.96).

Significance: UV-C technology effectively inactivates pathogens in highly opaque fluids such as PBBs. UV-C technology represents a sustainable and effective solution for processing plant-based beverages, addressing consumer demands for high-quality, minimally processed.

Award Application: Developing Scientist Award

P2-61: THE EFFECTS OF ROASTING TIME AND DEFATTING METHODS ON THE PHYSICOCHEMICAL PROPERTIES AND SHELF-STABILITY OF INSTANT GROUNDNUT SOUP POWDERS

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: The drudgery, long processing time, and limited storability associated with the traditional method of groundnut soup preparation in Nigeria warrants more research.

Purpose: This study aimed to develop an instant groundnut soup powder (IGP), offering a more convenient and shelf-stable alternative to the use of raw groundnut in soup preparation.

Methods: Raw groundnut seeds were processed into five unique IGPs by combining different roasting times (5 and 15 min) and defatting methods (mechanical and/or solvent extraction). Flour functionality, as well as the sensory properties of cooked reconstituted IGPs, were evaluated. Rancidity indicators and microbial counts were also explored during 4 weeks of ambient storage. Data from duplicate experiments were subjected to Analysis of Variance at $p \leq 0.05$.

Results: Both prolonged roasting (15 min) and defatting operations led to significantly higher water absorption capacity (0.89–1.08 ml/g), oil absorption capacity (0.93–1.49 ml/g), and bulk density (0.72–0.88 g/cm³), compared to the application of short-time (5 min) roasting alone (0.54 ml/g, 0.42 ml/g, 0.51 g/cm³). All IGPs exhibited similar moisture contents (5–6%), but defatted samples were relatively denser in proteins (26.10–38.77%) than the undefatted sample (24.34%). Sensory evaluation revealed no significant difference between raw groundnut- and IGP-based soups. During storage, iodine values steadily dropped from 78.77–91.57 g/100 g iodine to 76.04–85.39 g/100 g iodine, while the rise in free fatty acid and peroxide values was most minimized by combined prolonged roasting and solvent-enhanced defatting. Throughout storage, total bacterial counts of all samples were within the permissible limits ($<5.0 \times 10^5$ CFU/g) for soup powders requiring additional heating.

Significance: The study highlights the roles of roasting and defatting in developing an acceptable IGP, representing enhanced convenience and value addition to groundnut soup preparation. Physicochemical and microbial analyses during extended storage periods are recommended to further assess shelf-stability.

Award Application: Developing Scientist Award

P2-63: EVALUATION OF THE EFFICACY OF UV-C (254 NM) ON LISTERIA INNOCUA AND NATURAL MICROFLORA FROM COFFEE BEANS IN COLD BREW COFFEE

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Cold brew coffee, made by steeping coffee in water for 6–24 hours at low temperatures, lacks a ‘kill’ step to destroy microorganisms. Sampling studies of commercial cold brew suggest a risk of spoilage and foodborne pathogens.

Purpose: To evaluate the antimicrobial efficacy of UV-C light (254 nm) against *Listeria innocua* and natural microflora derived from green coffee beans in cold brew coffee.

Methods: Cold brew coffee was prepared at a concentration of 55 g/L using ground coffee (<1000 μm) in deionized water and steeped for 18 hours at room temperature. To obtain naturally occurring microflora, 0.5 g of green coffee beans were incubated in 10 mL DI water at 25°C for 72 hours. *Listeria innocua* was cultured in TSB at 37°C for 24 hours. Both *L. innocua* and microflora were centrifuged and resuspended in 10 mL buffered peptone water. Coffee samples (29 mL) were inoculated with 1 mL of each culture. Samples were treated with UV-C dosages (0.3, 0.6, 1.2, 1.8 mJ), serially diluted, and plated on TSA. Chemical analyses were also performed. Studies were conducted in duplicate (n = 4).

Results: UV-C treatment resulted in dose-dependent reductions of *L. innocua*: 0.3 ± 0.2 , 0.8 ± 0.2 , 1.7 ± 0.4 , and 2.5 ± 0.9 log CFU/mL, and natural microflora: 0.1 ± 0.2 , 0.6 ± 0.0 , 2.1 ± 0.1 , and 3.2 ± 0.4 log CFU/mL for 0.3, 0.6, 1.2, and 1.8 mJ, respectively. Microbial reductions were positively associated with increasing UV-C dosage.

Significance: UV-C shows promise as a non-thermal intervention for cold brew coffee. Determining optimal dosages supports the development of a ‘cold pasteurization’ method to enhance microbial safety without heat processing.

Award Application: Developing Scientist Award

P2-64: SYNERGISTIC EFFECT OF MILD HEAT AND OLIVE POMACE EXTRACT FOR INACTIVATION OF BIOFILM FROM FOOD CONTACT SURFACES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Biofilms are a persistent risk factor in the food processing environment. The level of inactivation of bacteria in biofilms is significantly sub-optimal despite significant developments in antimicrobial sprays and coatings. This study evaluates a synergistic processing approach that combines moderate heat (sourced from low-grade waste heat) with aqueous extracts obtained from agricultural byproducts to effectively improve the inactivation level of bacteria in biofilms from food contact surfaces.

Purpose: To evaluate the efficacy of synergistic inactivation of bacteria in biofilms on food-contact surfaces using moderate heat and food-grade compounds

Methods: Biofilms formed by *Escherichia coli* 609 (environmental strain), *Listeria innocua* rifampicin resistant on polypropylene and stainless-steel surfaces were treated using water heated to 55°C. Olive pomace extract (OPE) at a concentration of 0.2 mg GAE/ml was added to the water heated to 55°C, and biofilm inactivation was evaluated. Biofilm inactivation was evaluated after 5 and 10 minutes of treatment time. These results were compared against conventional heat treatment and chlorine-based sanitation methods. The inactivation mechanism was evaluated using metabolic assays and scanning electron microscopy images.

Results: The treatment with OPE plus mild heat at 55°C for 5 minutes achieved over 5 log inactivation of bacteria in the biofilm formed by *E. coli* and *L. innocua* on both plastic and stainless-steel surfaces within 10 min. Mild heat treatment (55 C) alone resulted in significantly reduced levels of inactivation within 10 minutes. For *E. coli* only 0.96 ± 1.33 CFU of bacteria were inactivation, while for *L. innocua*, the inactivation levels were 1.05 ± 0.27 after 5 minutes and 3.91 ± 0.88 after 10 minutes. initial investigation into mechanisms involved in biofilm inactivation suggests enhanced membrane damage and metabolic inhibition of bacterial cells with the synergistic treatment.

Significance: This study highlights a novel approach to sanitation that aligns with the goals of enhancing biofilm inactivation for improved food safety while saving energy and reducing chemical use.

P2-65: OHMIC HEATING INACTIVATION OF ALICYCLOBACILLUS ACIDOTERRESTRIS IN APPLE AND CRANBERRY JUICE

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: *Alicyclobacillus acidoterrestris*, a thermophilic, spore-forming bacterium, poses a significant challenge to the fruit juice industry due to its resistance to conventional pasteurization, making it a critical concern for juice manufacturers. This study is investigating Ohmic heating (OH) as an alternative to conventional heating (CH).

Purpose: This study aimed to evaluate the efficacy of OH in inactivating *A. acidoterrestris* spores in apple and cranberry juices and compare its performance with CH.

Methods: An OH system was developed comprising a power supply, a function generator, and an ohmic heating chamber with platinized titanium electrodes. Juice samples inoculated with *A. acidoterrestris* spores were heated at varying field strengths (30, 40, and 50 V/cm) to target temperatures (90°C, 100°C, 110°C). CH experiments were performed using the same setup to match the heating rates of OH. Spore inactivation kinetics were evaluated using four mathematical models (log-linear, Weibull, Modified Gompertz, and empirical sigmoid).

Results: OH consistently outperformed CH in spore inactivation across all field strengths and temperatures. At 110°C, OH reduced spore levels to undetectable (< 5 log CFU/mL inactivation) for both apple and cranberry juices across all tested field strengths. In contrast, CH resulted in residual spore counts of 1.86 ± 0.17 and 1.94 ± 0.23 log CFU/mL in apple and cranberry juices, respectively. The Modified Gompertz model emerged as the most accurate for describing OH-mediated spore inactivation kinetics. Additionally, OH demonstrated enhanced inactivation at lower temperatures (90°C and 100°C) at higher field strengths (50 V/cm), a phenomenon not observed with CH. These findings underscore the

synergistic effects of rapid heating and electric fields in OH.

Significance: This study establishes OH as a superior alternative to CH for juice pasteurization. By achieving efficient spore inactivation with reduced thermal impact, OH offers a promising solution to produce safer, higher-quality juice products. The findings have significant implications for scaling OH in commercial juice processing, addressing industry and consumer demands for minimally processed beverages.

P2-66: DEVELOPMENT OF A NEW FERMENTATION METHOD (HELENIZATION) TO IMPROVE FOOD SAFETY IN FERMENTED VEGETABLES

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Traditionally fermented vegetable products are susceptible to high prevalence of antibiotic resistance (AR) and opportunistic pathogens.

Purpose: This study aimed to develop a novel fermentation process (Helenization), to address the inherited food safety and quality challenges associated with fruit and vegetable products by natural fermentation.

Methods: Three batches of kimchi and one batch of sauerkraut, with duplicates, were made with (1) Helenization, involving pre-treatment with an organic acid sanitizer followed by fermentation with dedicated OSU starter cultures (*Leuconostoc mesenteroides* and *Pediococcus spp.* strains susceptible to antibiotics), or (2) traditional spontaneous fermentation (control). The outcomes of the Helenization versus traditional fermentation were evaluated by pH, AR bacteria via culture method, microbial profiles, and AR gene pool via metagenomic assessments.

Results: pH dropped significantly lower in fermented kimchi produced by Helenization at Day 2 ($p < 0.001$), and Helenization took half of the time in both kimchi and sauerkraut to reach desired pH than those by spontaneous fermentation. Helenization significantly reduced ampicillin resistant bacteria in kimchi products by about 2.8 logs (from 5.54 ± 1.13 to 2.74 ± 0.63 , $p < 0.05$) by plate counting method. Helenization reduced the abundance of AR genes in sauerkrauts by 84% compared to parallel spontaneous samples (from 0.142 ± 0.037 to 0.023 ± 0.001) and by 53% to 76% in kimchi, compared to three retail products. Helenization batches also exhibited more uniform microbiota dominated by the starter cultures, whereas spontaneous fermentation exhibited variability, including the presence of opportunistic pathogens or spoilage bacteria such as *Escherichia*, *Pseudomonas*, *Pantoea*, etc. AR lactic acid bacteria such as *Leuconostoc mesenteroides*, *Latilactobacillus curvatus*, and *Pediococcus pentosaceus* were further isolated from the spontaneous fermented vegetables.

Significance: The results illustrated the efficacy of Helenization in improving the

food safety, efficiency and consistency of fermented vegetable products, including but not limited to addressing the challenge of AR.

Award Application: Developing Scientist Award

P2-67: WHAT IS THE PREVAILING FOOD SAFETY CULTURE AT A HOSPITAL CATERING?

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Food safety must be reinforced in hospitals due to the health vulnerability of patients to foodborne diseases.

Purpose: To evaluate the Food Safety Culture (FS-culture) at a hospital catering.

Methods: in this case study, a mixed-method approach assessed FS-culture in a hospital catering. The quantitative (questionnaires for managers and food handlers and two checklists based on risk assessment and infrastructure evaluation) and qualitative methods (participant observation) were applied according to eight FS-culture elements: leadership, knowledge, commitment, communication, risk perception, work pressure and normative beliefs, work environment, and management system, styles and processes. The method already described in the literature for food services was tested in the hospital context. Data were triangulated using a scoring system on an interpretation grid, which typified the prevailing FS-culture and its elements from reactive (score-1) to proactive (score-3). This research was approved by the Brazilian Ethics Committee (79676724.3.0000.5440).

Results: During 18 days, 108 individuals were assessed. The managers (15) had an average age of 41 years (SD: 14.94) and 93% had a bachelor's degree. The food handlers (93) had an average age of 47 years (SD: 13.82); 44% completed high school, but 43% had no training in food safety. The

Results showed a “reactive to active” (score-1-2) prevailing FS-culture. The element “work pressure and normative beliefs” showed the highest score (3-proactive), while “leadership”, “knowledge”, “work environment”, “risk perception”, and “management systems, styles, and process” indicated the lowest scores (1-2-reactive to active).

Significance: The method was useful to assess FS-culture in hospital catering. The findings emphasize the importance of fostering a proactive FS-culture through risk

perception, leadership and management system to ensure food safety. Furthermore, the results highlight a need for training for food handlers as a legal requirement in Brazil, and as a booster for increasing food safety knowledge.

Award Application: Developing Scientist Award

P2-68: COMPARATIVE EFFICACY OF HAND WASH LATHER TIMES OF 5 TO 20 SECONDS BY AN IN VIVO CROSS-CONTAMINATION TEST METHOD

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: Hand hygiene is fundamental in managing food safety risk. Understanding the efficacy of hand hygiene protocols is crucial for risk assessments, choosing interventions and establishing effective guidance. There is little published data measuring microbial reduction on hands after different hand wash durations.

Purpose: This study evaluated the in vivo effectiveness of a typical foaming hand wash at three different wash durations in reducing bacterial load and transfer to food items.

Methods: A leading commercially available non-antibacterial foam handwash was tested with one dose as delivered from an automatic wall mounted dispenser using ASTM E2784 ("Standard Test Method for Evaluation of the Effectiveness of Handwash Formulations Using the Paper Towel (Palmar) Method of Hand Contamination"). All twelve subjects performed the "Core Handwash" technique for 5-, 15- and 20-seconds with no other variables in a cross over design. Mean log₁₀ reductions of *Escherichia coli* (ATCC #10536) on hands as well as bacterial counts transferred to melon balls were measured. Analysis of variance was used to compare treatments.

Results: No significant difference ($p > 0.05$) in efficacy between lathering scrub times of 5-, 15- and 20-seconds was observed. The mean log reduction was 2.95 after 5-seconds, 2.86 after 15-seconds and 3.00 after 20-seconds. The mean log transfer to melon was also not different ($p > 0.05$): 4.85 after 5-seconds, 4.76 after 15-seconds and 4.64 after 20-seconds.

Significance: This study demonstrates that requiring hand wash times of at least 20-seconds as currently prescribed in most food safety guidance (including the FDA Food Code) may be unnecessarily long. A well-formulated hand wash product can provide substantial bacterial reduction in the common observed wash time of 5-seconds. Allowance of shorter wash times by foodservice employees could increase the number of hand washes per shift, thereby reducing overall foodborne illness risk.

P2-69: LESSON FROM THE STUDIES OF MICROBIAL ECOLOGY OF FERMENTED FOODS AND BEVERAGES

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Advances in sequencing technology and the relatively easy access to the use of bioinformatics tools to profile microbial community structures have facilitated a better understanding of both culturable and non-culturable microbes in different ecosystems. Many industries in the Western Hemisphere depend on the use of very few microbes for fermentation and bioprocessing, which neglects other important microorganisms with health-beneficial, sensory, and nutritional properties.

Purpose: This article highlights the importance of profiling both the microbiota and microbiome of fermented foods and beverages to maximize their benefits in food safety, quality, and gut health.

Methods: Two sequencing approaches are commonly used to study microbial community structures in fermented products: (1) 16S rRNA gene amplicon sequencing targeting variable regions, and (2) whole-genome metagenomic sequencing using shotgun technology. The process involves sampling during fermentation, followed by DNA or RNA extraction, amplification of target genes, high-throughput sequencing, sequence assembly, binning, annotation, and statistical analysis.

Results: Around the world, very few fermented foods are made using standardized starter cultures. As a result, many microbial *consortia* associated with traditional fermented products remain poorly understood. Metagenomic profiling enables a clearer understanding of *in situ* microbial dynamics and helps identify spoilage organisms, off-flavor contributors, and beneficial microbes through microbiome and metabolomic analyses.

Significance: Metagenomic methods offer a powerful approach to monitor microbial relative abundance, alpha and beta diversity during fermentation, providing valuable insights into how diverse microbes influence fermented food safety and quality.

P2-70: GENETIC CHARACTERIZATION OF A *CRONOBACTER SAKAZAKII* STRAIN FROM PARSLEY

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Category: Laboratory and Detection Methods

Abstract:

Introduction: *Cronobacter sakazakii* is an emerging opportunistic, Gram-negative, rod-shaped, facultative anaerobic, foodborne bacterium that can cause life-threatening meningitis and necrotizing enterocolitis in neonates, aging, and immunocompromised individuals. It can survive in extreme dry conditions and has been linked predominantly to contaminated powdered infant formula.

Nevertheless, it has also been isolated from a wide variety of foods. To date, whole genome sequencing (WGS) is established as a contemporary and complete tool for bacterial typing. It is now routinely used to analyze samples collected during foodborne outbreak investigations. In our previous studies, we have identified several unique *C. sakazakii* strains from fresh produce, spice powder, and ready-to-eat food samples by performing WGS analysis. Here, we describe the draft genome sequence of a *C. sakazakii* strain isolated from parsley.

Purpose: The objective of this study was to identify and characterize a *C. sakazakii* strain recovered from parsley by performing WGS analysis.

Methods: A *C. sakazakii*-like bacterial strain from parsley (SRL-87) leaf sample grown in Northwestern Europe was isolated and analyzed. Initial identification was completed using VITEK 2, real-time PCR assay, and MALDI-TOF MS analysis, following FDA's BAM techniques. WGS was performed on an Illumina MiSeq system using a Nextera XT DNA library preparation kit and a 250-bp paired-end read MiSeq Reagent v2 kit (500-cycle), following the manufacturer's suggested protocols.

Results: MALDI-TOF MS analysis provided species identification with high confidence value (>99%) for the *C. sakazakii* strain SRL-87. The genome sequence of SRL-87 was 4,334,236 bp in length, distributed across 60 contigs. WGS analysis revealed the sequence type as ST23 for the genome of *C. sakazakii* strain SRL-87.

Significance: The *C. sakazakii* ST23 strain has been isolated from whole milk powder before and reported to have strong desiccation tolerance. WGS methods can be applied for precise strain identification of foodborne *C. sakazakii*.

P2-71: AN ENRICHMENT-FREE METHOD FOR QUANTIFYING SALMONELLA IN RAW CHICKEN

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella* contamination in poultry poses challenges to public health. Enrichment-based detection methods introduce biases, compromise quantification accuracy, and extend time-to-results.

Purpose: This study evaluates an enrichment-free platform integrating the Pathotrak Next-Generation Extraction (NGE) system and droplet digital PCR (ddPCR) for the quantification of *Salmonella* in poultry.

Methods: Raw chicken samples were inoculated with varying levels of *Salmonella* and processed using the Pathotrak NGE system for bacterial separation and concentration. Whole-cell preparations and extracted DNA were compared for detection efficiency. Assay flexibility was tested across multiple poultry matrices, including chicken breast, wings, and thighs, with data benchmarked against conventional microbiological plating. Statistical methods were used to correlate results with theoretical inoculation levels. Performance across matrices and contamination levels were evaluated to validate the platform's accuracy and robustness.

Results: Whole-cell preparations consistently detected more copies of *Salmonella* than extracted DNA at the same inoculation levels in both media and poultry

samples. Recovery rates across various raw poultry matrices ranged from 7.1% to 20.8%, demonstrating the assay's ability to accommodate a variety of matrices. Correlation between ddPCR measurements and theoretical inoculation levels was observed ($R^2=0.903$, slope=0.99). Statistical analysis confirmed the method's accuracy and robustness across varying contamination levels and sample types.

Significance: This enrichment-free method reduces detection time significantly, processing 96 samples in approximately 6 hours. By eliminating the enrichment step, the platform reduces bias introduced by competitive growth of bacteria, and it opens the possibility for multiplexing. Furthermore, the results were found to be comparable to the Most Probable Number (MPN) method, demonstrating the platform's potential for regulatory and industrial applications.

P2-72: ASSESSMENT OF ISO QPCR METHOD FOR DETECTING CAMPYLOBACTER IN SURFACE WATER

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Surface water used for irrigation has been found to contain pathogenic *Campylobacter* spp. that could potentially be transferred to food crops and thus humans or domestic animals. Thermotolerant *Campylobacter* spp., specifically *C. jejuni*, *C. coli*, and *C. lari*, account for most cases of human campylobacteriosis. In 2023, International Organization for Standardization (ISO) approved PCR-based methods for the detection and confirmation of thermotolerant *Campylobacter* spp. recovered from meat, produce, and raw milk. A method extension for surface water is needed.

Purpose: To validate ISO real-time PCR methods for the detection and confirmation of *Campylobacter* spp. in surface water.

Methods: Surface water samples (100 L) were spiked with 9, 17, and 43 CFU of *C. jejuni* ATCC 33291 (n = 3 per spike level) and filtered using dead-end ultrafiltration. Ultrafilters were backflushed and eluates were centrifuged. Recovered pellets were enriched in Preston broth under microaerobic conditions. DNA samples extracted from aliquots of the enrichments were used as templates for the ISO *Campylobacter* 16S rRNA real-time PCR assay. In parallel, the enrichments were plated on selective agar to recover single colony isolates to identify *Campylobacter* species using the ISO multiplex real-time PCR assay.

Results: The real-time 16S rRNA PCR was able to detect *Campylobacter* spp. in the primary enrichments. At the low spike level (9 CFU), two of the three samples were PCR-positive whereas all samples were PCR-positive at the higher spike levels (17 and 43 CFU) and this observation corroborated with positive colonies on

an isolation agar. Although false negatives can be a challenge, due to insufficient enrichment or background inhibition, this assay is useful for screening samples. Further, the multiplex PCR method was discriminatory and was able to correctly identify the spiked *Campylobacter* spp. A validation of the targets using exclusivity and inclusivity panels is in progress.

Significance: The real-time PCR assay(s) will provide a faster and more robust platform for the identification and detection of thermotolerant *Campylobacter* spp. recovered from surface water. This method can also be used as an alternative to biochemical-based methods.

P2-73: A LIQUID CRYSTAL BASED TEST KIT FOR ESCHERICHIA COLI O157 IN VARIOUS FOODS

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: A low infectious dose and high number of associated foodborne illness make *Escherichia coli* O157:H7 one of the most important targets in pathogen testing.

Purpose: in this study, a rapid detection assay, using the optically captured birefringence of the light path through a liquid crystal array, was developed for a sample-to-result robotic platform to identify *E. coli* O157:H7 in meat and fresh produce.

Methods: For inclusivity and exclusivity tests, 42 strains of *E. coli* O157 and 45 different bacteria were tested. For food sample testing, 325g, 325g, and 200g of raw ground beef, beef trim, and raw spinach, respectively, were inoculated with a high level (3.4 to 10.1 CFU) and a low level (0.5 to 1.5 CFU) of *E. coli* O157. inoculated food products were enriched with appropriate amount of modified Tryptic Soy Both with novobiocin (mTSB+n) (1L for meat and 600 mL for spinach) for 9.5 to 18 hours. Samples of enrichment were collected and loaded into the Crystal Diagnostics Accupath™ with APECO157 test kit and processed according to the user's manual. Chrom™ O157 agar was used for culture confirmation for all food samples with positive or negative results.

Results: The results for testing *E.coli* O157 in raw ground beef, beef trim, and raw spinach were 100%, 98%, and 100% accurate, according to the comparison with culture method. The developed test kit was also able to correctly identify all 42 inclusivity strains and 45 exclusivity stains. Test results were deemed positive when all three replicate tests were positive.

Significance: The Crystal Diagnostics™ AccuPath™ APECO157 test kit was proved to be an rapid and reliable method to test for *E.coli* O157 in food samples.

P2-74: VALIDATION OF SALMONELLA RECOVERY USING DEUF IN ENVIRONMENTAL WATERS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Dead-End Ultrafiltration (DEUF) filters are widely used in environmental food safety studies to recover pathogenic bacteria like *Salmonella* from irrigation and surface waters. Bacteria are eluted from the filters using a backflush solution (BF). Understanding survivability on/in the filters and BF is important to ensure accurate detection of live pathogens.

Purpose: To assess *Salmonella* survival in BF solutions and DEUF filters stored at 4°C over time, focusing on solution composition, natural contamination, and filter effects.

Methods: Daily plate counts on modified tryptic soy broth-yeast extract (mTSA-YE) were performed for all studies. BF Composition: five BF solutions (complete BF, Tween80, NaPP, Y-30, and DI-H₂O) were inoculated with *Salmonella* Gaminara GFP 5695 and plated daily to assess survival. inoculated BF: BF eluate from surface water DEUFs (n=3) as well as one control (DI-H₂O) were inoculated with GFP *Salmonella* and stored at 4°C and monitored daily. Filter storage: DEUFs (n=30) filtering surface water were inoculated with *Salmonella* Gaminara (10⁶ CFU/mL) and stored at 4°C. Filters were backflushed daily (three positive, one control) and eluate plating was conducted over six days.

Results: BF Composition: Two-way ANOVA revealed no significant effects of treatment (p=0.907), time (p=0.266), or their interaction, suggesting stable *Salmonella* concentrations in all BF solutions. inoculated BF: Time significantly affected *Salmonella* concentration (p=0.014), showing a log reduction from Day 2 (5.31) to Day 8 (3.70). Background bacterial counts also declined significantly, with nearly a full log reduction from Day 2 (5.61) to Day 8 (4.73). Filter storage: Gaminara showed consistent log CFU/mL (average=3.74), while background flora declined over time (average=3.14).

Significance: These findings demonstrate *Salmonella*'s stability during storage on DEUF filters and in complete BF solution in contrast to the background microflora, giving more flexibility of storage time before analysis for live pathogen detection.

Award Application: Developing Scientist Award

P2-75: BACGENE GO FOR SALMONELLA DETECTION FROM FOOD AND ENVIRONMENTAL SURFACES

Primary Author:

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Salmonellosis is one of the most common foodborne diseases and besides the severe effects on public health, the presence of *Salmonella* in food has a significant effect on the economy. Well over 2000 different serovars of *Salmonella* have been described and regulations consider each one as a potential human pathogen, therefore the need for rapid, accurate and reliable test methods is higher than ever.

Purpose: This study presents a validation of the first in a new generation of real-time PCR kits designed to improve user convenience and reduce errors. Method acceptance requires that the new method performs at least as well as the recognized reference method.

Methods: The performance of the BACGene GO *Salmonella* method was validated according to current AOAC OMA Appendix J Guidelines and best practices, and in harmonization with ISO 16140-2:2016 guidelines. The reference method was ISO 6579-1:2017. The validation comprised inclusivity and exclusivity testing; matrix studies for 25 g of deli salad, raw ground beef, bagged lettuce, and infant formula with probiotics, 25 mL of raw milk, and 10 cm x 10 cm of stainless steel, and ceramic tile, as well as robustness, product consistency and cyler 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 cyler variability tests all gave the anticipated results, showing the method to give reliable answers in all tested circumstances.

Significance: An easier-to-use method with all reagents ready-to-use and an automated result interpretation for detection of *Salmonella* offers potential to contribute to reducing the incidence of salmonellosis while helping to improve efficiency in food safety laboratories.

P2-76: NEW GRAM-POSITIVE LYSIS BUFFER FOR THE ISOLATION OF LISTERIA DNA

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The digestion of Gram-Positive bacteria such as *Listeria* is particularly challenging due to the thick multilayered and crosslinked peptidoglycan cell wall. Most current methods rely on heat lysis at 95°C or mechanical lysis to overcome this difficulty, but these solutions require extra equipment to perform: a heating apparatus or bead beater respectively. We developed a lysis buffer for gram-positive bacteria that uses an enzymatic lysis to isolate *Listeria* DNA with a simple incubation step.

Purpose: To develop a robust lysis method for Gram-Positive Bacteria.

Methods: Pure cultures of *Listeria monocytogenes* were tested to determine the lysis assay parameters. Cultures were inoculated with *L. mono* and incubated in 225L of Actero *Listeria* media at 30°C for 22h. Enzyme concentration was determined by comparing 1:5, 1:10, 1:12.5, 1:15, 1:20, 1:17.5, 1:20, and 1:40 dilutions of enzyme with a no-enzyme control. Results were measured by qPCR and delta Ct was calculated. The timing of the incubation was optimized by testing incubation at 0m, 15m, and 30m. A surfactant was added to stabilize the reaction and further aid in the breakdown of the *Listeria* cell wall. The new lysis buffer was tested against the current lysis buffer in a paired study design in salmon and spinach food matrices to demonstrate efficacy.

Results: The final formulation was determined and a 15-minute incubation at 60°C was selected. A range of incubation times from 15 minutes to 30 minutes was established. The enzyme mixture was shown to be stable at 25°C for 4 months. The new lysis buffer shows 3-8 Ct enhanced sensitivity over the original lysis buffer by qPCR analysis.

Significance: These data demonstrate that we can lyse *Listeria* in 15 minutes at 60°C with our new buffer, allowing for detection by qPCR and enhancing the

sensitivity of our pathogen detection assay.

P2-77: ENRICHMENT-FREE DETECTION AND DISCRIMINATION OF LISTERIA SPP., LISTERIA MONOCYTOGENES AND SALMONELLA SPP. FROM ENVIRONMENTAL SPECIMENS OBTAINED BY STANDARDIZED SPONGE COLLECTION

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Listeria* and *Salmonella* contamination pose a significant risk to food safety and have been the focus of enhanced regulatory scrutiny within the USDA. Enrichment based approaches to detect and discriminate *Listeria* and *Salmonella* are labor intensive, can lead to bias, and require off-site testing that limits turnaround time. To mitigate these challenges, PathogenDx developed and validated Enviro^{X-F} (PTM 092001), an enrichment-free, high throughput, array based nucleic acid test for the simultaneous detection and discrimination of *Listeria* spp, *Listeria monocytogenes* and *Salmonella* spp.

Purpose: The purpose of this study was to extend the matrices to include sponges that are more typically used in environmental monitoring.

Methods: To perform the matrix extensions, stainless steel and plastic surfaces were inoculated at three levels (0 CFU, 0.2-2 CFU, and 5-10 CFU/1sq.ft.) with *Listeria monocytogenes* and *Salmonella typhimurium*, then allowed to air dry. Samples were then recovered by sponging with a moistened 10mL World Bioscience EZ Reach Sponge. Then placed in 20mLs of HiCap™ Neutralizing Broth. The sponge was stomached, and the entire unenriched volume was concentrated by centrifugation and the resulting pellet lysed at 95C in 50µL of a lysis buffer. Multiplex PCR amplification was performed with 5µL of template, followed by automated array hybridization and autonomous array data analysis. The reference method for the matrix studies was the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (BAM) Chapter 5 for *Salmonella* and BAM

Chapter 10 for *Listeria* and *Listeria monocytogenes*.

Results: According to AOAC Appendix J, statistical analysis was performed using Probability of Detection (POD) statistical model. Results indicated no statistically significant difference was observed between the candidate and reference method.

Significance: An unenriched, multiplex array-based assay that has been designed to provide fast (unenriched, sample-answer in <6hrs), automated, inexpensive, high throughput detection and discrimination of *Listeria* and *Salmonella* in food processing operations.

P2-78: EVALUATION OF NEXT GENERATION SEQUENCING ASSAYS FOR DETECTION OF BACTERIAL PATHOGENS IN PROBIOTIC PRODUCTS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: With the growing use of probiotics products, a key challenge is ensuring their safety by detecting trace amounts of harmful microbes in the presence of high levels of the microbial components.

Purpose: To evaluate accuracy of three next generation sequencing assays in detecting low levels of spiked microbial pathogens in probiotics.

Methods: Probiotic products which contained combinations of varying bacterial species were spiked separately with *Salmonella enterica*, *Escherichia coli*, and *Listeria monocytogenes* strains at levels ranging from 20 to 2×10^9 CFUs. Probiotic 1 contained only *Lactobacillus rhamnosus*, probiotic 2 contained four species, and probiotic 3 contained fifteen species. We used three sequencing assays: Illumina's NexteraXT for whole metagenome sequencing (WMS), a Microbial ID assay (CDMI) which is a highly multiplex PCR method designed to target the V3 and V4 regions for over 2000 bacterial genomes, and a custom pathogen targeted amplicon sequencing (TAS) panel. Respective libraries were sequenced using Illumina's MiSeq platform. Bioinformatic workflows were customized for each method in GalaxyTrakr.

Results: TAS allowed for detection of the pathogens in all the products down to 10 CFU. WMS and CDMI detected pathogens down to 1000 to 100 CFU depending on the complexity of the product tested. WMS data showed about one log higher sensitivity than CDMI. Both CDMI and TAS allow for multiplexing without losing sensitivity when compared with WMS.

Significance: We have evaluated three sequencing methods for use in routine

surveillance of live microbial products. While WMS has the advantage of profiling the microbial content of a sample, for the specific purpose of detecting low levels of pathogens in probiotic products, CDMI and TAS are better suited, with TAS being the most sensitive.

P2-79: VERIFICATION OF A RAPID SCREENING METHODOLOGY FOR BRINE AND PROCESS WATER SAMPLING VIA THE VIDAS® UP LISTERIA ASSAY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Brine and process water that contacts post-lethality ready-to-eat (RTE) products should be monitored for *Listeria* to ensure the effectiveness of process control and safety of RTE foods, while complying regulations (9 CFR 430). A rapid screening methodology needs to be validated for this testing in addition to the FSIS MLG 8.14 Guidance.

Purpose: To develop and validate the methodology for sampling brine and process water in RTE foods for *Listeria* detection via the VIDAS® UP Listeria (LPT, Ref 30126) assay.

Methods: Sterile 15% NaCl and 1% NaCl solutions were prepared to represent brine and process water, respectively, and inoculated with 5% NaCl-adapted *Listeria* cocktail at a final cell density of 10 – 100 CFU/mL. Un-inoculated samples were included as negative controls. With the filtration method, 500 mL of 15% (n = 10) and 1% NaCl (n = 10) samples were filtered through a membrane (pore size 0.4 µm), and the filter was transferred to a sterile bag for incubation with 100 mL LPT broth for 20 – 26 h at 30°C. With the direct enrichment method, 25 mL of 15% (n = 10) and 1% NaCl (n = 10) samples were transferred to a sterile bag and enriched similarly as above. The enrichments were run using the LPT assay to detect the presence or absence of *Listeria*; enrichments were also streaked to MOX agar for confirmatory results.

Results: All inoculated 15% and 1% NaCl samples were detected to be positive for *Listeria* by the VIDAS® LPT assay and confirmed with visible growth on MOX. All controls were negative for *Listeria* by the LPT assay with no growth on MOX. Both filtration and direct enrichment were appropriate methods to detect *Listeria*.

Significance: This study verified the testing procedures for monitoring *Listeria* within process water and brine solutions that satisfy the FSIS regulations.

P2-80: AI-ENABLED IMAGING FOR PATHOGEN DETECTION UNDER STRESS CONDITIONS: A SYSTEMATIC REVIEW

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Advances in pathogen detection by incorporating artificial intelligence (AI) can give insights on bacterial physiology in challenging environmental conditions that traditional methods may not detect.

Purpose: The aim of this systematic review was to evaluate the application, efficacy, and technological advancements of AI-enabled imaging for pathogen detection, including its impact on speed, accuracy, and predictive modeling under stress conditions.

Methods: The review analyzed studies from five electronic databases (PubMed, IEEE, Scopus, Web of Science Core Collection, and ACM) using key search terms such as artificial intelligence, pathogen, detection, imaging. These terms were systematically replaced with alternatives to ensure comprehensive retrieval. Inclusion criteria, guided by the PICOS framework (populations, interventions, comparators, outcomes, and study design), focused on microscopy-based pathogen detection enhanced by AI. Data extraction followed PRISMA guidelines, covering aspects such as biological sample preparation, AI-enabled data analyses, and performance metrics. The review evaluated how these methods addressed challenges like biological noise and pathogen viability.

Results: of 2,736 citations retrieved, 119 were reviewed in full and 27 studies met the inclusion criteria. These studies represented over 40 different pathogens, most focusing on major foodborne pathogens such as *Salmonella* and *E. coli*. Of the studies, 2 focused on bacteria under stress conditions characterized by antibiotic treatment or bacteria in a viable but non-culturable state. However, many studies lacked the use of comparators to benchmark AI techniques, highlighting a gap in standardization. Additionally, inconsistencies in reporting experimental data collection such as incubation conditions, imaging settings, and bacterial concentrations hindered reproducibility and study comparisons.

Significance: This study provides a comprehensive overview of current methods in

AI-enabled pathogen detection from both biological and computational perspectives. The findings also highlight the need for standardized benchmarks and uniform reporting in AI-enabled pathogen detection studies to enhance the credibility of AI methodologies and improve their reproducibility.

Award Application: Developing Scientist Award

P2-81: VALIDATION OF QPCR ASSAYS TO UPDATE BAM CHAPTER 4A FOR THE SCREENING OF E. COLI O157 AND NON-O157 STEC

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: When the BAM qPCR assay for O157 STEC was designed in 2009, the diversity in Shiga toxin (Stx) subtypes with currently 4 stx1 [stx1a, stx1c, stx1d, stx1e] and 15 stx2 [stx2a to stx2o] was unknown. The stx primers were updated recently to capture all STEC subtypes. Two O157 markers were included: BAM CH 4A wzy in BLwzy assay and/or z3236 in BLz qPCR assay.

Purpose: The performance of the updated multiplex qPCR assays was compared to the BAM chap 4A qPCR assay in mixed cultures.

Methods: After 24 hr-enrichment following BAM CH 4A, cultures from lettuces [romaine and iceberg] and baby spinach were mixed with 2-fold or 3-fold serial dilutions of various STEC cultures, boiled and stored to -20°C prior to qPCR analyses. A non-toxigenic strain of *E. coli* O157, EC150, was co-inoculated in some samples prior to lysis to examine potential competition between the amplification of stx and O157 targets. Samples were run in triplicate on the ABI 7500 Fast system. To determine LOD95, amplification in 12 technical replicates defined our lowest dilution.

Results: The enrichment cultures varied from 3.2×10^7 CFU/ml to 8.3×10^8 CFU/ml. No false amplification was observed with the BLstx1 and BLstx2 confirming their specificity. Nevertheless, the z3236 marker in BLz multiplex assay was at least 250-fold less sensitive than the BAM wzy marker in BLwzy multiplex to detect O157 in presence of competitive DNA. No competition was seen between

wzy and stx detection. BLwzy LOD95 in mixed cultures was 5.9 CFU per reaction for wzy, similar to BAM qPCR, and 2 CFU per reaction for stx1a and stx2a. Unlike BLwzy the BAM STEC qPCR assay failed to detect stx2b and stx2f.

Significance: BLwzy is more efficient than the current BAM STEC qPCR assay to screen for *E. coli* O157 and non-O157 STEC in produce enrichment samples.

P2-82: GROWTH OF CRONOBACTER IN POWDERED INFANT FORMULA DURING ENRICHMENT IN BUFFERED PEPTONE WATER

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Evaluation of the recovery and growth of different *Cronobacter* species in powdered infant formula (PIF) during enrichment provides guidance to further optimization of detection methods.

Purpose: This study aimed to determine the growth of healthy and lyophilized *Cronobacter* cells in PIF during primary enrichment in buffered peptone water (BPW).

Methods: Healthy and lyophilized cells of seven *Cronobacter* species were inoculated into 100 g samples of Brand A PIF at an initial inoculation level of 3–15 CFU per sample. The inoculated samples were thoroughly mixed with BPW in a 1:10 ratio and incubated at 36 °C. Bacterial growth was determined at 18 and 24 h using differential agar plates.

Results: For PIF inoculated with healthy cells, the levels of *Cronobacter* ranged from 7.20 to 9.26 log CFU/mL at 18 h. *C. universalis* was the only species below 8 log CFU/mL at 18 h and below 9 log CFU/mL at 24 h, and it was the only species showing growth of >1 log CFU/mL from 18 h to 24 h. Other species reached ~9 log CFU/mL at 18 h and thus did not show significant growth afterwards. For PIF inoculated with lyophilized cells, *Cronobacter* levels at 18 h ranged from 7.04 to 8.89 log CFU/mL. *C. universalis* and *C. condimenti* were the only species below 7.5 log CFU/mL at 18 h, and *C. universalis* remained below 7.5 log CFU/mL at 24 h. *C. muytjensii* declined from 8.89 to 6.18 log CFU/mL between 18 and 24 h. All other species grew from ~8.8 to ~9.5 log CFU/mL between 18 and 24 h.

Significance: Understanding the levels of *Cronobacter* after BPW enrichment are

critical to optimization of subsequent cell concentration, DNA extraction, qPCR screening, and any metagenomic-based assays. It also contributes useful information for evaluating the performance of secondary enrichment broths.

P2-83: COMPARISON OF NEWLY DEVELOPED AND/OR MODIFIED (PRE-)ENRICHMENT BROTHS FOR DETECTION OF *LISTERIA MONOCYTOGENES* IN DIFFERENT MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Recovering sub-lethally injured *Listeria monocytogenes* (Lm) from environment and food is critical for detection, emphasizing the need to evaluate enrichment schemes.

Purpose: To compare several (pre-)enrichment broths, including those in reference methods and newly developed and/or modified broths, for recovering Lm in environmental and food matrices.

Methods: A stress (heat or desiccation)-exposed Lm strain was inoculated onto environmental surfaces, avocado skins, and alfalfa sprouts. Samples were enriched in Buffered Listeria Enrichment Broth (BLEB), University of Vermont Modified (UVM) Listeria Enrichment Broth, Half Fraser (HF), modified Half Fraser (mHF), LESS+ and 24-Listeria Enrichment Broth (24-LEB) for 24 h at their recommended incubation temperatures. At 24 h, enrichments (except BLEB) were transferred to Fraser Broth for an additional 24 h incubation at 36°C. Qualitative analysis was performed at 24 and 48 h and quantitative analysis was performed at 24 h.

Results: in environmental samples, Lm levels at 24 h, from lowest to highest, were: UVM < HF < BLEB < mHF. At 48-h, Lm was detected in all 6 replicates by BLEB, UVM, and mHF. For alfalfa sprouts, at 24 h, levels were: mHF < BLEB < UVM < HF. Lm was detected at 48 h in all replicates by BLEB and UVM. For avocado skins, levels were: UVM < BLEB < HF < mHF, and Lm was detected in all six replicates in HF. in the second comparison, levels in environmental samples were: BLEB < LESS+ < mHF < 24-LEB, and Lm was detected in all replicates in BLEB at

48 h. For alfalfa sprouts, only LESS+ was able to recover Lm at 24 h and at 48 h, Lm was detected in all replicates by LESS+. Avocado skins were not used in the second comparison.

Significance: Our data shows that (pre-)enrichment schemes require further evaluation to determine the proper enrichment broth for detecting Lm.

Award Application: Undergraduate Student Award

P2-84: A NEW CHROMOGENIC PLATING MEDIA - R & F® BACILLUS CEREUS GROUP (BCG) AGAR ALLOWED FOR IMPROVED ISOLATION AND QUANTIFICATION OF SPECIES BELONGING TO THE B. CEREUS GROUP INCLUDING B. CYTOTOXICUS

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Paul Nguyen, R & F Products

Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The limited selectivity of MYP against other Gram-positive bacteria can lead to an underestimation of *B. cereus*. Commercially available chromogenic media have greatly improved the selection and differentiation of *B. cereus* from food. However, reduced sensitivity for *B. cytotoxicus* with some chromogenic media have been reported. For a better understanding of the expected real-world performance of BCG, a reference method comparison study was needed.

Purpose: Purpose of this study was to compare the performance of BCG to the ISO 7932:2004 reference method.

Methods: A method comparison study was conducted using the accuracy profile protocol as described in ISO 16140-2 using a paired study design and incubation at both 30 and 35°C. A variety of food matrices were inoculated with *Bacillus cereus* group species [Powdered infant formula (PIF), potato flakes - *B. cytotoxicus*, Ultra-high temperature (UHT) milk, rice flour - *B. cereus*, and ice cream - *B. weihenstephanensis*]. To further challenge the method, a coinoculation study was also performed with UHT inoculated with *B. cereus* and *E. faecium* at 102 and 104 CFU/mL, respectively.

Results: Using the accuracy profile statistical method, all Results except for *B. cytotoxicus* inoculated PIF, were within the acceptability limits. Further statistical analyses by ANOVA followed by the post hoc Tukey test determined the *B. cytotoxicus* bacterial counts were, (i) significantly higher on BCG compared to MYP, and (ii) significantly higher when incubated at 35°C compared to 30°C ($p < 0.05$). When high background levels of *E. faecium* were present, *Bacillus* colonies were easier to enumerate from BCG compared to MYP.

Significance: The data presented here supports (i) BCG would improve the

sensitivity of the reference method when analyzing food products contaminated with *B. cytotoxicus*, (ii) the performance of BCG is equivalent to the reference method when incubated at either 30 or 35°C.

P2-85: INVESTIGATING SOURCE-SPECIFIC VARIATIONS IN HYPERSPECTRAL SIGNATURES OF *SALMONELLA* *INFANTIS*

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Hyperspectral microscope imaging (HMI) is emerging as a rapid pathogen detection method, but identifying stress-resistant serovars like *Salmonella* *infantis* remains challenging due to their environmental adaptability.

Purpose: This study investigates whether *S. infantis* isolates exhibit shifts in hyperspectral signatures by analyzing high-dimensional data to uncover source-dependent spectral differences.

Methods: Six isolates (bovine, bovine feces, pig ears, produce farm drag swab, human clinical, and dog food outbreak) were cultured in trypticase soy broth with yeast extract at 32°C for 16 h, washed, and resuspended in sterile deionized water. For HMI, 2 µL of each suspension was pipetted onto a glass slide, air-dried for 15 min, then overlaid with 1 µL of deionized water and a cover slip. Three slides were prepared per biological triplicate, capturing non-overlapping regions and yielding 50 datasets per isolate, using a darkfield hyperspectral microscope with a 100× objective. Single-cell spectral signatures were extracted using a U-Net based segmentation algorithm. Principal component analysis, a statistical technique that transforms data into principal components (PCs), was applied to reduce the dimensionality with a threshold of 0.99 cumulative explained variance per isolate and identify key wavebands.

Results: The number of PCs required to explain 99% of variance ranged from 10 (bovine feces, dog food outbreak, pig ears) to 16 (bovine). one-way ANOVA revealed differences in scores for PC1-PC3 across isolate sources ($p < 0.05$). Examination of the top 10 PC's loadings identified distinct wavebands contributing most strongly to each isolate's spectral profile: 535-540 nm for bovine, 570-580 nm for bovine feces, 650-670 nm for dog food outbreak, 540-560 nm for human clinical and produce farm drag swab, and 580-600 nm for pig ears.

Significance: These findings highlight how source-specific spectral shifts can complicate *S. infantis* identification while demonstrating HMI's potential to capture

subtle variations, providing a foundation for improving pathogen detection algorithms under environmental adaptation.

Award Application: Undergraduate Student Award

P2-86: DEVELOPING A MICROSCOPY-BASED METHOD TO QUANTIFY BIOFILM REMOVAL

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: As biofilm removal is critical in maintaining a safe food production environment, there is a need to develop standard tools that can assess the performance of antimicrobial and cleaning products used in food production. Traditionally, microscopy has been a reliable qualitative tool for assessing removal, but with software advancements, it can be a quantitative tool as well.

Purpose: Develop an accurate and reliable method to assess biofilm removal using fluorescent microscopy and image analysis software.

Methods: The CDC biofilm reactor was used to generate *Pseudomonas aeruginosa* biofilms on glass carriers. Rods containing carriers with biofilm were removed from the reactor and suspended in hard water (control) or various treatments (e.g., peracetic acid) for 10 minutes under shear. In some instances, multiple treatments were applied. After treatment and post-rinsing, the carriers were removed and stained with SYTO™ 9. A Leica DM6 microscopy system with a GFP filter cube was used for imaging. Five image z-stacks were taken at 40x for each treatment group. The images were analyzed using Leica LAS X image analysis software. Removal was calculated based on the total volume measurements in each image stack in relation to the control volume. Results from each image were averaged to determine the percent biofilm removal per treatment group.

Results: The reactor produced a *P. aeruginosa* biofilm with a log density of between 9.0 – 9.5 log₁₀ CFU/carrier. The biofilm contained multiple microcolonies ranging in diameter from 19 to 190 µm and depth from 13 to 16 µm. To date, a limited number of products have been evaluated, including peracetic acid and PerQuat™-based chemistries. Results from this analysis have shown removal ranging from <1% to >60%.

Significance: Using fluorescence microscopy and imaging software is a promising tool for quantitatively assessing the biofilm removal capabilities of antimicrobial and cleaning products used in the food industry.

P2-87: VERIFICATION OF THE ASSURANCE® GDS FOR SALMONELLA TQ KIT FOR DETECTING SALMONELLA IN DAIRY AND CHOCOLATE-BASED PRODUCTS FROM INDIA

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: India is the world's largest dairy producer, accounting for 23% of global milk production. Dairy products from India can be dissimilar from other world regions and should be verified to allow rapid detection by PCR of *Salmonella* strains. *Salmonella* can persist in dairy matrices. Moreover, dairy and milk-based chocolate products are known to contain PCR inhibitory components. Six different dairy products from India were analyzed per ISO 16140-3 using Assurance® GDS for *Salmonella* Tq (GDS *Salmonella*).

Purpose: To verify a method to detect *Salmonella* in Indian dairy and chocolate products using BPW enrichment broth followed by GDS *Salmonella* detection assay.

Methods: Implementation and food item verification studies were conducted by an external expert laboratory following ISO 16140-3. Dairy matrices (25 g/mL test portion) were inoculated by *Salmonella* strains (1 - 6 CFU per sample) and enriched for 18-24 h at 34-38°C in BPW (1:10 dilution ratio). Matrices analyzed were UHT milk, butter, cheese curd, milk peda dessert, cheese dip and chocolate containing raisins and nuts. Following ISO 16140-3, per matrix, seven replicates were inoculated, and one sample was not inoculated. Detection is performed using GDS *Salmonella* method followed by a confirmation step. Confirmation following the reference method (ISO 6579-1/A1) was conducted on all samples.

Results: Forty-eight (48) samples were analyzed for the verification study. The results met the Acceptance Criteria of an ISO 16140-3 study, in which all inoculated samples were positive by GDS *Salmonella*. No significant differences were observed between the ISO reference method and the GDS *Salmonella* alternative one.

Significance: These data verified the detection of *Salmonella* in dairy and

chocolate products manufactured from india using Assurance® GDS for *Salmonella* Tq.

P2-88: RAPID QUANTIFICATION OF SALMONELLA TYPHIMURIUM IN GROUND CHICKEN USING IMMUNOMAGNETIC CHEMILUMINESCENT ASSAY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Many countries have implemented regulations to monitor and reduce *Salmonella* contamination in poultry products. Rapid quantitative analysis enables poultry processors to detect contamination early, take corrective actions to lower *Salmonella* levels, and improve product safety.

Purpose: This study aims to develop an Immunomagnetic Chemiluminescent Assay (IMCA) for quantifying *Salmonella* Typhimurium in ground chicken.

Methods: Immunomagnetic microbeads coated with monoclonal antibodies were used to capture and concentrate *Salmonella* from ground chicken samples. A biotin-conjugated antibody, followed by an avidin-horseradish peroxidase conjugate, was applied to bind the captured bacteria and initiate a chemiluminescence reaction catalyzed by the peroxidase. Light intensity was measured in relative light units (RLU) using a portable luminometer. Ground chicken samples (32.5 g) were inoculated with a cocktail of four *S. Typhimurium* strains, ranging from 0 to 3.5 log CFU/g. Contamination levels were confirmed using the most probable number (MPN) method. Samples were enriched in buffered peptone water (BPW) and BAX MP Supplement for 6 and 8 hours at 42°C before being analyzed with IMCA.

Results: Linear regression analysis showed that the optimal fit for *Salmonella* quantification (0 to 3.5 log CFU/g) occurred at the 8-hour enrichment time ($R^2 \geq 0.89$), compared to the 6-hour enrichment ($R^2 \geq 0.52$). The limit of quantification (LOQ) for IMCA was determined to be less than 1 CFU/g. A strong positive correlation ($R^2 \geq 0.81$) was found between IMCA and MPN

Results, indicating consistency between the two

Methods.

Significance: These results demonstrate that IMCA is an effective method for the rapid detection and quantification of *Salmonella* in ground chicken. Providing results within a day, IMCA enhances testing efficiency and contributes to the production of safer, higher-quality poultry products, reducing the public health

risks associated with *Salmonella* contamination.

Award Application: Developing Scientist Award

P2-89: SURVIVAL OF SEVEN SPECIES OF CRONOBACTER IN POWDERED INFANT FORMULA: A STUDY TO DETERMINE INOCULATION LEVELS IN METHOD VALIDATION

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: During validation of methods to detect *Cronobacter* in powdered infant formula (PIF), lyophilized *Cronobacter* cells were used to artificially inoculate the samples before aging. Since cells die off during the aging period of PIF required by validation protocols, it is critical to adjust *Cronobacter* levels and lengths of aging to achieve fractional positive outcomes.

Purpose: Survival curves of thirteen *Cronobacter* strains of seven species in PIF were constructed to identify optimal inoculation and aging parameters for method validation.

Methods: Thirteen strains from *C. sakazakii*, *C. malonaticus*, *C. dublinensis*, *C. turicensis*, *C. condimenti*, *C. muytjensii*, and *C. universalis* were selected for this study. Freshly cultured cells were resuspended in 10% non-fat dry milk and freeze-dried on Genesis pilot lyophilizer. The resulted lyophilizates were ground into fine powders and thoroughly mixed with milk-based PIF. The *Cronobacter*-PIF mixture were aged at room temperature and cell counts were determined periodically by direct plating on brain heart infusion agar (BHIA, a differential agar for *Cronobacter*) and chromogenic *Cronobacter* isolation agar (CCI).

Results: After freeze-drying and mixing with PIF, *Cronobacter* cell count levels ranged from 5 to 7 log CFU/g on day 0. Within 21 days, viable cell counts decreased by 2 to 3 log CFU/g for most strains. After 21 days, *C. universalis* E797, *C. dublinensis* E791, and *C. turicensis* E625 continued to decline sharply till below the limit of enumeration at 40 to 70 days; *C. sakazakii* E477, *C. sakazakii* E899, and *C. dublinensis* E515 levels stabilized with slow decline until day 120. The die-

off of *C. malonaticus* E615 slowed down after 14 days and had a stabilization phase until 155 days.

Significance: The data provide guidance to achieve appropriate inoculation levels with specific *Cronobacter* strains for method validation and insights to interspecies variation in *Cronobacter* survival in PIF.

P2-90: MATRIX EXTENSION STUDY OF FDA REAL-TIME PCR FOR SCREENING SALMONELLA IN FOODS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The Bacteriological Analytical Manual (BAM) *Salmonella* chapter 5 has incorporated an FDA *Salmonella* qPCR assay for screening *Salmonella* in validated food matrices. A matrix extension study is needed for adding new food items into the validated matrix list.

Purpose: To validate 25 more food matrices for the FDA *Salmonella* qPCR method by using the same DNA templates collected from a matrix extension study of the FDA *Salmonella* LAMP (loop-mediated Isothermal Amplification) assay.

Methods: A matrix extension study for the FDA *Salmonella* LAMP assay to validate 25 new food matrices was conducted by nine state laboratories through FDA's Laboratory Flexible Funding Model Cooperative Agreement ProGram (LFFM CAP). Following FDA's Microbiological Method Validation Guidelines, 3rd Edition, each matrix extension was a single-laboratory validation study with 5 uninoculated controls, 20 replicates of fractional inoculation level, and 5 replicates of high inoculation level. All 30 test portions of each matrix were analyzed by BAM culture method and LAMP assay in 24 h pre-enriched culture. The DNA templates used for LAMP assay from each matrix were saved and later shipped to FDA lab for the qPCR matrix extension study.

Results: All 25 matrices were successfully validated by qPCR method as evidenced by fractional recovery, all 5 high-level test portions tested positive (except cocoa powder and alfalfa sprout with 4 out of 5 positives), with no cross-contamination in uninoculated test portions. The relative level of detection (RLOD = LOD50, qPCR/LOD50, culture method and RLOD = LOD50, qPCR/LOD50, LAMP) was in the range of 0.6 – 1.2 within the acceptability limit of 1.5 for paired studies per ISO 16140-2.

Significance: This study provided more validated food matrices for the FDA qPCR

assay to screen *Salmonella* in human food and animal food, expanding the toolbox for testing of more product types to better ensure food safety and public health.

P2-91: A COMPLEMENTARY APPROACH TO MONITOR FOOD SAFETY, QUALITY AND REMAINING SHELF-LIFE OF SPINACH JUICES BY USING CHEMICAL AND MICROBIAL FINGERPRINTING

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Chemical (excitation-emission matrices or EEMs) and microbial (RNA sequencing) fingerprinting provide complementary comprehensive tools to scout progressive quality and safety risks in food products.

Purpose: This study assessed the complementarity of using EEMs and RNA sequencing to report on the safety and quality of spinach juices and subsequently, their remaining shelf life.

Methods: Fresh organic baby spinach was blended with deionized water (1:1 w/w) to prepare juice-like products. Each sample was divided into three aliquots: 1- untreated (UJ), 2-pasteurized (72°C, 1 min (PJ)), 3- treated with gallic acid (8 mg/mL) (GJ) and stored at 4 and 15°C for 7 days. Samples were collected daily for EEMs ($\lambda_{\text{ex}}=250\text{-}530\text{nm}$, $\lambda_{\text{em}}=270\text{-}750\text{nm}$) using a spectrophotometer. DNA was also extracted from the juices using the Qiagen DNeasy Kit. 16S rRNA sequencing was performed on the Illumina MiSeq and analyzed using bioinformatic pipelines.

Results: Three main EEMs regions exhibited the most changes during storage for treated (n=28) and untreated (n=14) samples, depending on treatment type and testing time. Culture-independent 16S rRNA amplicon sequencing yielded more

bacterial genera than culture-dependent. Higher abundance of *Pseudomonas*, *Exiguobacterium* and *Erwinia* was observed in untreated samples. PJ and GJ showed inhibition of *Aeromonas*, *Chryseobacterium*, *Delftia*, *Enterobacteriaceae*, *Exiguobacterium*, *Paenibacillus*, and *Sphingobacterium*, which may contain pathogenic and/or spoilage microbial species. Higher abundance of *Bacillus* was seen in PJ, while *Pseudomonas* was more prevalent in GJ. Using an ANN model to analyze the changes in intensity collected from the EEMs and microbial growth allowed for the correct classification of >90% of samples as “fresh” vs. “spoiled.”

Significance: PJ and GJ were effective in inhibiting microbial communities and had unique EEMs. Analyzing the fingerprinting data using an ANN model allowed identifying early spoilage and quality changes in juices. This may improve food safety, quality and shelf-life reporting.

Award Application: Developing Scientist Award

P2-92: DEVELOPMENT OF A MULTIPLEX RECOMBINASE POLYMERASE AMPLIFICATION COUPLED WITH LATERAL FLOW DIPSTICKS FOR THE SIMULTANEOUS RAPID DETECTION OF SALMONELLA SPP., SALMONELLA TYPHIMURIUM AND SALMONELLA ENTERITIDIS

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella* spp. is one of the leading foodborne pathogens globally, and its rapid detection is essential to ensure food safety. Traditional detection methods can be time-consuming and labor-intensive, prompting the need for more efficient alternatives.

Purpose: This study aimed to establish a visual, rapid, and highly specific detection method for *Salmonella* spp., including major serovars *Salmonella* Enteritidis and *Salmonella* Typhimurium, using recombinase polymerase amplification (RPA) coupled with lateral flow dipsticks (LFD).

Methods: A multiplex RPA-LFD assay was developed and optimized at a reaction volume of 25 μ L and a temperature of 38°C. The detection process was completed within 25 minutes. The assay was tested against standard strains and artificially contaminated chicken and retail food samples. The assay's specificity was validated by testing for cross-reactivity with other common foodborne bacteria. The limit of detection (LOD) was determined for *Salmonella* spp., *S. Enteritidis*, and *S. Typhimurium* under both pure and contaminated sample conditions.

Results: The LODs were 2.8×10^2 , 5.9×10^2 , and 7.6×10^2 CFU/mL for *Salmonella* spp., *S. Enteritidis*, and *S. Typhimurium*, respectively. No cross-reactivity was observed. In artificially contaminated chicken samples, the LODs were 2.8×10^3 , 5.9×10^3 , and 7.6×10^3 CFU/mL for *Salmonella* spp., *S. Enteritidis*, and *S. Typhimurium*, respectively. The method effectively detected the pathogens in retail food samples.

Significance: The multiplex RPA-LFD method developed in this study offers a sensitive, rapid, and specific tool for the detection of *Salmonella* spp. and its key serovars in food. This approach has strong potential for routine food safety

screening, especially in resource-limited or high-throughput environments.

Award Application: Undergraduate Student Award

P2-93: FIBER OPTICS SERS SENSOR FOR RAPID AND MULTIPLEX DETECTION OF SALMONELLA IN TURKEY RINSATE

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella* is a major contributor to foodborne illnesses in the U.S., with millions of infections annually and a \$4.1 billion economic burden. Conventional detection methods remain slow, expensive, and labor-intensive, underscoring the need for more efficient technologies for rapid detection.

Purpose: Introduce a transformative fiberoptics-based Surface Enhanced Raman Spectroscopy (SERS) sensor for rapid and multiplex detection of *Salmonella* serotypes in turkey rinsate samples.

Methods: The sensor integrates highly uniform nanoantenna arrays on a side-polished multimode optical fiber core, significantly enhancing the reflected light intensity. Additive manufacturing is used to print a 3D microstructure with tilted cylindrical ports to optimize reflected light, a groove to support fiber core polishing, and a sample compartment for testing. A novel low-cost microsphere photolithography method was employed to create nanoantenna arrays with disk diameters and periodicities of 675 nm and 0.77 μm , and 680 nm and 1 μm . This design presents a new solution by increasing the sensing surface area, reducing the periodicity, and thus achieving a high signal-to-noise ratio.

Results: The sensor demonstrated high specificity and multiplex detection of *Salmonella* serotypes Typhimurium and Enteritidis, achieving a limit of detection

of 0.3–0.5 CFU/mL in 10 minutes. It also effectively differentiated live (1 CFU/mL) from dead *Salmonella* Typhimurium cells (334 CFU/mL), including those treated with peracetic acid (10 ppm) or heat (95°C for 0.5 hour). A study on detection time confirmed that 10 minutes is optimal for accurate *Salmonella* detection. Each sample was tested at least 4 times, using 3–4 sensors for accuracy.

Significance: This research develops a portable, low-cost SERS sensor for rapid, multiplex detection of *Salmonella* serotypes in poultry, enabling swift interventions to improve food safety. With results in just 10 minutes, the technology has the potential to transform food safety testing across various industries. It ensures regulatory compliance with the proposed USDA FSIS standard for *Salmonella* testing.

Award Application: Developing Scientist Award

P2-94: A NOVEL LAMP-BASED QUANTITATIVE SALMONELLA ASSAY FOR POULTRY CARCASS RINSE

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella* is the leading cause of food-borne illness, and poultry is recognized as the primary reservoir for various *Salmonella* serotypes. Accurate quantification of *Salmonella* in poultry products is crucial for assessing potential risks to public health and implementing effective intervention strategies to reduce contamination and ensure food safety.

Purpose: To evaluate a novel loop-mediated isothermal nucleic acid amplification method (LAMP) and algorithm for quantification of *Salmonella* in whole carcass rinses and compare it to the traditional most probable number (MPN) method.

Methods: Each chicken carcass was rinsed with 400 mL of nBPW or BPW. The rinsate was spiked with *Salmonella* at four contamination levels and held in the refrigerator for 2-3 days. For LAMP method, 30 mL rinsate was enriched with 30 mL of a proprietary media, incubated for 5-6 h at 42°C. An aliquot of 1.5 mL enrichment broth was processed and analyzed using MDA2QSAL. The concentration of *Salmonella* was automatically reported with the built-in model. The same rinsate was also analyzed using the MPN technique. Two samples paired t-test was conducted to compare the difference in means between the two methods. The limit of quantification was assessed based on analyzing the results of spiking different levels of *Salmonella* Typhimurium in the nBPW or BPW rinses.

Results: The rapid LAMP method successfully quantified *Salmonella* in chicken carcass rinses after 5-6 h enrichment. The t- test shows no significant differences between the LAMP and MPN methods in all inoculation levels evaluated for *Salmonella* quantification ($p < 0.05$). The limit of quantification of the method is at 1 CFU/mL.

Significance: This novel LAMP method provides a simple, fast, and accurate

method to determine the concentration of *Salmonella* in chicken rinses, allowing poultry producers to institute appropriate early interventions to reduce *Salmonella* contamination and illness associated with raw poultry products.

P2-95: COMPARATIVE EVALUATION OF A SUITE OF HLYA-BASED LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAYS FOR DETECTING LISTERIA MONOCYTOGENES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Multiple loop-mediated isothermal amplification (LAMP) assays for detecting *Listeria monocytogenes* in foods have been reported in the literature but independent evaluation of these assays has not been done.

Purpose: This study aimed at evaluating the performance of 13 hlyA-based LAMP assays for *L. monocytogenes*, along with two newly designed LAMP assays targeting the same gene.

Methods: We comparatively evaluated the sensitivity (limit of detection) and specificity (inclusivity/exclusivity) of these 15 sets of LAMP assays. All assays were performed on the OptiGene Genie II platform using the GspSSD2.0 Isothermal Mastermix. The limits of detection were determined using a ten-fold serially diluted reference *L. monocytogenes* culture (ATCC 19115), and inclusivity and exclusivity were determined with a panel of 50 *L. monocytogenes*, and 30 non-*L. monocytogenes* *Listeria* and 30 non-*Listeria*, respectively.

Results: Among the 15 LAMP assays, all except two previously published sets achieved sensitive detection of the reference *L. monocytogenes* strain down to 10 cells per reaction. Five assays could detect down to the 1 cell per reaction level. The thirteen assays including the two newly designed ones had comparable inclusivity and exclusivity with the extensive collection of strains (n = 120) where no false-negative or false-positive

Results were observed. The assay time varied among the 15 LAMP sets, ranging from 5 to 17 min (8-9 min for the two newly designed assays) for the reference

strain tested at 10⁴ cells/reaction.

Significance: Rapid and reliable detection of *L. monocytogenes* in foods plays a critical role in implementing timely control measures to reduce illnesses and fatalities associated with this prominent foodborne pathogen in susceptible populations, ensuring food safety and protecting public health.

P2-96: COMPARATIVE ANALYSIS OF INOCULATION AND RECOVERY TECHNIQUES FOR BETTER RECOVERY OF LISTERIA MONOCYTOGENES FROM READY-TO-EAT (RTE) BULK TURKEY AND ROAST BEEF

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: According to the CDC, out of 31 known foodborne pathogens, *Listeria monocytogenes* stands out as responsible for the highest mortality rate of 19%. A quantitative risk assessment by the US FDA and USDA has classified RTE deli meats as high risk for listeriosis. Published studies focusing on inoculation and recovery methods for *L. monocytogenes* from bulk deli RTE meats are limited, and additional studies are needed.

Purpose: This study aims to compare different combinations between two inoculation methods (brushing and spreading) and two methods of recovering (stomaching and shaking) *L. monocytogenes* from bulk turkey and roast beef over 7 days.

Methods: Dual-antibiotic-resistant *L. monocytogenes* at 6 log CFU/g were inoculated on 5 sides of the bulk meat sample (top, left, right, bottom, center) using either brushing (silicone brush) or spreading (disposable cell spreader) methods. *L. monocytogenes* in the meat samples were then recovered with buffered peptone water using either stomaching or shaking methods. Sampling for enumeration of *L. monocytogenes* in the meat samples was done on days 0, 3, and 7. Samples were plated on tryptic soy agar with antibiotics.

Results: Approximately 3 to 4 logs CFU/g of *L. monocytogenes* were recovered

from both bulk meat samples. in turkey meat, the brushing and stomaching (BST) combination was demonstrated to show a slightly higher recovery (average range 3.60-3.99 log CFU/g) of *L. monocytogenes* than the spreading and shaking (SSH) combination (average range 3.32-3.93 log CFU/g) over 7 days. in roast beef, shaking was demonstrated to have a slightly higher recovery (average range 3.75-4.01 log CFU/g) overall of *L. monocytogenes* than stomaching (average range 3.71-3.95 log CFU/g) over 7 days. However, no clear inoculation method that worked better for roast beef could be determined.

Significance: The

Results can help determine the best combined method that can be used to efficiently recover *L. monocytogenes* from RTE meats.

P2-97: VERIFICATION OF THE ASSURANCE® GDS FOR SALMONELLA TQ KIT FOR DETECTING SALMONELLA IN INDIAN SPICES AND SPICE MIXTURES SNACKS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Detection of food pathogens in the Spice food category is difficult due to several reasons, such as dried conditions, strong antimicrobial properties, etc. Over the years *Salmonella* has been identified as one of the main pathogens leading to spice-related recalls and outbreaks. Moreover, specific, and sensitive detection of *Salmonella* for the Spice category from pathogen method protocols are not well developed in many of the commercially available detection kits. Our *Salmonella* assay is a highly sensitive and specific qPCR-based method. This methodology uses a two-prong approach – 1) proprietary probes and specific primers and 2) antibody-based immunomagnetic separation (IMS) to concentrate the target microorganisms and remove potential cross-reactors. Thus, this QPCR assay offers accuracy in difficult food categories such as Spices.

Purpose: To verify an alternative protocol to detect *Salmonella* in Indian Spices and Spice Mixtures using QPCR based *Salmonella* detection method.

Methods: 25 g spice mixtures added to 225 ml BPW media (BPW+0.5% Tween 80 for spiced peanuts, or BPW+0.5% K₂SO₃ for panchrathan, channa dal, and Khatka meetha). For saffron, 100 mg sample was added to 0.9 mL BPW in a sterile tube. Samples were homogenized using a masticator and sample bags were spiked with *Salmonella* at 3-5 CFU. *Salmonella* was grown at 36± 1 °C for 18-24 h. Following ISO 16140-3, per matrix, seven replicates were inoculated, and one sample was not inoculated. *Salmonella* was detected using qPCR-based assay followed by conventional confirmation methods.

Results: Sixty-three (63) samples were analyzed in this study and results were comparable to ISO 16140-3. All spiked samples were positive. These results demonstrate our assay was able to detect *Salmonella* in all spices used in this study with 100% specificity, 100% sensitivity, 0% false-positive and 0% false-negative results.

Significance: These data demonstrate application of Assurance® GDS for *Salmonella* Tq for detecting *Salmonella* accurately and consistently in the difficult food category of Spices.

P2-98: DETECTION OF BACILLUS CEREUS SENSU LATO GROUP FROM A BROAD RANGE OF FOODS USING A NOVEL DRY-FILM INDICATOR ENUMERATION METHOD

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Bacillus cereus* is a Gram-positive, spore-forming organism commonly found in the environment and various dried, raw, and processed foods. The *Bacillus cereus* sensu lato group comprises several species of closely related organisms that have been linked to food poisoning of the emetic and enteric type due to inadequate storage or incorrect preparation of foods.

Purpose: Evaluate the performance of a new dry-film indicator method for the detection of *Bacillus cereus* sensu lato group organisms for inclusivity, exclusivity, and matrix performance in comparison to the procedures specified in the ISO 7932 standard and FDA BAM Chapter 14.

Methods: Inclusivity for 38 strains within the *Bacillus cereus* sensu lato classification and exclusivity for 32 non-target species were assessed with pure culture testing. Method comparison was performed with portions of dried, ready-to-eat, raw meat, and dairy matrices (n=120 samples). Samples were inoculated with low levels of the target organism and plated onto the novel dry film method and the reference agar medium specified in ISO 7932. The dry films were incubated at both 30°C and 35°C for 20–24 hours.

Results: 100% inclusivity for strains within the *Bacillus cereus* sensu lato group was determined with the dry film method and 100% exclusivity was determined for non-target organisms. Performance of the method for matrices showed no significant difference (ANOVA $P > 0.05$) at 30°C ($P = 0.09$) or 35°C ($P = 0.95$) when compared to the reference standard across the 120 foods tested within the broad range of food categories.

Significance: The novel dry-film design offers an efficient and specific approach for the detection of *Bacillus cereus* sensu lato group organisms in a ready-to-use

format. This offers food manufacturers and commercial laboratories a convenient alternative method to evaluate the microbiological quality and safety of a broad range of foods.

P2-99: MALDI-TOF AND COMPACTDRY™ : A STREAMLINED APPROACH TO MICROBIAL IDENTIFICATION

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Optimizing workflows for microbial isolation and identification is essential in microbiological testing. The combined use of CompactDry™ dehydrated media plates and MALDI-ToF mass spectrometry enhances identification accuracy, achieving results comparable to traditional methods.

Purpose: This study evaluated MALDI-ToF identification accuracy when using microorganisms cultured on non-traditional culture media (dehydrated media plates).

Methods: Twelve formulations of dehydrated media plates were inoculated, incubated, and evaluated using the Autof ms1000 MALDI-ToF system (Autobio Diagnostics Co., Ltd.). Test organisms, representative of those commonly found on each plate type, were cultured on traditional non-selective agar from frozen stock vials. Suspensions were prepared and inoculated onto the respective dehydrated media plates. Traditional non-selective agar plates served as controls. Growth conditions and MALDI-ToF identification procedures were tailored to each plate. Three preparation methods were used: Extended Direct Transfer Method, Swab Suspension Transfer Method, and Filamentous Fungi Extraction Method. Plate counts, preparation methods, identification results, and confidence scores were evaluated and compared to controls.

Results: Most of the dehydrated media plates tested demonstrated successful identification rates comparable to controls. Organisms tested from *Bacillus cereus* (BC), Coliforms (CF), *Escherichia coli* (EC), Enterobacteriaceae (ETC), *Pseudomonas aeruginosa* (PA), *Salmonella* (SL), Total Count (TC), Total Count Rapid (TCR), and *Staphylococcus aureus* (X-SA) plates achieved identification rates equal to or higher than controls. Identification success was slightly lower for *Listeria* (LS) plates, and inconclusive results were observed for Total Yeast & Mold (YM) and Total Yeast & Mold Rapid (YMR) plates, warranting further

troubleshooting. Overall, the study suggests that MALDI-ToF identification directly from dehydrated media plates is feasible and accurate across a broad bacterial population.

Significance: Combined use of MALDI-ToF technology and dehydrated media plates highlights the potential for more convenient and efficient microbial identification in various applications.

P2-100: SWAB DIVERSITY STUDY - FDA/GENOMETRAKR COLLABORATION TO DETERMINE OPTIMAL ISOLATE PICKS FOR CAPTURING STRAIN DIVERSITY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Identifying pathogens in food samples, a resource-intensive activity crucial to surveillance and outbreak response, depends greatly on picking and sequencing colonies from selective media as prescribed in the FDA Bacteriological Analytical Manual (BAM).

Purpose: To determine if enhanced sequencing of *Salmonella* spp. and/or *Listeria* spp. per food sample leads to the capture of more unique strains.

Methods: Ten GenomeTrakr labs collected and sequenced between 2 and 22 *Listeria* or *Salmonella* isolates per sample from selective agars during routine food sample analysis, with 11 isolates collected from most samples. Data were analyzed to determine if: 1) number of strains per sample increased with the number of colony picks per sample, and 2) genus impacted the number of strains per sample. Strain level identification from assembled genomes was done using ANI (Average Nucleotide Identity) and CFSAN SNP pipeline to capture the SNP distance between the group of picks per sample. Pairs or groups of isolates within 20 SNPs of one another were identified as the same strain.

Results: Labs sequenced 785 colony picks from 77 routine food samples. A preliminary power analysis estimated 11 picks per sample would capture at least 3 strains with 95% confidence. Using Bayesian Poisson regression and other methods, the likelihood of observing 3 or more strains was quadruple with >11 picks vs <11 picks (14.3% and 3.6%, respectively). After accounting for number of picks, *Listeria*+ samples presented significantly more strains than *Salmonella*+ samples. The likelihood of observing 3 strains increases substantially (doubles) when the number of picks increases from 6 to 11. The likelihood of observing at least four strains more than doubles when the number of picks increases from 11 to 16.

Significance: These findings suggest that picking and sequencing additional colonies during food sample analysis results in a greater diversity of strains captured, increasing the probability of finding a high-impact strain during outbreak response and surveillance.

P2-101: RAPID MULTI-LOCUS TNGS-BASED GENOTYPING OF LISTERIA MONOCYTOGENES BY THE CLEAR SAFETY AUTOMATED PLATFORM TO IDENTIFY OUTBREAK-RELEVANT ISOLATES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: An automated tNGS platform can amplify and sequence multiple loci in the *Listeria* genome to rapidly and easily speciate and strain type *Listeria* isolates.

Purpose: We evaluate if the multi-locus based tNGS genotyping method has discriminatory power to distinguish *Listeria monocytogenes* strains associated with different outbreaks.

Methods: Sixteen *Listeria monocytogenes* strains isolated from clinical and food sources related to three different outbreaks (Nova Scotia, 1981; Massachusetts, 1983; and California, 1985), and 9 ATCC control *Listeria* spp. strains were grown in 2mL of Clear *Listeria* Media incubated at 35°C overnight. Fifty µL of overnight cultures were added to 450µL of Clear Prep Solution in duplicate and loaded onto the Clear Safety automated platform that performs cell lysis, live/dead cell screening, PCR amplification of multiple *Listeria* loci, library preparation, and sequencing on an Oxford Nanopore Technologies flowcell. Amplicon sequences are demultiplexed, quality-filtered, and mapped to a reference database to confirm the species and strain type of the *Listeria* isolates.

Results: The multi-locus tNGS genotyping method correctly identified the species of 25 *Listeria* isolates tested. For 24 of the *Listeria* isolates tested and typable by the system (*Listeria grayii* was excluded), a unique identification number was assigned based on the pattern of SNPs detected in the set of loci that were sequenced. Statistical analysis comparing the agreement between genotyping data and outbreak collection metadata indicated excellent concordance, with a

Cohen's Kappa of 0.891. The Hunter Gaston Discriminatory index, indicated a 0.8369 probability of distinguishing randomly selected strains or moderately high discriminatory power.

Significance: Current *Listeria* strain typing methods (WGS, PFGE, MLVA) require colony isolation, experienced lab personnel, and several days to provide results. The multi-locus tNGS-based genotyping method is rapid, easy-to-use with a single touchpoint on the fully automated platform, and provides sufficient discriminatory power to identify strains derived from the same outbreak.

P2-102: KANAMYCIN INDUCES PHENOTYPE CHANGES OF SALMONELLA ON XLD AGAR AND INHIBIT CARBOHYDRATE FERMENTATION

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Traditional detection and identification of *Salmonella* spp. involves plating onto selective agars, such as XLD agar. Production of sulfhydryric acid (H₂S), by the bacteria originate a distinctive black colony phenotype. However, addition of antibiotics to culture media can cause phenotype changes in *Salmonella* that could cause misinterpretations.

Purpose: To determine the effect of kanamycin on phenotype changes of *Salmonella* on XLD agar and carbohydrate fermentation.

Methods: *Salmonella* strains ATCC-12325, 14028 and 19040 and 22 *Salmonella* wild type isolates were analyzed. These were inoculated on XLD agar containing kanamycin (6, 12, 25 or 50 µg/ml) and incubated at 37°C/24h to determine changes in the phenotype of the colonies. Bromocresol purple broth (BCP) with these kanamycin concentrations was used to evaluate *Salmonella* lactose fermentation; whereas fermentation of glucose, sucrose, mannitol, arabinose, inositol, melobiose, maltose, rhamnose and galactose was determined at 50 µg/mL. Qualitative color changes on media were observed.

Results: Kanamycin at 6, 12 and 25 µg/ml on XLD agar provoked changes in *Salmonella* colonies, as consequence, 32%±4 (8±1/25) of strains grew as black colonies; 12%±4 (3±1/25) were yellow, and 24%±12 (6±3/25) showed both phenotypes. in contrast, kanamycin at 50 µg/mL produced black colonies in 16% (4/25) of isolates, whereas yellow colonies increased to 52% (13/25). interestingly, kanamycin at 6 µg/mL produced 44% (11/25) of colorless colonies, and 12 and 25 µg/mL inhibited growth of 30%±10 (7±2/25) of isolates. An average of 28%±4 (7±1/25) of strains fermented lactose after exposure to all kanamycin

concentrations, compared to 84% (21/25) without antibiotic. Also, kanamycin at 50 µg/mL reduced 37%±31 of strains that fermented seven carbohydrates compared to 86%±14 without antibiotic. Sucrose and inositol fermentation were inhibited by kanamycin in all treated bacteria.

Significance: Caution should be taken when adding kanamycin to this culture media to avoid misidentification of *Salmonella* isolates.

Award Application: Developing Scientist Award

P2-103: HARMONIZED VALIDATION STUDY: NEOGEN® PETRIFILM® BACILLUS CEREUS (BC) COUNT PLATE FOR QUANTITATIVE DETERMINATION OF THE BACILLUS CEREUS GROUP IN A BROAD RANGE OF FOODS AND ANIMAL FEED

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: The Neogen® Petrifilm® *Bacillus cereus* Count Plate is a selective and differential, sample-ready, culture medium system for quantitative determination of the *Bacillus cereus* group (*Bacillus cereus sensu lato*) in a broad range of foods.

Purpose: To validate the performance of the alternative method according to ISO 16140-2:2016 Microbiology of food chain- Method Validation- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method and AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces ("Appendix J") for submission to MicroVal and AOAC Performance Tested MethodsSM certification proGrams, respectively.

Methods: The validation study consisted of a method comparison study, including inclusivity and exclusivity and matrix study testing. For the inclusivity and exclusivity study, 51 *Bacillus cereus* group isolates and 34 non-*Bacillus cereus* group isolates were evaluated. in a paired matrix study, five categories of foods were evaluated and compared to the ISO 7932:2004 Microbiology of Food and animal feeding stuffs- Horizontal method for the enumeration of presumptive *Bacillus cereus*- Colony count technique at 30°C and FDA Bacteriological Analytical Manual Chapter 14 *Bacillus cereus* reference method using mannitol-egg yolk-polymyxin Agar.

Results: In the inclusivity and exclusivity study, all 51 of the *Bacillus cereus* group isolates were detected, and all 34 exclusivity isolates produced negative results.

In the matrix study, the alternative method demonstrated equivalence when compared to both reference methods for all matrices with incubation at either 30°C or 35°C.

Significance: The alternative method is an accurate method for the quantitative enumeration of *Bacillus cereus* group in all matrices evaluated while allowing for fast and reliable enumeration of the *Bacillus cereus* group in an easy-to-use platform.

P2-104: ONT FLONGLE SEQUENCING FOR THE RAPID DETECTION OF FOODBORNE PATHOGENS IN WHOLE CHICKEN RINSATE IN THE PRESENCE OF NATURAL MICROFLORA

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Approximately 48 million cases of foodborne illness occur annually in the United States and hence rapid detection is desirable.

Purpose: To investigate ONT Flongle sequencing for the rapid detection of foodborne bacterial pathogens (*Campylobacter* species, *Salmonella enterica*, *Listeria monocytogenes*, and *E. coli* O157:H7) in whole chicken rinsate in the presence of natural microflora.

Methods: Ten randomly selected retail whole chicken carcasses were rinsed with 400 ml sterile buffered peptone water. Half of the prepared chicken rinsate was spiked with predetermined inoculum concentrations of the tested pathogens, while the other half of the rinsate was left unspiked. Both spiked, and unspiked rinsates were mixed with an appropriate volume of enrichment media for targeted pathogens and incubated for 24 hours at proper conditions. 2 ml of each enrichment sample was collected and used for DNA isolation. Native Barcoding ONT libraries were prepared and loaded on the Flongle flow cell (R10.4.1). Using Fast Basecalling, bacterial species were identified using the WF-metagenomics workflow available through EPI2ME. Traditional culture methods were used as a control.

Results: Using ONT sequencing, species level identification of all targeted pathogens was successful in all the ten spiked samples and confirmed by culture methods. However, the cutoff value for the species level identification was less than 1% minimum abundance for some samples due to the presence of untargeted bacterial species as natural background. In the unspiked samples ONT Flongle sequencing was able to detect more *Escherichia coli* and *Salmonella enterica* than those detected by the culture method. Overall, the majority of untargeted reads were assigned to *Enterococcus* species, *Ligilactobacillus* species, and other natural microflora.

Significance: The success of ONT Flongle sequencing in accurately identifying

multiple targeted foodborne pathogens in chicken rinsate in the presence of natural microflora is very valuable and could replace the laborious time-consuming conventional culture methods.

P2-105: VERIFICATION TESTING OF REAL-TIME PCR KITS WITH VEGETABLE AND NUT MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Recent outbreaks of *Listeria monocytogenes*, *Salmonella*, and Shiga toxin-producing *Escherichia coli* (STEC) linked to fresh produce such as onions, carrots, and nuts highlight the need for food safety testing methods with rapid turnaround times.

Purpose: Produce samples were inoculated with *Salmonella* Typhimurium, *Listeria monocytogenes*, or *Escherichia coli* O157:H7 to determine the viability of Real-Time PCR kits for detecting pathogens in vegetables and nuts.

Methods: Twenty matrices were divided into seven 25 g portions, enriched with Buffered Peptone Water Standard or Listeria Special Broth II, and inoculated with low levels of target bacteria (3–10 CFU) and competing bacteria (100–500 CFU), high levels of target bacteria (100–1,000 CFU) and competing bacteria (>1,000 CFU), or left uninoculated. After an 18-hour enrichment, samples were tested with Real-Time PCR kits every 1–2 hours until a positive result was obtained or the maximum incubation time was reached. If results were not obtained, different enrichment ratios, longer enrichment times, and pre- and post-extraction dilutions were tested as needed.

Results: All matrices tested achieved the desired results with a 1:4 or 1:9 enrichment ratio for 18 hours, with the following exceptions. When tested for *Salmonella*, dried black beans required an additional 1:9 post-extraction dilution and spirulina required a 1:19 enrichment ratio with a 1:9 pre-extraction dilution. When tested for *Salmonella* or STEC, compost required a 1:19 enrichment ratio and a 1:9 pre-extraction dilution. When tested for *Listeria monocytogenes*, spirulina required a 1:39 enrichment ratio for 30 hours and a 1:9 pre-extraction dilution, and compost required a 1:29 enrichment ratio for 20 hours and a 1:9 post-extraction dilution.

Significance: The results of this study show the iQ-Check PCR kits are useful tools for the detection of pathogens in vegetable and nut matrices.

P2-106: A NOVEL PCR-NANOPORE CHECKPOINT SEQUENCING METHOD FOR SALMONELLA DETECTION

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Nanopore sequencing has been increasingly used for pathogen detection in the food industry in recent years. Although the method provides advantages over next-generation sequencing, such as long-read capabilities and reduced alignment errors, this enzyme-based approach is limited by a low sequencing accuracy.

Purpose: This study aims to design an enzyme-free nanopore sensor for detecting *Salmonella* marker genes by accurately reading specific checkpoints in the attached ID sequence, therefore eliminating the need to sequence an entire marker gene.

Methods: ID-tagged primers specific for the *Salmonella* Typhimurium (ST) marker gene, STM4200, were designed, with an oligo-ethylene glycol (OEG) spacer incorporated between the ID tag and the primer sequence. Conventional PCR amplified the ST marker gene, generating amplicons with ID tags containing two hybridization sites for a universal encoder oligonucleotide. The MspA nanopore sequentially unzipped the ID/encoder duplex and the amplicon, producing two distinct and tandem nanopore current states from checkpoint sequences preceding the duplexes. This unique current pattern served as a molecular fingerprint, enabling visual identification of single ST marker gene molecules with high specificity and sensitivity.

Results: The ST marker gene was validated using a QIAcuity digital PCR, and ID-tagged amplicons were confirmed via DNA electrophoresis. Purified amplicons, annealed with the universal encoder at 0.5 ng/μL in the nanopore chamber, produced a nanopore current signature at 0.6 signatures per minute. These signatures exhibit three tandem current states corresponding to the two

checkpoint sequences and amplicon unzipping. Control experiments confirmed this pattern as a single-molecule fingerprint for ST detection.

Significance: This study demonstrates PCR-Nanopore Checkpoint Sequencing as a rapid, sensitive, and cost-effective method for detecting *Salmonella* marker genes at a single-molecule resolution. It lays the groundwork for multiplexed pathogen detection through automated nanopore fingerprint recognition, offering a transformative advancement in food safety diagnostics.

P2-107: DEVELOPMENT OF SELECTIVE MEDIA FOR THE ENUMERATION OF CHLORELLA VULGARIS AND LISTERIA MONOCYTOGENES FROM LEAFY GREEN NUTRIENT SOLUTION AND BIOFILMS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Algae and foodborne pathogens can persist in hydroponic leafy green systems, posing production and safety risks. Algal biofilms may host multiple microbial species, including foodborne human pathogens such as *Listeria monocytogenes*. The interactions between *L. monocytogenes* and algal biofilms in hydroponics are poorly documented. Understanding these interactions is important for the development of fresh produce safety mitigation strategies.

Purpose: To develop an in vitro protocol with optimal selectivity and sensitivity for the enumeration of *Listeria monocytogenes* and *Chlorella vulgaris* from mixed cultures.

Methods: Leafy greens agar (LGA), LGA amended with chloramphenicol (LGAchlor) or ampicillin (LGAamp), and LB amended with nalidixic acid (LBna) were tested for their selectivity to *L. monocytogenes* and *C. vulgaris* at 20–24°C and 35°C. *L. monocytogenes* (~10⁸ CFU/mL) and *C. vulgaris* (~10⁷ CFU/mL) were grown in BHI and leafy green nutrient solution (LG), respectively. Mixed cultures were grown in LG. Stock inocula were serially diluted, plated onto each medium, and incubated at 20–24°C or 35°C. *L. monocytogenes* was incubated for 48 h, *C. vulgaris* for 3–4 days, and CFU/mL was calculated.

Results: *L. monocytogenes* enumeration on LBna was 100% of the stock inoculum at 35°C and 84% at 20–24°C. On LGA, enumeration was 89% at 35°C and 42% at 20–24°C. *L. monocytogenes* did not grow on LGAchlor or LGAamp at either temperature. *C. vulgaris* enumeration on LGA, LGAchlor, and LGAamp was >99% of the stock inoculum at both temperatures, but *C. vulgaris* did not grow on LBna. LBna was selected for *L. monocytogenes* enumeration, and LGchlor for *C. vulgaris*. In mixed cultures, *C. vulgaris* was consistently enumerated on LGchlor, while *L. monocytogenes* enumeration on LBna was inconsistent.

Significance: Selective media were developed to enumerate *C. vulgaris* and *L. monocytogenes* from pure and mixed cultures. Further optimization is needed to enhance *L. monocytogenes* detection sensitivity from LG onto LBna medium.

Award Application: Developing Scientist Award

P2-108: FLAVOBACTERIUM COVAE: A FASTIDIOUS BACTERIUM - A LITERATURE REVIEW ON GROWTH MEDIA

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Flavobacterium covae* is a significant pathogen in the Channel Catfish industry, as it is a primary bacterium responsible for causing columnaris disease, which threatens both fish health and the sustainability of aquaculture businesses, thereby impacting food security.

Purpose: This systematic literature review aims to identify, based on existing research, the most effective media and methods for promoting optimal growth of *Flavobacterium covae*, considering it's a fastidious bacterium that presents challenges for cultivation.

Methods: A systematic literature review was conducted using the MSU library database to identify articles published between 2022 and 2024 on the isolation or growth of *Flavobacterium covae*. Each article was carefully analyzed to extract methodologies for isolating, activating, or promoting the growth of *F. covae*. Selected publications were in English, and the keyword "*Flavobacterium covae*" was used. A matrix chart was created to organize and compare the methods, helping identify patterns and evaluate the effectiveness of different approaches, thus guiding future research on optimizing growth conditions for *F. covae*.

Results: A total of 22 articles were identified in the review. Of these, 16 articles (73%) utilized the modified Shieh agar or broth as the culture medium. This medium was composed of the following ingredients: 0.5% tryptone, 0.2% yeast extract, 45.6 μM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.1 mM KH_2PO_4 , 1.2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 3.6 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The second most used medium was Flavobacterium Columnare Growth Media, which appeared in 5 articles (23%). The composition of FCGM included: 8 g tryptone, 0.8 g yeast extract, 1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.74 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 5 g NaCl, 1.5 g sodium citrate, and 1 L of distilled deionized water (ddH₂O). Compounds like tobramycin and neomycin were common, suggesting roles in promoting *F. covae* growth.

Significance: This review aids in optimizing *Flavobacterium covae* growth, essential for studying and preventing columnaris disease in aquaculture, ensuring food safety.

Award Application: Developing Scientist Award

P2-109: EVALUATION OF A PCR WORKFLOW FOR DETECTION OF *S. AUREUS* FROM RTE FOODS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Ready-to-eat (RTE) foods have potential for causing foodborne illness as they can support the growth of pathogenic bacteria without requiring any further heat treatment prior to consumption. Molecular workflows for detection of foodborne pathogens are rapid and can help manufacturers make usage decisions on RTE foods faster in their supply chain.

Purpose: The purpose of this study was to verify the performance of the Thermo Scientific™ SureTect™ *Staphylococcus aureus* PCR Assay workflow for the detection of *S. aureus* from RTE foods.

Methods: An unpaired Probability of Detection (POD) study was conducted on cooked chicken comparing performance of the PCR workflow (100 g samples) to the ISO 6888-3 culture media reference method (10 g samples) for detection of *S. aureus*. A total of thirty samples were evaluated per method (20 low-spiked, 5 high-spiked and 5 unspiked). Performance was compared by POD analysis to identify any statistically significant differences between the methods. A sensitivity study, comprising thirty-six 100 g samples including five different food matrices (cooked chicken, cooked ham, cooked prawns, pasta, potato salad) and eight strains were evaluated, comparing performance of the PCR workflow and confirmation (culture media) workflow. All matrices for the sensitivity study were seeded with 1–7 CFU per sample and stored at 2–8°C for 72 hours before enrichment and testing with both the PCR and confirmation workflows.

Results: The POD analysis for the matrix study demonstrated no significant difference in performance between the candidate and reference method. The sensitivity study showed that all eight strains of *S. aureus* were successfully detected from the five matrices after 22-hour enrichment, with statistical analysis showing no significant difference between the PCR workflow and confirmation workflow.

Significance: The data shows that the *Staphylococcus aureus* PCR workflow is a rapid and reliable alternative method for the detection of *S. aureus* from RTE foods.

P2-110: PERFORMANCE OF A SALMONELLA PCR ASSAY ACCORDING TO NPIP

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Annually, *Salmonella* causes greater than 200 million infections globally, with a resulting 93 million cases of gastroenteritis and 155,000 deaths. The National Poultry Improvement ProGram (NPIP) is a federal proGram focusing on improvement of poultry and poultry products throughout the United States. The proGram tests and monitors poultry diseases, including Salmonellosis, at the farm level through analysis of primary production samples (PPS) to limit transference of *Salmonella* isolates of concern from flocks to food processing plants.

Purpose: To evaluate a *Salmonella* PCR assay according to NPIP regulations, for detection of *Salmonella* from poultry PPS as an indicator of flock health.

Methods: A total of 75 PPS samples (25 positive and 50 negative) at three NPIP authorized laboratories were compared against the NPIP culture reference method. All positive samples were naturally contaminated with *Salmonella*, and full serology testing was conducted. A Limit of Detection (LOD) study was conducted using target and a non-target organism to ascertain the impact of non-target organism interference at differing contamination levels of target analyte. Additionally, a panel of 26 *Salmonella* inclusivity isolates were tested, with a total of 60 exclusivity isolates to test specificity.

Results: The study demonstrated comparable performance to the NPIP culture method across all three NPIP authorized laboratories. The LOD study showed no interference of non-target organism in the performance of the assay, and the inclusivity and exclusivity study

Results adhered to NPIP requirements.

Significance: The *Salmonella* PCR Assay constitutes a rapid and reliable workflow for the detection of *Salmonella* from PPS adhering to NPIP regulatory guidelines,

giving accurate and reliable data on the health of poultry flocks to indicate areas for potential *Salmonella* contamination at upstream processing plants.

P2-111: DETECTION OF CONTAMINATION MIXTURE OF SALMONELLA AND CRONOBACTER FROM PIF

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: From late 2021 until 2022, the US experienced severe shortages of powdered infant formula (PIF), driven by supply chain issues and product recalls due to contamination and resulting neonatal illness caused by *Cronobacter sakazakii*. Ensuring PIF is free from *Salmonella* and *Cronobacter* is a vital measure needed to reduce dangerous infection in infants.

Purpose: To evaluate the harmonized enrichment protocol utilized by the Thermo Scientific™ SureTect™ *Cronobacter* species PCR Assay and Thermo Scientific™ SureTect™ *Salmonella* species PCR Assay with PIF to determine whether recovery, growth, and detection are comparable between single and co-inoculated samples.

Methods: *Salmonella* Newport and *Cronobacter sakazakii* cultures were used to prepare three replicates of single and co-inoculated 375 g PIF samples according to AOAC Appendix J guidelines. Samples were diluted 1-in-6 in pre-warmed BPW and incubated at 34–38 °C for 18–26 hours. After incubation, PCR was conducted using the SureTect PCR Assays, whilst target-specific selective plating media were used to assess growth levels. Results were confirmed by testing samples according to ISO 22964:2017 and FDA BAM Chapter 29 for *Cronobacter* spp. and ISO 6579-1:2017 and FDA BAM Chapter 5 for *Salmonella* spp.

Results: There were no statistically significant differences in the detection of *Salmonella* and *Cronobacter* between single and co-inoculated samples. Positive results detected by the SureTect methods were confirmed by ISO 22964:2017 and FDA BAM Chapter 29 reference methods for *Cronobacter* spp. and by ISO 6579-1:2017 and FDA BAM Chapter 5 for *Salmonella* spp.; there were no false negatives.

Significance: Even when challenged with a contamination mixture, dual detection of *Salmonella* and *Cronobacter* is possible following the harmonized enrichment protocol utilized by the SureTect methods. The harmonized enrichment is simple, with a low dilution ratio, and enables rapid and accurate detection of *Salmonella* and *Cronobacter* from PIF.

P2-112: STEC AND SALMONELLA DETECTION IN BEEF TRIM SAMPLING CLOTHS USING REAL-TIME PCR

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Ensuring the safety of beef trim is crucial for public health and industry compliance, as mandated by United States Federal Regulations. These regulations require rigorous testing for pathogens such as *Escherichia coli* O157:H7, non-O157 Shiga toxin-producing *E. coli* (STEC) serotypes, and *Salmonella* species to prevent foodborne illnesses and maintain quality standards.

Purpose: To assess the performance of the SureTect PCR assays relating to *E. coli* O157:H7, STEC, and *Salmonella* species detection from beef trim sampling cloths from a single enrichment.

Methods: The study was conducted according to the rules described in AOAC Appendix J. The SureTect PCR assays for *E. coli* O157:H7 and STEC detection were compared to USDA FSIS MLG 5C.03, and for *Salmonella* species detection, the SureTect assay was compared to USDA FSIS MLG 4.14. Beef trim sampling cloths were tested with and without neutralizing Buffered Peptone Water (nBPW). Co-inoculation of the cloths with both *Salmonella* and *E. coli* O157:H7 simulated worst-case, real-world scenario.

Results: The difference in probability of detection (dPOD) for the unpaired studies at the fractional level was within ± 0.10 for all the assays. All the dPOD values met AOAC acceptability limits and demonstrated that there was no statistically significant difference in performance between the SureTect

Methods and the relevant reference method.

Significance: The statistical analysis of the study results demonstrated that the SureTect PCR Assays provide accurate and reliable detection of *E. coli* O157:H7, non-O157 STEC serotypes, and *Salmonella* species from beef trim. The co-

inoculation of sampling cloths with *Salmonella* and *E. coli* O157:H7 simulated real-world worst-case conditions, further demonstrating the reliability of the SureTect , methods.

P2-113: TESTING 125G OF DAIRY AND MULTI-COMPONENT FOODS FOR LISTERIA USING A REAL-TIME PCR BASED METHOD

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Listeria monocytogenes* is a major foodborne pathogen with a significant impact on public health. Detecting *L. monocytogenes* in foods before it can cause infection is crucial to reducing the impact to public health. Current *Listeria* detection methods involve testing a 25 g food sample, however there are benefits to testing larger sample sizes. These benefits include increased sensitivity due to testing a larger portion of a batch, or being able to pool smaller sample sizes together, reducing the cost per test. It can also help laboratories meet applicable regulatory requirements. Purpose: To validate the performance of the Thermo Scientific™ SureTect™ *Listeria monocytogenes* PCR Assay and Thermo Scientific™ SureTect™ *Listeria* species PCR Assay using a 125 g sample size for dairy products and multi-component food products. Methods: The studies were conducted according to ISO 16140-2:2021 and AOAC Appendix J. In the unpaired study, 125 g samples were tested for the SureTect methods and 25 g samples were tested for the reference methods. For dairy products, the SureTect method was compared to the ISO 11290-1:2017 and FDA BAM Chapter 10 reference methods. For multi-component foods, the SureTect methods were compared to ISO 11290-1:2017 and USDA FSIS MLG 8.15 reference methods. Results: The sensitivity of the SureTect *Listeria monocytogenes* method was 91.2%, versus 90.0% for the reference method. For SureTect *Listeria* species it was 91.0%, versus 88.7% for the reference method. The RLOD for dairy and multi-component foods was 0.843 and 1.935 respectively for *L. monocytogenes*, and 0.843 and 0.780 respectively for *Listeria* species. Significance: The SureTect *Listeria* PCR Assays are accurate and reliable methods for the detection of *L. monocytogenes* and other *Listeria* species from large sample sizes of dairy products and multi-component foods. The option to use the larger sample size ensures applicable regulatory requirements are met, improves sensitivity, or reduces the cost per test when pooling samples.

P2-114: PERFORMANCE OF THERMO SCIENTIFIC™ SURETECT™ PCR KITS FOR THE DETECTION OF MULTIPLE FOODBORNE PATHOGENS IN A MEAT PRODUCTION FACILITY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Diverse foodborne pathogens can be present in a meat manufacturing facility, including: *Salmonella*, *Listeria*, and Shiga toxin-producing *E. coli* (STEC). Rapid detection

Methods with harmonized workflows to simplify processing in the laboratory are critical for streamlined testing to make batch-release and quality control decisions.

Purpose: The

Purpose of this study was to verify performance of the Thermo Scientific™ SureTect™ PCR kits for detection of *Salmonella* spp., *Listeria* spp., *L. monocytogenes*, *E. coli* O157:H7, and STEC from diverse meat and production environment products.

Methods: Ten matrix-pathogen combinations were tested, comprising: 25 g and 375 g raw meat (*Salmonella*, *E. coli* O157:H7 and STEC), 25 g raw meat (*Listeria* spp. and *L. monocytogenes*), up to 150 g animal feed (*Salmonella*), and environmental sponges (*Salmonella*). All samples for each matrix were artificially contaminated with a non-target challenge organism and half of the samples were contaminated with a target pathogen to generate 100 negative and 100 positive samples. All samples were evaluated with the PCR workflow following the appropriate enrichment conditions per pathogen of interest.

Results: All 200 samples generated data as expected, resulting in 100% sensitivity, 100% specificity, and 100% accuracy. Raw meat workflows generated PCR data within 10 hours of sample preparation and had completely harmonized enrichment conditions for *Salmonella*, *E. coli* O157:H7 and STEC. *Listeria* and *L.*

monocytogenes also had harmonized enrichment workflows and PCR results were available within 24 hours.

Significance: The data shows that all of the PCR workflows were suitable alternative

Methods for the detection of diverse foodborne pathogens from samples taken from a meat and production environment products.

P2-115: HIGH-FAT FOOD SALMONELLA SPP. DETECTION WITH LAMP - BIOLUMINESCENT ASSAY

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Category: Laboratory and Detection Methods

Abstract:

Introduction: High fat-content matrices can be challenging for pathogen detection; therefore, specific sample preparation methods and analytical tools are needed to identify pathogens. Choosing a simple-to-use, rapid and accurate *Salmonella* spp. detection method is important for monitoring intervention effectiveness and for taking action to avoid product contamination.

Purpose: Determine the difference of probability of detection (dPOD) of the *Salmonella* Loop-Mediated Isothermal Amplification (LAMP)-Bioluminescent assay in high fat content matrices compared to ISO 6579-1:2017 confirmation.

Methods: Food matrices of five high fat contents (n=35) from a Brazilian food industry were analyzed in a paired testing comparing the rapid method to ISO 6579-1:2017. Samples were margarine with 80% of fat (n=7), margarine with 50% of fat (n=7), seasoned margarine with 77% of fat (n=7), pasteurized whole liquid egg (n=7), and skimmed milk with 27% of fat with 10% of fat (n=7). Fractional artificial contamination was used. One sample of each matrix was blank and the strain (*Salmonella* Typhimurium ATCC 14028) was spiked at two levels: low (n=5, N1=0.2–2 CFU/test portion) and high (n=1, N2=5 CFU/test portion), for each matrix, in test portions of 25 g. Samples were enriched with BPW ISO (225 mL), incubated (37°C/24 hours), and analyzed with the LAMP-Bioluminescent assay and by ISO 6579-1:2017. The difference in probability of detection (dPOD) for the candidate method was determined by comparison with the reference method.

Results: The difference between the alternative LAMP-Bioluminescent method and the reference method showed a result probability of detection of 0.00, indicating no statistical difference between methods.

Significance: The Neogen® Molecular Detection Assay 2 – *Salmonella* enabled reliable and rapid detection of *Salmonella* spp. in high fat content foods.

P2-116: RAPID DETECTION OF SALMONELLA SPP. USING THE LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY - BIOLUMINESCENT IN BOVINE BLOOD FLOUR FROM 3 REGIONS OF BRAZIL

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Brazil is one of the most important beef producers globally. Blood is a significant and sustainable natural protein source that offers economic feasibility and nutritional benefits. Choosing a rapid and accurate *Salmonella* spp. detection method in blood flour samples is important for monitoring intervention effectiveness and for taking action to avoid product contamination.

Purpose: Determine the sensitivity, relative trueness (RT), Negative Deviation (ND) and Positive Deviation (PD) of the *Salmonella* LAMP-Bioluminescent assay in bovine blood flour compared to ISO 6579-1:2017.

Methods: Bovine blood flour samples (n=60) from 3 Brazilian regions (west-center, north and southeast), were paired tested comparing a rapid method to ISO 6579-1:2017. Fractional artificial contamination was used, five samples from each region were blank and the strain (*Salmonella* Typhimurium ATCC 14028), was spiked in 2 levels, low (n=15, N1=5 CFU/test portion, west-center) and high (n=30, N2=15 CFU/test portion) and all samples were contaminated with 2-3 log₁₀ CFU/test portion above of *Escherichia coli* ATCC 25922. Samples were enriched with BPW ISO (475mL), incubated (37°C/24 hours) and analyzed with LAMP-Bioluminescent assay and by ISO 6579-1:2017. Parameters required by ISO 16140-2:2016 for method comparison were determined.

Results: The alternative LAMP-Bioluminescent presented sensitivity, RT of 100% for both, and ND-PD and ND+PD of zero.

Significance: The Neogen® Molecular Detection assay 2 – *Salmonella* enabled reliable and rapid detection of *Salmonella* spp. in bovine blood flour.

P2-117: DETECTION OF LISTERIA FROM 125 G HAM STEAKS USING THE HYGIENA BAX® SYSTEM REAL-TIME PCR ASSAY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Deli meats and other Ready-to-Eat (RTE) meats pose the highest risk of illness from *Listeria monocytogenes* and are responsible for the majority of human listeriosis cases in the United States. In the last 2 decades, both regulators and industry have made continuous efforts to enhance food safety standards and implement more controls in processing to ensure the absence of *L. monocytogenes* from these products.

Purpose: This study was designed to evaluate the performance of a commercial real-time PCR assay compared to the USDA FSIS reference standard for the detection of *Listeria* from 125 g test portions of RTE ham steak.

Methods: Unpaired test portions were inoculated with *Listeria monocytogenes* at a low level expected to yield fractional positives (n=20) and a high level expected to be all positive (n=5). Five additional samples were left uninoculated for controls. Samples were acclimated at 2–8 °C for 72 hours prior to enrichment. Test samples were homogenized with Actero *Listeria* Enrichment Media (ALM), incubated at 35 °C for 24–48 hours, and tested by real-time PCR and culture. Reference samples were enriched and confirmed according to the USDA FSIS MLG method.

Results: For the test method, 7/20 low inoculated and 5/5 high inoculated samples were detected by real-time PCR and confirmed by culture. The reference method also confirmed 7/20 low inoculated and 5/5 high inoculated samples. Statistical significance was determined using the probability of detection (POD), which indicated no differences between the test and reference methods.

Significance: The study results demonstrate that the BAX System Real-Time PCR assay is sensitive, specific, and accurate for the detection of *Listeria* in 125 g samples of RTE ham steak in 24–48 hours.

P2-118: RAPID SCREENING OF MICROORGANISMS FROM CHICKEN BONE BROTH, CHICKEN STOCK, AND CHICKEN BROTH USING HYGIENA'S ATP-BASED INNOVATE™ RAPID MICROBIAL SCREENING SYSTEM

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Aseptic processing and packing is a widely used method for foods and beverages to produce a commercially sterile, shelf-stable product. This process is designed to eliminate harmful and spoilage organisms, yet contamination can still occur.

Purpose: The objective of these studies was to evaluate a rapid screening method utilizing ATP bioluminescence for the detection of spoilage organisms in broths and stocks compared to standard agar plate methods.

Methods: Six bacterial organisms: *Bacillus cereus* (spores), *Clostridium sporogenes*, *Cronobacter sakazakii*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Staphylococcus aureus* were prepared to inoculate chicken bone broth, chicken stock and chicken broth in separate studies. Organisms were added to triplicate packs at a level of 10-100 CFU, incubated and tested across a 7-day period. Additional packs were left uninoculated for controls. Each day, 50 µL aliquots were transferred to a microtiter plate to detect microbial ATP using the RapiScreen™ Dairy assay. At the same time, samples were plated onto appropriate agars for confirmation.

Results: Pre-established thresholds obtained from the uninoculated packs were used to determine positive

Results with the RapiScreen assay. ATP measurements from all three products tested were equivalent to plating for all days measured but with at least a 24-hour time savings. The probability of detection for all organisms was 100%, with the exception of *Clostridium*. For *Clostridium* only, there was fractional detection and growth in chicken broth and no detection or growth in chicken stock. In all cases, there was method agreement.

Significance: This study demonstrates that the innovate System is sensitive and robust for the detection of microbial ATP from contaminated products, allowing manufacturers to confirm inventory quality for fast and efficient decisions on

product release compared to plating methodologies.

P2-119: VERIFICATION OF A COMMERCIAL REAL-TIME PCR *LISTERIA MONOCYTOGENES* AND *LISTERIA* SPECIES DETECTION METHOD ON INDIVIDUALLY QUICK-FROZEN VEGETABLE MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: With increasing concerns and rising numbers of product recalls associated with pathogens in produce, most major PCR testing platforms are validated for detection of *Listeria* in fresh produce. However, validations for other produce matrices are sparse, particularly for frozen matrices which can severely reduce initial temperatures of enrichment broths.

Purpose: To verify the ability to detect *Listeria monocytogenes* and *Listeria* species in frozen vegetable matrices utilizing real-time PCR.

Methods: Twenty-three different frozen vegetable matrices were tested, including cauliflower, carrots, various vegetable medleys, and chopped spinach: each with seven replicates (125g) inoculated with *L. monocytogenes* at ca. 7.5 CFU /sample. All samples were enriched with 1,125 mL of BACGro Ultra *Listeria* Broth™ (BULB) and incubated at 37°C for 21 ± 3 hours. Following incubation, samples were tested for *Listeria monocytogenes* and *Listeria* spp. using BACGene™ real-time PCR kits according to the manufacturer's instruction. All proprietary media and tests kits were supplied by Gold Standard Diagnostics™.

Results: All seven replicates returned a presumptive positive result for *Listeria monocytogenes* and *Listeria* spp. for each of the 23 matrices, giving a total of 322 presumptive positive PCR results. The FDA microbiological method validation guidelines require 7/7 (or 19/20) presumptive results to consider a method extended to a new matrix, with no statistical analysis necessary. This standard was met 46 times in this study for both *Listeria* spp. and *Listeria monocytogenes*.

Significance: These data demonstrate that this method can reliably detect *Listeria monocytogenes* and *Listeria* spp. in a wide range of frozen vegetable matrices.

P2-120: VERIFICATION OF A COMMERCIAL REAL-TIME PCR SALMONELLA DETECTION METHOD ON INDIVIDUALLY QUICK-FROZEN VEGETABLE MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: With increasing concerns and rising numbers of product recalls associated with pathogens in produce, most major PCR testing platforms are validated for detection of *Salmonella* in fresh produce. However, validations for other produce matrices are sparse, particularly for frozen matrices which can severely reduce initial temperatures of enrichment broths.

Purpose: To verify the ability to detect *Salmonella* in frozen vegetable matrices utilizing real-time PCR.

Methods: Twenty-three different frozen vegetable matrices were tested, including cauliflower, carrots, various vegetable medleys, and chopped spinach: each with seven replicates (375g) inoculated with *S. typhimurium* at ca. 7.5 CFU/sample. All samples were enriched with 3,375 mL of BACGro™ Buffered Peptone Water (BPW) and incubated at 37°C for 21 ± 3 hours. Following incubation, samples were tested for *Salmonella* spp. using BACGene™ real-time PCR kits according to the manufacturer's instruction. All proprietary media and tests kits were supplied by Gold Standard Diagnostics™.

Results: All seven replicates returned a presumptive positive result for *Salmonella* spp. for each of the 23 matrices, giving a total of 161 presumptive positive PCR results. The FDA microbiological method validation guidelines require 7/7 (or 19/20) presumptive results to consider a method extended to a new matrix, with no statistical analysis necessary. This standard was met for all 23 matrices in this study.

Significance: These data demonstrate that this method can reliably detect *Salmonella* spp. in a wide range of frozen vegetable matrices.

P2-121: VERIFICATION OF A COMMERCIAL REAL-TIME PCR SHIGA-TOXIN PRODUCING E. COLI (STEC) DETECTION METHOD ON INDIVIDUALLY QUICK-FROZEN VEGETABLE MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: With increasing concerns and rising numbers of product recalls associated with pathogens in produce, most major PCR testing platforms are validated for detection of STEC in produce. However, validations for other vegetable matrices are sparse, particularly for frozen matrices which can severely reduce initial temperatures of enrichment broths.

Purpose: To verify the ability to detect STEC in frozen vegetable matrices utilizing real-time PCR.

Methods: Twenty-three different frozen vegetable matrices were tested, including cauliflower, carrots, various vegetable medleys, and chopped spinach: each with seven replicates (125g) inoculated with *E. coli* O111 at ca. 30 CFU /sample. All samples were enriched with 1,125 mL of BACGro™ modified tryptic soy broth (mTSB) and were incubated within the AOAC validated enrichment time window of 10-24 hrs. Following incubation, samples were tested for STEC using BACGene™ real-time PCR kits according to the manufacturer's instruction. All proprietary media and tests kits were supplied by Gold Standard Diagnostics™.

Results: All seven replicates returned a presumptive positive result for STEC for each of the 23 matrices, giving a total of 161 presumptive positive PCR results. The FDA microbiological method validation guidelines require 7/7 (or 19/20) presumptive results to consider a method extended to a new matrix, with no statistical analysis necessary. This standard was met for all 23 matrices in this study.

Significance: These data demonstrate that this method can reliably detect STEC in a wide range of frozen vegetable matrices.

P2-122: APPLICATION OF A HIGH AFFINITY PHOTOREACTIVE DNA BINDING DYE TO PREVENT INTERFERENCE FROM DEAD CELL DNA DURING CRONOBACTER DETECTION DURING MOLECULAR DETECTION

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Cronobacter* can be present in various raw materials used to manufacture powdered infant formula (PIF). Although *Cronobacter* is effectively killed during processing, residual DNA from dead cells may remain. If this DNA is amplified during the amplification step, it could result in a non-cultured positive result. This could delay decision-making regarding the sample, impacting laboratory and manufacturing productivity. intercalating dyes (ID) can covalently bind to free DNA and DNA from dead cells without affecting live cells.

Purpose: To evaluate the efficacy of a proprietary intercalating dye (PMAxx™, Biotium) in treating PIF enriched for detection of *Cronobacter* before molecular DNA amplification step utilizing a loop mediated isothermal amplification-based method.

Methods: The efficiency of the intercalating dye was tested on enriched PIF samples containing free DNA from *Cronobacter*. Additionally, enriched PIF samples containing DNA from dead *Cronobacter* cells and heat-shocked live *Cronobacter* cells were assessed. The limit of detection of the LAMP-based method to detect *Cronobacter* after enrichment using pre-treatment with ID was determined using a paired study. A total of 60 samples were analyzed with samples artificially inoculated with heat-shocked *Cronobacter* at ~5CFU/25g, 1CFU/25g and 0CFU/25g. LAMP-based method and a reference culture base method were used for analysis.

Results: The intercalating dye used in this study successfully eliminated the signal of up to 108copies of free DNA/mL and up to 105CFU/mL of dead *Cronobacter* cells. The limit of detection did not show significant differences between the LAMP method pre- and post-ID treatment, and it was not significantly different when compared to a culture base method. Probability of detection analysis provided an interval coefficient that contain zero, indicating no significant difference between

the two methods.

Significance: For molecular assays, pre-treatment of enriched samples with an intercalating dye is an effective method for reducing interference from DNA of dead cells.

P2-123: EVALUATION OF PERFORMANCE OF THE NEOGEN MOLECULAR DETECTION ASSAY 2 - SALMONELLA AS A METHOD TO ANALYZE 375 G TEST PORTIONS OF EGG PRODUCTS WITH A NEXT DAY RESULT COMPARED TO USDA, FSIS MLG 4.14

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Increasing sample size from traditional 25g test portions may affect method sensitivity as well as the recovery of a target pathogen, thus comparing performance of a larger sample size to the sample size defined in a reference method is critical. In this study we evaluated the performance of a loop mediated isothermal amplification and bioluminescence reporting method, when increasing sample size from 100 up to 375g. The method was compared to the United States Department of Agriculture FSIS MLG 4.4 for detection and isolation of *Salmonella*.

Purpose: To determine the performance of the Neogen Molecular Detection Assay 2 - *Salmonella* (MDA2SAL) to detect *Salmonella* in 100 or 375g test portions of different egg products compared to a reference method following ISO 16140-2 guidelines.

Methods: Liquid, hard-cooked, pre-cooked, dried egg and French toast were tested following USDA FSIS MLG 4.4 using 100g test portions and following the Neogen MDA2SAL method using 375g test portions. For each matrix and method n=5 test portions were spiked with 10CFU/sample, n=20 with ~1CFU/g and n=5 with 0CFU/g. N=328 test portions evaluated. All samples analyzed with MDA2SAL were cultured confirmed USDA FSIS MLG 4.4. Probability of detection, construction of 95% confidence interval and calculation of detection limit according to ISO 16140-2 were determined.

Results: For the five types of egg products tested using 325g test portions, the interval coefficient contained zero indicating that the evaluated method was not significantly different from the USDA FSIS MLG 4.4 and the relative limit of detection following an unpaired comparison was below 2.5 which is the

acceptability limit according to ISO 16140-2.

Significance: Increasing test portion to 375g provides an optimized testing solution to laboratories in egg manufacturing facilities which enables faster detection of *Salmonella* as part of their finished product testing scheme.

P2-124: EVALUATION OF PERFORMANCE OF THE NEOGEN MOLECULAR DETECTION ASSAY 2 - LISTERIA MONOCYTOGENES AS AN ALTERNATIVE METHOD TO ANALYZE EGG PRODUCTS WITH A NEXT DAY RESULT COMPARED TO ISO 11290-1:2017

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Category: Laboratory and Detection Methods

Abstract:

Introduction: With the growing use of probiotics products, a key challenge is ensuring their safety by detecting trace amounts of harmful microbes in the presence of high levels of the microbial components.

Purpose: To evaluate accuracy of three next generation sequencing assays in detecting low levels of spiked microbial pathogens in probiotics.

Methods: Probiotic products which contained combinations of varying bacterial species were spiked separately with *Salmonella enterica*, *Escherichia coli*, and *Listeria monocytogenes* strains, at levels ranging from 20 to 2×10^9 CFUs. Probiotic 1 contained only *Lactobacillus rhamnosus*, probiotic 2 contained four species, and probiotic 3 contained fifteen species. We used three sequencing assays: Illumina's NexteraXT for whole metagenome sequencing (WMS), a Microbial ID assay (CDMI) which is a highly multiplex PCR method designed to target the V3 and V4 regions for over 2000 bacterial genomes, and a custom pathogen-targeted amplicon sequencing (TAS) panel. Respective libraries were sequenced using Illumina's MiSeq platform. Bioinformatic workflows were customized for each method in GalaxyTrakr.

Results: TAS allowed for detection of the pathogens in all the products down to 10 CFU. WMS and CDMI showed detection of the pathogens to as low as 1000 to 100 CFU depending on the complexity of the product tested. WMS data showed about one log higher sensitivity than CDMI. Both CDMI and TAS allow for multiplexing without losing sensitivity when compared with WMS.

Significance: We have evaluated three sequencing methods for use in routine

surveillance of live microbial products. While WMS has the advantage of profiling the microbial content of a sample, for the specific purpose of detecting low levels of pathogens in probiotic products, CDMI and TAS are better suited, with TAS being the most sensitive.

P2-125: PERFORMANCE OF A SINGLE-SHIFT *SALMONELLA* QUANTIFICATION IN GROUND POULTRY MEAT USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION AND BIOLUMINESCENCE

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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. The United States Department of Agriculture has proposed a framework in which establishment of process control monitoring and final product standards are critical components. These require quantification of defined levels of *Salmonella* and/or identification of specific serotypes.

Objective: To evaluate the performance of the Molecular Detection Assay 2Q – Quantitative *Salmonella*, based on loop-mediated isothermal amplification and bioluminescence reporting, to quantify *Salmonella* in ground poultry matrices.

Materials and Methods: Portions of 325 g of ground chicken and ground turkey meat were spiked at 0 CFU/g, 1 CFU/g, 10 CFU/g, and 100 CFU/g (n = 20 samples per inoculation level and poultry type; N = 160) using a cocktail of four *Salmonella* serotypes. Samples were homogenized with 400 mL of Buffered Peptone Water. An aliquot of 30 mL of homogenate was enriched with 30 mL of a proprietary media and a second aliquot was used to determine MPN. Homogenates were incubated for 5–6 hours. An aliquot of 1.5 mL was processed to be analyzed using LAMP. LAMP-bioluminescence graphic output is analyzed by a model that reports the

concentration of *Salmonella*. Variance comparison, paired t-test, and log difference to MPN were calculated. Robustness to typical laboratory parameters was evaluated.

Results: There was no significant difference between the LAMP and MPN methods in all inoculation levels evaluated ($p > 0.05$). The paired t-test with MPN at each level had a p-value > 0.05 . The log mean difference at each level was within $+0.5$ log CFU/g. LAMP was robust to operators, hardware, and lot-to-lot variations. The method consistently quantifies samples with 1 CFU/g.

Significance: The LAMP offers a cost-effective, accurate, and rapid method for quantification of *Salmonella* to support poultry producers in verifying *Salmonella* intervention effectiveness and evaluating final product safety.

P2-126: ACCURATE DETECTION OF FOODBORNE PATHOGENS: CHROMOGENIC MEDIA INNOVATION FOR BACILLUS CEREUS, STAPHYLOCOCCUS AUREUS, AND CLOSTRIDIUM PERFRINGENS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens* are prevalent pathogens that can contaminate a wide range of processed foods, leading to foodborne illnesses characterized by diarrhea and emesis.

Purpose: in this study, three newly developed chromogenic selective media—BCCA (*B. cereus* Chromogenic Agar), SAS (*Staphylococcus* spp. Chromogenic Sensorgen Agar), and CPS (*C. perfringens* Chromogenic Sensorgen Agar)—were evaluated for their effectiveness in detecting and isolating these pathogens.

Methods: To assess the inclusivity of these media, 10 strains of each pathogen, including both reference and food-isolated strains, were selected. Exclusivity was evaluated by streaking 10 strains of various foodborne pathogens on the chromogenic agar plates. Mannitol egg Yolk Polymyxin agar, Baird-Parker agar, and *Clostridium perfringens* agar, Korean Food Code standards, were also used under identical conditions.

Results: The results of our study on the three types of chromogenic media were evaluated by Korean accredited testing institution. The media were assessed as positive when colored colonies and yolk lecithinase or yolk lipase were

simultaneously formed. For 40 selected strains, BCCA, SAS, and CPS demonstrated 100% inclusivity and exclusivity, respectively.

Significance: Our results contribute to public health by preventing foodborne illnesses for effective novel advancement in foodborne pathogen detection.

P2-127: DEVELOPMENT OF REAL-TIME PCR ASSAY WITH RISK MONITORING CAPABILITIES FOR THE DETECTION OF E. COLI O157:H7 IN BEEF AND PRODUCE

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The beef industry is continuously seeking solutions to produce quick and predictable results that accurately identify the presence of adulterant *E. coli* O157:H7. To control and potentially minimize the risk of an *E. coli* O157:H7 outbreak, a real-time PCR assay for the detection of *E. coli* O157:H7 was developed with an additional target. The additional target serves as an indicator of conditions favorable for an O157:H7 contamination event and can be used as a risk monitoring tool.

Purpose: To demonstrate the utility of the BAX® Real-time *E. coli* O157:H7 Exact plus S Assay for the detection of *E. coli* O157:H7, and as a process risk monitoring tool in relevant food samples.

Methods: Over 80 samples were tested using this assay, utilizing enrichments according to BAX® testing procedures with comparisons to USDA MLG reference methods. The matrices tested included ground beef (375g), beef trim (375g), MicroTally® on beef trim (375g), and leafy greens (375g). Enrichment samples were inoculated with levels between 0.44 to 1 CFU per mL. Additionally, industry partners submitted samples for testing and evaluation of the assay.

Results: Fractional detection levels were achieved at target sensitivity for each matrix (30%), with plating results in agreement. Samples from industry partners performed as expected, with evidence suggesting that a higher rate of S-target positives indicates an increased chance of pathogenic *E. coli* contamination in subsequent days.

Significance: The results of this study support that adulterant *E. coli* O157:H7 can be quickly detected in beef products. The assay enables the industry to make quick decisions regarding their beef product(s), minimize the potential for illness to consumers, and actively monitor conditions that could lead to unwanted

outbreaks or contamination.

P2-128: EVALUATION OF RAPID ALTERNATIVE METHOD OF COMMERCIAL STERILITY TESTING ON LONG SHELF-LIFE FOODS WITH SOLERIS® METHOD

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Commercial sterility testing for long shelf-life foods requires 13 days, making it critical to shorten this timeline for timely production decisions in the food industry.

Purpose: To develop and evaluate an alternative method with shorter incubation and detection time for long shelf-life food products compared to the traditional method.

Methods: Commercial sterilized food product Galbitang (Korean beef rib soup) was spiked with two different microorganisms: *Staphylococcus capitis* and *Streptococcus oralis*, at three different levels: none (n=3), low (<10 CFU/g, n=20), and high (>100 CFU/g, n=3). Each sample was incubated at 36 °C for 3 days as pre-enrichment and microbial detection was performed using Soleris® NF-105 vials, resulting in 24-hour detection. For the reference method, pre-incubation was performed for 10 days at 36 °C, followed by 1 day incubation at room temperature (24–26 °C). One mL of sample was transferred to Fluid Thioglycollate medium and incubated at 36 °C for 48 hours for microbial detection (K-Food Code). Statistical comparison was performed using paired qualitative analysis.

Results: All uninoculated samples showed negative results. In the paired study, the alternative method demonstrated 100% agreement with the traditional method, indicating that the alternative detection method is compatible with traditional sterility testing and enhances pre-incubation time needed for detection. The analytical performance of the alternative method is equivalent to the traditional K-Food Code while providing results 9 days earlier.

Significance: This alternative method for long shelf-life food products shortened the detection time to 36 hours of incubation and 24 hours of detection, which can save approximately 9 days until product release.

P2-129: COMPARATIVE PERFORMANCE OF EASY PLATE AC-R FOR RAPID AEROBIC BACTERIA COUNT IN MULTIPLE FOOD TYPES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The aerobic plate count is crucial in food microbiological testing, but traditional methods such as Plate Count Agar (PCA) take 48 hours for enumeration. The newly developed ready-to-use (RTU) media for aerobic bacteria count, Easy Plate AC-R (RTU-A) by Kikkoman Biochemifa Company, are convenient and environmentally friendly, and allow for rapid enumeration in just 24 hours.

Purpose: The enumeration performance of RTU-A, PCA, and three other RTU media (RTU-X, RTU-Y, RTU-Z) was compared using various commercially available food samples.

Method: Sixty-four food matrices, including meats, seafoods, vegetables, fruits, dairy products, and prepared foods such as RTE foods and dried foods, were used for evaluation. Each medium was inoculated with 1 mL of food dilution and incubated at 35 ± 1 °C. Colony counts were performed visually at 24 ± 1 hours for RTU media and at 48 ± 2 hours for PCA. Counts for RTU-A were performed not only visually but also using Colony Counting System (CCS).

Result: The correlation coefficients between RTU-A and PCA, as well as RTU-X, RTU-Y, and RTU-Z, were 0.986, 0.990, 0.993, and 0.996, respectively. RTU-A demonstrated equivalent enumeration performance to PCA at 48 hours incubation, with comparable results achieved in just 24 hours. In addition, the correlation coefficient between visual colony counts and those obtained by CCS for RTU-A was 0.979. CCS tended to detect higher numbers of colonies compared to visual counting, confirming its ability to accurately count small and pale colonies.

Significance: The newly developed RTU-A has demonstrated the ability to perform aerobic bacteria count in significantly less time than conventional PCA methods. When combined with CCS, it is expected to further reduce operator workload, standardize processes and improve traceability.

P2-130: HIGH-THROUGHPUT RAPID ISOLATION OF SALMONELLA ON AN AUTOMATED PLATFORM MAGICYTE MB

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Conventional methods for isolation of *Salmonella* from food are slow, resource intensive, and low-throughput. In addition, colony picking based on endpoint observation cannot determine if a colony is discrete.

Purpose: This work aims to demonstrate a fully automated workflow for high-throughput rapid isolation of discrete *Salmonella* colonies on MagiCyt MB, a benchtop robot that integrates the functions of sample processing, liquid handling, agar dispensing, microplating, incubation, time-lapse imaging, real-time image analysis, result reporting, and colony picking.

Methods: Sixteen *Salmonella* Typhimurium-inoculated onion powder and spinach samples were added into a 96-well “sample” plate with anti-*Salmonella* antibody-conjugated magnetic beads. The sample plate was then loaded into the MagiCyt MB instrument, along with an empty 96-well “culture” plate, an empty 96-well “output” plate, pipette tips, and a trough of molten XLT4 agar. Inside MagiCyt MB, bead-bound *Salmonella* cells in each of the 16 samples were isolated by a magnet array and then serially diluted into 6 wells in the sample plate. All diluted samples were then plated in the “culture” plate filled with XLT4 agar and incubated for 12 hours. During incubation, MagiCyt MB took an image of each well every hour. Time-lapse images of each well were automatically analyzed for MagiCyt MB to detect microcolonies and rank them based on growth, morphological change, and discreteness. After incubation, MagiCyt MB aspirated top-ranking colonies with pipette tips and transferred them to wells in the “output” microplate. Before and after picking each colony, MagiCyt MB took an image to evaluate the success of colony picking. Sixteen randomly selected colonies in the output plate were subcultured on chromogenic agar for *Salmonella* colony confirmation.

Results: MagiCyt MB identified 9 to 24 discrete colonies and then picked 6 colonies from each sample. At least 5 colonies were successfully picked from each sample based on pre- and post-picking image analysis. All sixteen subcultures

were *Salmonella* positive.

Significance: This method addresses a bottleneck in high-throughput *Salmonella* testing workflow and makes discrete colonies available for downstream analyses days earlier.

P2-131: HIGH-THROUGHPUT RAPID PHENOTYPIC SCREENING OF SALMONELLA

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Conventional culture methods for detection of *Salmonella* from food are labor intensive and take four days to get results. As an alternative, labs often use molecular methods (e.g., PCR) for rapid screening and then culture presumptive positive samples for downstream confirmation. But such an approach could lead to discrepancies between genotypic and phenotypic results.

Purpose: This study aimed to demonstrate a novel high-throughput “sample-to-answer” method for rapid phenotypic screening of *Salmonella* from food.

Methods: Two brands of spinach were bought from grocery stores. Twelve samples (25g each) were inoculated with 5 CFUs of *Salmonella*; twelve samples (25g each) were inoculated with 5 CFUs of *Salmonella* and 5 CFUs of *E. coli*; three samples were inoculated with 5 CFUs of *E. coli*; and three samples were uninoculated. After 22 hours of pre-enrichment at 42°C, a 10 µL aliquot of each sample was mixed with anti-*Salmonella* antibody-conjugated beads in a 96-well “sample” plate and then loaded into the MagiCyte MB for analysis. MagiCyte MB is a benchtop robot that integrates the functions of sample processing, liquid handling (e.g., dilution, washing, mixing), agar dispensing, microplating, incubation, time-lapse imaging, real-time image analysis, result reporting, and colony picking. Inside MagiCyte MB, bead-bound *Salmonella* cells in each sample were isolated by a magnet array, plated in the “culture” plate filled with XLT4 agar, and incubated at 37°C for 12 hours. During incubation, MagiCyte MB took an image of each well every hour. Time-lapse images of each well were automatically analyzed by the MagiCyte MB algorithm to identify *Salmonella* based on colony morphology and metabolic activities.

Results: Colonies grew in all 27 samples inoculated with bacteria. Only the 24 samples inoculated with *Salmonella* were identified by the MagiCyte MB algorithm as *Salmonella* positive. MagiCyte MB obtained screening results of all 30 samples with 100% accuracy and a turnaround time of 36 hours.

Significance: MagiCyte MB provides a novel solution for rapid phenotypic

screening of *Salmonella* from food. The fully automated “sample-to-answer” workflow is well suited for high-throughput settings..

P2-132: EXPLORING THE POTENTIAL OF DEEP EUTECTIC SOLVENTS IN CONCENTRATING NON-ENVELOPED VIRUS FOR IMPROVING RAPID VIRUS DETECTION

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Deep Eutectic Solvents (DESs) are an emerging class of environmentally friendly reagents that are composed of Lewis or Bronsted acids and bases that form eutectic mixtures with adjustable properties, depending on the ratios of the components in the solvent. They have the potential to serve as reagents for concentration of noroviruses.

Purpose: The purpose of this study was to examine the ability of DESs to concentrate bacteriophage MS2.

Methods: MS2 was diluted to 10^5 PFU/mL in PBS, vortexed at a range of times with different DESs (Cobalt-, Nickel-, and Dysprosium-based), and separated using a magnet. The supernatant was removed and samples were washed to remove unbound virus. Captured MS2 was eluted by vortexing with modified Luria Broth and recovered virus was quantified by RT-qPCR. Infectivity testing was performed by completing a plaque assay.

Results: The cobalt-based DES displayed 68.8% (SD 15.6%) capture and 32.9% recovery (SD 9.1%); the nickel-based DES displayed 60% capture (SD 7.8%) and 36% recovery (SD 6.2%); and the dysprosium-based DES displayed 99% (SD 0.4%) capture and 2% recovery (SD 0.8%) (n=3). All experiments were performed with 30 seconds of dispersion. DESs displayed promising capture results, though recovery was notably lower for all DESs—especially dysprosium—suggesting that further optimization of elution conditions could improve recovery. Increasing optimization time raised capture percentages for cobalt (79.7%, 2 mins) and nickel (97.2%, 2 mins), but no change was observed for dysprosium. Plaques were recovered, indicating that intact virus was recovered without damaging the

capsid.

Significance: These results indicate that DESs have the potential to recover significant amounts of non-enveloped viruses and could serve as green, cost-effective reagents for virus concentration from food and environmental samples.

Award Application: Developing Scientist Award

P2-133: MICROBIOMES OF LETTUCE GROWN ADJACENT TO CAFO REVEAL PRESENCE OF PEN SOIL

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Concentrated animal feeding operations (CAFOs) and fresh produce growing fields are in proximity with each other in some agricultural settings, creating a possibility for STEC contamination of fresh produce by aerial deposition of cattle pen soil.

Purpose: To elucidate the impact of distance on fugitive dust deposition between a large CAFO and produce fields, microbial source tracking was applied to outer romaine lettuce leaf microbiomes.

Methods: Metagenomic sequencing was performed on soil (n=449), irrigation water (n=70), and composite outer lettuce leaf (n=199) samples collected from five experimental plots in different growing seasons and located 0.26 to 1.97 miles from a CAFO (>100,000 cattle) in the southwestern US, as well as on cattle pen soil (n=8) from a cattle feedlot in the southwest. For each field separately, proportions of source bacterial communities (pen soil, field soil, and water) were computed for individual lettuce bacterial communities using SourceTracker2. Lettuce metagenomes were also queried for cattle mitochondrial DNA (mtDNA) using exact matching with a privately curated database.

Results: The median percent lettuce bacterial communities similar to cattle pen

soil ranged from 1.6% to 35.9%, with lettuce grown a greater distance from the CAFO having lower proportions of pen soil (Wilcoxon rank, $P < 0.0001$). In addition, the relative abundance (RA) of *Corynebacterium maris*, a bacterial species associated with cattle, found in the lettuce metagenomes followed the same trend (Wilcoxon rank, $P < 0.0001$) with median RA values of 1.0% and 22.9% for 1.97 and 0.26 miles, respectively. While cattle mtDNA was detected in 65.6% of the lettuce samples located 0.26 miles from the CAFO, a prevalence rate of only 7.8% was observed at 1.97 miles.

Significance: This work highlights the importance of including the potential for airborne transmission of pathogens in risk assessments for contamination of fresh produce grown in proximity to CAFOs.

P2-134: INFLUENCE OF POSTBIOTIC INCLUSION ON FECAL SHEDDING OF SALMONELLA ENTERICA IN BEEF-ON-DAIRY CROSS CATTLE IN A TEXAS FEEDLOT PROXIMAL TO HARVEST

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: The “beef-on-dairy” crossbreeding strategy is a recent phenomenon creating economic value for dairy producers. *Saccharomyces cerevisiae* fermentation-derived postbiotics (SCFP) support rumen development, health, and performance and may support reducing colonization potential of *Salmonella enterica*.

Purpose: This trial evaluated *Salmonella* fecal shedding in beef-on-dairy cattle supplemented with or without SCFP postbiotics from birth to proximal harvest weight.

Methods: A convenience sampling of pens containing Angus x Holstein crossbred cattle, supplemented with or without SCFP (LiquiCare and NaturSafe, Diamond V, Cedar Rapids, IA, USA) from birth to harvest, were selected at the feedlot to include heifers and steers (n=10 pens/sex). Enrolled pens were sampled on three occasions from October to December of 2023. On each visit, n=20 fresh fecal pats were randomly sampled aseptically (20-25g), immediately placed into a cooler, and transported to the laboratory. Upon receipt, 10g of feces were diluted 1:10 in 90mL of pre-warmed 42°C BAX MP media, a retain reserved, overnight incubation at 42° ± 1°C, and screening by the BAX system real-time *Salmonella* detection assay. Retains from positive samples were subject to *Salmonella* enumeration by the BAX SalQuant® test according to manufacturer’s directions.

Results: Overall *Salmonella* prevalence in the feces of SCFP fed cattle was 5.53% (95% CI; 3.84 – 7.89), significantly (P=0.0002) lower than the Control cohort at 14.12% (95% CI; 11.38 – 17.39). Within sampling rounds, prevalence in the SCFP cohort was consistently lower than Control for October (6.00% vs. 14.50%), November (7.00% vs. 12.50%) and December (4.00% vs. 15.50%), respectively. Of *Salmonella* positive samples, 51.2% of Control were enumerable with a mean load

of 1.30 log₁₀ CFU/g as compared to 38.2% of SCFP samples with a mean load of 0.98 log₁₀ CFU/g.

Significance: These data suggest SCFP dietary inclusion may aid in the reduction of *Salmonella* colonization in beef-on-dairy crossbred cattle and have utility as a preharvest food safety intervention.

P2-135: BIOCHAR TYPE AFFECTS E. COLI REMOVAL FROM IRRIGATION WATER BY SAND FILTRATION

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction; Fresh produce recalls and foodborne illness outbreaks continue to occur, and contaminated irrigation water has been implicated in some outbreaks. Biochar has been previously used in filters to remove bacteria from water, although little work has compared different types of biochar to optimize bacterial filtration.

Purpose: To compare the effectiveness of various biochars to remove *E. coli* from simulated irrigation water.

Methods: Biochar-sand filters were constructed from 108 or 216 mm (length) x 52.5 mm (inner diameter) PVC pipe at 50:50 (wt.:wt.) biochar:sand ratios (either wet-packed or dry packed). Various biochars (n=24) were compared, including those pyrolyzed from hardwood; softwood; switchgrass; miscanthus, wheat, or oilseed rape straw; walnut, almond, cacao, or rice husk; guayule leaf; paper; corn stover; and horse litter. Biochar was pyrolyzed in-house or acquired from other sources, including commercial manufacturers. Simulated irrigation water was inoculated with 4.5 log CFU/ml rifampicin-resistant generic *E. coli*, pumped through filters at 0.5 L/min, and *E. coli* populations determined in the effluent, by plating on chromogenic agar with 50 ppm rifampicin, as well as with Idexx Colilert.

Results: *E. coli* reductions ranged from 0.1-4.5 log, varying based on biochar type, filter length, and biochar:sand media moisture content used when packing filters (i.e., wet- or dry-packed). The 216 mm filters reduced significantly more *E. coli* (regardless of biochar type) as did wet-packed biochar filters when compared with dry-packed filters ($p < 0.05$). Biochar that effected < 0.78 log reductions included those pyrolyzed from cacao, horse litter, wheat straw, as well as hardwood and softwood pyrolyzed at $\leq 700^\circ\text{C}$. Biochars that removed > 1.0 log of *E. coli* included those pyrolyzed from oilseed rape and miscanthus straw, almond shell, paper, and guayule leaf. A commercial softwood biochar (pyrolyzed at $700\text{--}1,000^\circ\text{C}$) was the

most effective, consistently removing >4 log *E. coli* from irrigation water.

Significance: Results may be used to scale-up laboratory biochar:sand filters for field studies to remove *E. coli* from irrigation water.

P2-136: INVESTIGATING SALMONELLA CONTAMINATION OF ROMAINE LETTUCE PRODUCTION IN THE DESERT SOUTHWEST

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Between 1.4%-2.5% of *Salmonella* illnesses can be attributed to leafy green (LG) consumption. Many routes of preharvest contamination of LGs exist including contaminated agricultural water and fugitive dust from nearby animal operations. The desert southwest is a major producer of LGs in the US. Understanding the movement of pathogens in this region is critical to safe LG production.

Purpose: Routes and mechanisms of *Salmonella* contamination of Romaine lettuce grown in an agricultural environment in the desert southwest were assessed.

Methods: Research plots were established at two distances (~1 mi and ~2 mi) from a large (>80,000 animals) concentrated animal feeding operation (CAFO) over two growing seasons (2022-2023 and 2023-2024). Plots followed industry practices, with two plantings per season (fall and spring). Samples collected included soil, drag swabs, passive air, canal water, sediment, and lettuce tissue. Modified FDA-BAM

Methods were used to culture *Salmonella*, followed by whole genome sequencing

to identify serovars.

Results: Over 3,300 samples were analyzed, with an overall *Salmonella* prevalence of 2.5% (range: 1.3% in spring 2023 to 4.7% in fall 2022). Fall plantings had higher prevalence and serovar diversity compared to spring. Over 300 isolates and 34 serovars were recovered, with water samples showing the highest positivity rate (65% vs 12% all other samples). The same five serovars were found in water and lettuce tissue in fall 2022. only one passive air sample tested positive, with a serovar not matching others in the study. Typhimurium was recovered consistently, while Newport, Mbandaka, Tennessee, and Poona were found only in fall plantings.

Significance: The findings suggest that fugitive dust may not be a significant route of contamination, while water may serve as a reservoir for *Salmonella* and may contribute to lettuce contamination; this information offers insights for developing best management practices in the region.

P2-137: ASSESSMENT OF FLIES IN PROXIMITY TO LIVESTOCK AND PRODUCE PRODUCTION SYSTEMS IN TEXAS: INVESTIGATING THEIR ROLE AS SENTINELS OF ANTIMICROBIAL RESISTANCE IN *SALMONELLA* ENTERICA

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Flies are frequently associated with food spoilage because they thrive in unsanitary environments. Their close association with livestock and food chains further increases contamination risks. Flies can also vector foodborne microbial pathogens such as *Salmonella enterica*, which frequently exhibit antibiotic resistance and pose a significant challenge to public health.

Purpose: This research examined spatial distribution and antimicrobial resistance (AMR) profiles of *Salmonella enterica* recovered from flies captured in high agricultural commodity-producing counties in Texas to evaluate their potential usefulness as AMR pathogen sentinels.

Methods: House flies and blow flies were gathered from six counties across central and south-central Texas. Four exterior areas were sampled 25.75–27.4 km from a central location in each county, with three traps positioned 100 meters apart at every location. Flies from a particular point/trap belonging to the same species were combined and homogenized for *Salmonella* detection and recovery. The presence of *Salmonella* spp. was confirmed using Neogen's 3M Molecular Detection System (MDS) following selective enrichment and plating. Confirmed *Salmonella* isolates were serotyped and antimicrobial susceptibility (AST) assessed using the SensiTitre AST system. Statistical analysis was conducted on categorical data.

Results: A total of 230 samples were collected and processed, of which 89 were positive for *Salmonella* spp. The predominant serovars identified were *Salmonella* infantis (n=32), Enteritidis (n=28), Muenster (n=12), Montevideo (n=10), Braenderup (n=5), Anatum, and Kentucky (n=1 each). Chi-square testing indicated significant differences in the distribution of antimicrobial sensitivity among serovars ($P<0.001$) and across six counties ($P<0.001$). Tetracycline,

nalidixic acid, streptomycin, ampicillin, amoxicillin/clavulanic acid, and azithromycin were drugs most frequently associated with AMR *Salmonella* isolates from sampling sites.

Significance: Flies captured near livestock and fresh produce farms can be informative surveillance tools for monitoring the presence and transmission of antimicrobial-resistant bacterial pathogens.

Award Application: Developing Scientist Award

P2-138: SURVIVAL DYNAMICS OF FOODBORNE PATHOGENS ON MICROGREENS UNDER DIFFERENT STORAGE TEMPERATURES

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: This study emphasizes the serious food safety issues associated with fresh microgreens consumption by highlighting the internalisation and persistence of foodborne pathogens on microgreens throughout storage.

Purpose: This study aims to determine the survival of *Escherichia coli* O157, *Listeria monocytogenes*, and *Salmonella enterica* on microgreens stored at various temperatures.

Methods: Standardised concentrations of each pathogen were applied to microgreen surfaces for inoculation. The samples underwent storage in controlled conditions and were analysed at predetermined intervals (Day 0, Day 2, Day 4, Day 7, Day 14). To assess pathogen survival, samples were homogenized, subjected to serial dilutions, and plated on selective media. Pathogen levels were quantified by enumerating colony-forming units per Gram (CFU/g), with subsequent 16S rRNA analysis. Control samples without inoculation were maintained to monitor natural microbial growth. The investigation also incorporated microscopy techniques to examine potential pathogen internalisation within plant tissues.

Results: Preliminary results show differential survival rates among the pathogens, with refrigeration significantly slowing but not eliminating microbial growth. For example, *Salmonella enterica* showed a reduction (2 log) by Day 14 under refrigeration, while *Listeria monocytogenes* demonstrated greater resilience. internalisation has been observed in microgreen tissues, underscoring challenges in decontamination and the associated risks for raw consumption.

Significance: This study contributes critical insights into the behavior of foodborne pathogens on microgreens during storage for the first time and helps develop targeted interventions to mitigate microbial risks. The findings stress the importance of tailored safety protocols for minimally processed produce and have significant implications for producers, regulators, and consumers in ensuring the safety of microgreens.

P2-139: EFFECT OF STORAGE TIME AND TEMPERATURE ON THE MICROBIAL LOAD IN COMPOST AND COMPOST TEAS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Compost and compost teas have gained popularity among farmers for their potentials to control plant diseases, enhance plant nutritional content, and support soil microbial communities. However, concerns over the microbial distribution in these materials necessitate investigation.

Purpose: This study aimed to evaluate the microbial load and nutrient composition in selected commercial composts and compost teas.

Methods: Duplicate samples of three solid composts and three compost teas of commercial origin were stored at 4°C and 22°C for 16 weeks and analyzed for nutrient composition and the populations of total aerobes (TA), total yeasts and molds (YM), total and fecal coliforms (TC and FC), Enterococci (EC), *Salmonella*, and *Listeria* spp. Data obtained were analyzed using Fisher's Least Significant Difference test with Statistical Analysis Software.

Results: Results showed that the mean YM counts in samples stored at the two temperatures were not significantly different ($P > 0.05$). The mean TA and TC counts in samples stored at 22°C were higher ($P < 0.05$) than those stored at 4°C, and the opposite phenomenon was observed with the mean *Listeria* counts. Mean count of each microbial group from all six composts fluctuated as storage time passed. Compost tea I had the highest TA, YM, and *Listeria* counts, whereas the three counts were the lowest in compost tea II. Solid compost II had the highest TA and *Listeria* counts and compost I had the highest YM and TC counts, while compost III had the lowest TA, YM, TC, and *Listeria* counts. No *Enterococci* and *Salmonella* were detected from all examined samples. The nutrient composition of all six samples remained steady during the storage period.

Significance: The microbial levels in evaluated composts and compost teas vary and are influenced by storage conditions. The study provides useful information to commodity growers interested in including composts or compost teas in agricultural production.

Award Application: Developing Scientist Award

P2-140: EFFICACY LEVELS OF ASCAROSIDE #18 IN CONTROLLING ENTEROHEMORRHAGIC E. COLI ON SEEDS AND SPROUTS OF VARIOUS PLANTS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Several previous studies revealed the efficacy of ascaroside #18 treatment for controlling *Salmonella enterica* and enterohemorrhagic *Escherichia coli* growth on alfalfa and fenugreek seeds and sprouts. The minimal and optimal concentrations of the treatment on alfalfa seed and sprouts have been determined by newly conducted research.

Purpose: This study assessed whether ascaroside #18 would be effective for controlling pathogen growth in other plants under similar experimental conditions.

Methods: Sanitized commercial fenugreek, spinach, radish, and lettuce seeds were exposed to 1 mM, 0.1 mM, 10 μ M, or 1 μ M of ascaroside #18 for 30 minutes before being immersed into each of four individual EHEC cell suspensions. The seeds were then sprouted on 1% water agar with or without the supplementation of an appropriate concentration of ascaroside #18. Inoculated seeds not treated with ascaroside #18 were sprouted on regular agar as treatment controls. On days 0, 1, 3, 5, and 7, EHEC populations in collected samples were enumerated. Data were analyzed using ANOVA in SAS.

Results: Plant type, treatment concentration, and sprouting time were the main effects ($P < 0.05$) influencing EHEC populations on seeds and sprouts. The mean EHEC population on spinach samples was the highest, while fenugreek samples had the lowest mean EHEC population. The minimal inhibitory concentration of ascaroside #18 was 10 μ M, and the most effective concentration was 1 mM when ascaroside #18 was included in water agar. Cell populations increased continuously during the seedling process.

Significance: This project confirmed that ascaroside #18 can be used in plants other than alfalfa. It identified the appropriate treatment concentrations. These findings suggest that ascaroside #18 treatment could be used as one of the interventions in agricultural practices, specifically for seeds and sprouts.

Award Application: Developing Scientist Award

P2-141: SPATIAL MODELING APPROACH FOR CHARACTERIZING WATER RUNOFF PATHWAYS TO IDENTIFY POTENTIAL PATHOGEN SOURCES AND SAMPLING LOCATIONS ASSOCIATED WITH NEARBY PRODUCE FARMS

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Category: Pre-Harvest Food Safety

Abstract:

Introduction: Pathogens can be transported to produce farms from nearby land through various pathways, including water runoff. Runoff water may originate from excessive rainfall, agricultural irrigation, overflowing rivers, streams, or sewer systems, and overwhelmed or poorly functioning septic systems.

Purpose: Our study developed an approach to quantitatively and geospatially characterize a sub-basin area potentially contributing water runoff to a given produce farm.

Methods: We randomly selected one farm among many located within a diverse 226 km² watershed in the Western United States as a case study to develop our runoff modeling approach. The watershed encompasses diverse land uses, including urban areas, riparian zones, farmland, grazing lands, and Confined Animal Feeding Operations (CAFOs). To construct the model, we used publicly accessible satellite imagery to identify potential pathogen sources near the farm and utilized 1- meter Digital Elevation Model (DEM) data to delineate watershed boundaries, water flow directions and generate the stream network.

Results: The runoff area draining into the targeted produce farm was ~3 km² and included several potential pathogen sources. of 10 nearby CAFOs, three were identified as likely contributors to pathogen contamination through runoff. A nearby river was determined to have a low potential for contributing runoff while three septic tanks, among many, were identified as potential contributors. The drainage endpoint was also identified.

Significance: Our water runoff modeling approach demonstrates that publicly available data can effectively delineate runoff areas relevant to a particular farm or farming area. This method can aid farmers and investigators in identifying (and mitigating) potential pathogen sources from nearby land/water. The modeling can

also identify strategic sampling locations, especially those associated with drainage endpoints. The information gained from such an analysis could be used to enhance food safety through strategic planning and inform root cause investigations during foodborne outbreaks or contamination events.

P2-142: EFFICACY OF PEROXYACETIC ACID-BASED SANITIZER TO INACTIVATE TULANE VIRUS USING A MODIFICATION OF THE EPA/FDA PROTOCOL FOR REDUCTION OF FOODBORNE BACTERIA IN AGRICULTURAL WATERFOR PREHARVEST

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Pre-harvest agricultural water (ag water) is a potential route of fresh produce contamination by viral and bacterial pathogens. The FDA allows treatment of non-compliant ag water; however, water treatments are registered based on efficacy against bacterial pathogens only, and their efficacy against viral pathogens is unknown.

Purpose: To determine the concentration of a commercial sanitizer containing 15% peroxyacetic acid (PAA) and 10% hydrogen peroxide necessary to achieve a 3-log reduction and the concentration to completely inactivate the human norovirus surrogate Tulane Virus (TV) in simulated pre-harvest ag water.

Methods: The EPA/FDA “Efficacy Protocol for Reduction of Foodborne Bacteria in Agricultural Water for Preharvest” was modified for use with TV. TV at 10^7 PFU/mL was inoculated into test ag water and equilibrated to test temperature (25°C) for 30 minutes. Sanitizer dose was solely based on PAA concentration. PAA was added to the test mixture to achieve a concentration of 0, 8, 15, 84, 110, 133, 167, or 334 ppm with a contact time of five or ten minutes, then neutralized with sodium thiosulfate ($n=6$ per concentration). Neutralized samples were enumerated by plaque assay on LLC-MK2 cells.

Results: PAA at concentrations of 110 ppm and above were sufficient to reduce TV by 3 log PFU/mL at five minutes. A concentration of 334 ppm was necessary to reduce TV below the limit of detection (2.32 log PFU/mL). The maximum allowable concentration of PAA in ag water, 133 ppm, was not sufficient for complete inactivation of TV at a five- or ten-minute contact. Treatment with 8 ppm of PAA resulted in a significant ($p<0.05$) reduction in TV but only achieved a 1-log reduction.

Significance: These data indicate that the methods for reducing risk of pathogen contamination of fresh produce by bacterial pathogens may not be sufficient for viral pathogens in agricultural water.

P2-143: INTERACTIONS OF FOODBORNE HUMAN PATHOGEN (E. COLI O157:H7) AND PECTINOLYTIC BACTERIA (DICEYA FANGZHONGDAI) ON DIFFERENT VARIETIES OF ONION (ALLIUM CEPA)

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Fresh produce including onions is widely consumed raw, with an average U.S. per capita consumption of 19.18 pounds. However, onions have been linked to foodborne illness outbreaks, particularly due to *E. coli* O157:H7, raising questions about bacterial contamination.

Purpose: This study investigated the influence of soft rot pathogens (SRP- *Dickeya fangzhongdai*) on the survival and proliferation of foodborne pathogens (*E. coli* O157:H7) in yellow, red, and green onion varieties.

Methods: *E. coli* O157:H7 was chromosomally labeled with mScarlet, and experiments were conducted by inoculating the foodborne pathogen alone and in the presence of SRP (*D. fangzhongdai*) in yellow, red, and green varieties of onion plants. The *E. coli* O157:H7 population in the onion plants was monitored at 7- and 14-days post-inoculation (dpi) by recovering and enumerating the pathogen from different sections of onion plants which also included onion bulbs (crown and middle section of bulb). Colonies were plated onto media, scanned under a fluorescence microscope, and confirmed via end-point PCR. Experiments were repeated three times with three replicates per onion variety, and data were statistically analyzed using ANOVA and Tukey's test ($\alpha < 0.05$).

Results: At 14 dpi, the plants co-inoculated with SRP showed significant populations of *E. coli* O157:H7 in different parts of yellow (up to $8.23 \pm 0.36 \log_{10}$ CFU/g), red (up to $8.26 \pm 0.29 \log_{10}$ CFU/g), and green onions (up to $8.50 \pm 0.18 \log_{10}$ CFU/g). In contrast, *E. coli* O157:H7 was not detected in any sections of onions that were not co-inoculated with SRP.

Significance: The presence of pectinolytic bacteria enhanced the survival and proliferation of *E. coli* O157:H7 in onion varieties, underscoring the importance of

plant pathogens in food safety and public health.

P2-144: INTEGRATED CROP-LIVESTOCK GRAZING EFFECT ON FOOD SAFETY OF SPINACH IN MARYLAND

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Expansion of integrated crop-livestock systems (ICLS) is often limited by concerns that livestock grazing may introduce pathogens into soil, which could subsequently transfer to produce.

Purpose: This study aimed to characterize the presence and environmental conditions influencing the transfer of selected foodborne pathogens—*Escherichia coli* O157, non-O157 *E. coli*, *Listeria monocytogenes*—and generic *E. coli* (gEc) from soil to spinach on Maryland's Eastern Shore.

Methods: A randomized complete block field experiment was conducted in 2021 and 2022 at the University of Maryland Eastern Shore with three treatments, four replications, and three composite samples per replicate. Targeted most probable number (MPN) enumeration and PCR were used to detect foodborne pathogens and gEc in soil and spinach following sheep grazing on winter cover crops (WCCG). Results were compared to two controls: tilled winter cover crops (WCCT, control 1) and winter fallow (WF, no cover crops or grazing, control 2).

Results: Generic *E. coli* concentrations in soil showed no significant differences across days following sheep grazing. Kruskal-Wallis analyses indicated that year, 30-day cumulative precipitation, sampling day, average soil temperature (20°C), and relative humidity over the 20 days prior to sampling were the most influential variables on gEc concentrations. Bacterial populations were detected in all treatments (LOD = 0.11 CFU/g soil), with detection in up to 8.3% (21/252) of samples 150 days post-grazing in 2021, and 6.9% (15/216) up to 120 days in 2022. Two spinach samples tested positive for *E. coli* O157:H7 on day 120 in 2021.

Significance: These findings contribute to understanding the food safety risks and

microbial dynamics associated with livestock grazing on cover crops. The data provide science-based evidence to inform evaluation of appropriate intervals between animal grazing and produce harvesting in ICLS systems.

Award Application: Developing Scientist Award

P2-145: SURVIVAL AND TRANSFER OF E. COLI TO FRESH PRODUCE IN ORGANICALLY MANAGED SOILS AMENDED WITH POULTRY LITTER COMPOST OR HEAT-TREATED POULTRY LITTER PELLETS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Poultry litter compost (PLC) and heat-treated PL pellets (HPP), widely used as organic fertilizer/soil amendments, are concerns due to the survival/persistence of foodborne pathogens and their transfer to fresh produce. **Objective:** Conduct a field-inoculated-challenge study to examine the persistence/transfer of non-pathogenic *E. coli* from soil to fresh produce using either 6-month-aged PLC or HPP as fertilizer in USDA-National Organic ProGram (NOP) certified soil.

Methods: A replicated (n=4) 2-year, randomized complete block field study with PLC and HPP, tilled/subsurface trenched into soil plots, was conducted to assess the survival/transfer of Ecrif-R to four crops 0-120 days-post-inoculation (dpi). Soils were sampled and analyzed monthly post-inoculation until harvest for *E. coli* presence.

Results: All amended, inoculated soils were positive for inoculated *E. coli* (6-log MPN/g) immediately after inoculation. At 90 dpi, all plot soils were positive for inoculated gEcrif-R; by 120 dpi, gEcrif-R concentrations in soil declined significantly ($p < 0.05$) both in 2018 and 2019. No other pathogens (*Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*) were detected at 0 or 90 dpi in plot soils. Generic Ecrif-R was detected on four of 16 cantaloupes, four of 16 cucumbers from APL plots, four of 20 radish bulbs from till-incorporated, and four from subsurface-applied APL tested positive for gEcrif-R at 90 dpi. By 120 dpi, two of 20 spinach were positive from subsurface PLC plots; no other pathogens or

gEcrif-R were recovered in 2018-2019.

Significance: Results confirm significant, consistent declines and decreasing survival of inoculated *E. coli* populations in soil throughout growing seasons, resulting in very low detection on any unwashed 90/120 dpi commodity in accord with NOP wait times. Combined environmental conditions (soil moisture, splash transfer of soil to produce, soil-air temperatures) impact *E. coli* in tilled and subsurface PL product-amended soils and on produce managed in compliance with NOP requirements.

P2-146: SURVIVAL DYNAMICS OF SALMONELLA TYPHIMURIUM IN HYDROPONICALLY GROWN BASIL: A SYSTEM-SPECIFIC ANALYSIS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: The rapid expansion of hydroponic farming for fresh produce, including culinary herbs, necessitates understanding associated food safety risks to protect public health.

Purpose: This study examined the survival dynamics of *Salmonella* Typhimurium in two common hydroponic systems: nutrient-film-technique (NFT) and deep-water-culture (DWC), during a 28-day basil production cycle.

Methods: A high-contamination scenario was simulated by inoculating nutrient solutions in both systems with *S. Typhimurium* at an initial concentration of 6 log CFU/mL. Pathogen levels were monitored in the nutrient solution (NS), roots, and leaves periodically (0, 1-, 3-, 6-, 12-, 24-, and 28- days post-inoculation) across four replicates and two trials, with uninoculated systems as controls. Basil was grown under standardized conditions: light PPFD of 275-278 $\mu\text{mol}/\text{m}^2/\text{s}$, electrical conductivity of 1.2-1.4, pH of 5.8-6.2, temperature of 25°C, and 75% relative humidity.

Results: Pathogen enumeration using selective media revealed persistent *Salmonella* survival in both systems, with significantly higher concentrations in DWC compared to NFT ($p < 0.05$). In NFT, pathogen levels in NS declined rapidly, dropping below 1 log CFU/mL within 12 days. Root contamination in NFT peaked at 6.2 log CFU/g within 24 hours post-inoculation, followed by an 84% reduction by harvest (day 28). DWC exhibited a slower pathogen decline, with NS contamination reducing to 2.7 log CFU/mL and root contamination peaking at 5.5 log CFU/g within 24 hours, followed by a gradual 45% reduction by day 28. While *Salmonella* was not detected on basil leaves in either system, there remains a

significant risk of cross-contamination during preharvest and harvest practices.

Significance: *Salmonella* persistence in NS and roots poses an environmental health concern regarding their disposal. This research provides a comparative analysis of pathogen persistence in hydroponic systems, highlighting the importance of system design in microbial safety and the need for tailored intervention strategies to ensure the safety of hydroponically grown produce.

Award Application: Developing Scientist Award

P2-147: PREVALENCE OF LISTERIA MONOCYTOGENES IN NATIVE MAINE LOW-BUSH BLUEBERRIES

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: This study provides insight into the prevalence of *L. monocytogenes* in the pre- and post-harvest environment of native Maine low-bush blueberries, with the goal of identifying contamination pathways and the impact of farming practices on berry safety.

Purpose: The aim of this study is to determine pathways for *L. monocytogenes* contamination during the processing of low-bush blueberries for fresh sale.

Methods: Samples were collected weekly during active harvest (2022-2024) across 6 farms (A-F) and sample types include: soil, fruit, and processing surfaces. To test for contamination, 25 g of sample was enriched for 24 hours in 225 g Universal Pre-enrichment Broth. DNA was extracted and screened by qPCR. Positive samples were enriched in BLEB to select for *L. monocytogenes*. Enrichments were cultured on PALCAM agar and characteristic colonies were isolated and identity-confirmed by qPCR. A Chi-square analysis was performed using RStudio Version 2024.09.0+375 to assess the relevance of farm and sample type on likelihood of *L. monocytogenes* isolation.

Results: During this study, there were a total of 635 samples, across 6 different farms and 8 sample types. From the 635 samples, 18 (2.8%) samples produced a positive result for *L. monocytogenes*. Both farm ($p < 0.001$) and sample type ($p = 0.043$) had significant impacts on isolation frequency. Out of the 18 positive samples, 83.3% of those came from a single farm and 44.4% came from soil samples, followed in frequency by mechanical harvesters and harvest bins.

Significance: This study suggests that soil is the most likely source of *L. monocytogenes* to low-bush blueberry fruits, but that pathogen transfer to fruit and likely mitigated by strict adherence to sanitary practices. Results highlight the variability of distinct ecosystems in terms of *L. monocytogenes* presence.

P2-148: LONGITUDINAL STUDY OF THE PREVALENCE OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI AND SALMONELLA IN PECAN ORCHARDS UNDER REGENERATIVE AGRICULTURE MANAGEMENT

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: With its positive impacts especially on soil and environmental health, adaptive multi-paddock (AMP) grazing, an emerging regenerative agricultural practice, could be adapted into native pecan production in the southern United States. However, the potential impact of AMP grazing on food safety risks associated with cattle grazing particularly in orchards with tree nut production, remains unclear.

Purpose: To evaluate the long-term impact of AMP grazing on the prevalence of foodborne pathogens of Shiga toxin-producing *E. coli* (STEC), and *Salmonella* in pecan orchards.

Methods: Each year (2022-2024), soil (April, July, and October) (n=129 per year) and in-shell pecan (December) (n=42 per year) samples were collected from pecan orchards under AMP management in Oklahoma. Detection and isolation of STEC and *Salmonella* were conducted by enrichment, selective isolation, and multiplex PCR. Pathogen detection rates were analyzed using the SAS program's general linear model (GLM) to perform variance analysis, correlation coefficient analysis, and mean comparisons.

Results: The prevalence of STEC in soil samples was significantly higher ($p < 0.05$) in 2023 (31.23%) and 2024 (22.57%) compared to 2022 (6.29%). In contrast, the prevalence of *Salmonella* differed significantly ($p < 0.05$) among all three years, at 40% (2023), 14.05% (2024) and 2022 (3.93%). For pecan samples, STEC prevalence was significantly higher ($p < 0.05$) in 2023 (52%) and 2024 (41.03%) compared to 2022 (14.39%), whereas *Salmonella* was significantly higher in 2023 (81%) compared to 2022 (12.61%) and 2024 (10.37%). The detection rate of both

pathogens in soil samples varied by season over the three years, with fall and spring showing a significantly higher rate than summer.

Significance: The observed up/down trends in the prevalences of foodborne pathogens in pecan orchards under AMP management may provide insights on the potential impact of AMP grazing on food safety risks associated with grazing.

Award Application: Developing Scientist Award

P2-149: OPTIMIZING TREATMENT SYSTEMS FOR WASTEWATER REUSE IN HYDROPONICS: A CASE STUDY ON ROMAINE LETTUCE PRODUCTION

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: The global population is projected to reach 9 billion by 2030, resulting in a 50% increase in food demand and straining existing agricultural systems. Hydroponics offers a sustainable solution with efficient water use and year-round production. However, challenges associated with it, such as water reuse and microbial contamination, must be addressed, as untreated wastewater poses environmental and public health risks.

Purpose: This study aimed to validate the effectiveness of filtration and UV-C treatment for safe water reuse in hydroponics.

Methods: Wastewater from a parent deep-water culture hydroponic system was treated using two methods: (1) Slow Sand Filtration (SSF) followed by UV-C treatment, and (2) SSF combined with biochar filtration and UV-C treatment. Both were operated at a flowrate of 4.33 GPM. Untreated wastewater served as the control. The study included five production cycles, each with three independent replicate systems per treatment (total of 12 systems). Four water samples were collected per cycle per system: before inoculation, after inoculation, after filtration, and after UV-C treatment. Water quality parameters (turbidity, EC, pH, ammonia, nitrites, nitrates, phosphates, and *E. coli*) were monitored biweekly. Plant growth (SPAD, shoot/root weights) and postharvest quality (vitamin C, chlorophyll) were also assessed.

Results: No statistically significant differences have been observed in SPAD values, shoot weight, root weight, vitamin C content, or chlorophyll levels across treatments ($P > 0.05$), indicating comparable plant growth between treated and untreated water. Microbial analysis has shown >4 -log reduction of *E. coli* per mL of water sample across both treatment systems, highlighting the effectiveness of both the filtration and UV-C methods.

Significance: These findings support the potential of treated wastewater for safe reuse in hydroponic systems. Continued assessment across all growth cycles will

provide further insights into long-term performance and optimization. This research contributes to sustainable food production by promoting water reuse, resource conservation, and public health protection.

Award Application: Developing Scientist Award

P2-150: MICROBIOME ANALYSIS OF MANURE-AMENDED SOILS AND CORRESPONDING PRODUCE FROM INTEGRATED CROP-LIVESTOCK FARMS ON THE EASTERN SHORE OF MARYLAND

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Biological soil amendments of animal origin (BSAAO) provide fertilizer for crops on sustainable integrated crop-livestock farms (ICLFs). However, BSAAO may alter soil microbiota and facilitate pathogen transfer to fresh produce, thereby increasing contamination potential.

Purpose: To analyze BSAAO's influence on ICLF-soil and -produce microbiomes relative to USDA-National Organic program's 90-/120-day application-to-harvest wait-period.

Methods: Using standard methods, 79 BSAAO soil and produce samples from three Maryland Eastern Shore ICLFs were collected pre-(D0A) and post-BSAAO (D0B) application and monthly for 180-days. Metagenomic DNA was extracted and shotgun metagenomic sequencing (Illumina NextSeq 2000) was employed to characterize sample microbiota.

Results: Bacteria dominated microbial communities across treatments (>91%), while fungi and viruses were detected less frequently. Viruses were observed on D90- and D120-produce. Pre-incorporation, ICLF-BSAAO harbored pathogens from the families Bacillaceae, Enterobacteriaceae, and Clostridiaceae (*Escherichia coli* , *Bacillus cereus*, and *Clostridium botulinum/perfringens*). from D0A (97.21% and 0.05%) to D0B (97.80% and 0.06%), BSAAO slightly increased the average bacterial and fungal abundances, respectively. BSAAO impacts on soil bacterial communities resulted in reduced pathogenic *Bacillus cereus* and *Salmonella enterica* prevalence from D0A to D0B; however, *Listeria monocytogenes* was detected on D0B. on D90 and D120, multiple pathogens spanning the Bacillaceae, *Campylobacteraceae*, Enterobacteriaceae, Staphylococcaceae, Clostridiaceae, and Listeriaceae families were present in soils and on produce. Alpha diversity was significantly lower on produce. Beta diversity showed significant differences in

microbial communities between BSAAO and soil groups ($p \leq 0.05$) and between soil and produce groups during different time points ($p \leq 0.05$).

Significance: Study findings provide insights on BSAAO effects on ICLF-soil and -produce microbiomes, and foodborne pathogen prevalence demonstrates ICLF food safety vulnerability. Future research should address the influence of microbial communities on soil health and the persistence of foodborne pathogens on sustainable farms.

Award Application: Developing Scientist Award

P2-151: COMPARING METHODS AND DURATION OF AIR SAMPLING TO QUANTIFY BACTERIAL POPULATIONS IN BIOAEROSOLS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Bioaerosols from adjacent animal operations have been identified as a potential risk for produce contamination. Isolation of foodborne pathogens and indicators from air can be challenging.

Purpose: To compare active and passive sampling methods to detect foodborne pathogens and indicators from air surrounding an animal operation.

Methods: Air samples were collected (n=6) near an animal operation. Active samples were collected onto Brilliance *E. coli*/coliform (BEC), and *Salmonella* (CS), and STEC (CX) chromogenic agar using andersen impact samplers (5, 10, and 20 min). Plates were incubated at 37°C for 48h. Passive air sampling was performed using cheesecloth filters (12 and 24h). Cheesecloth was stomached with 200ml 2% BPW+0.2% tween80, plated onto TSA and BEC, and incubated at 37°C for 24h. Remaining rinsate was combined with double-strength TSA, incubated at 37°C for 24h, and streaked onto SMAC and CX for STEC detection. For *Salmonella* detection, 9.0ml TT and 9.9ml RV broth were inoculated with 1.0 and 0.1ml TSB enrichment, respectively and incubated at 42°C. After 24h, broths were streaked onto HE, XLD, and CS. STEC and *Salmonella* suspect isolates were confirmed via qPCR and the presence of *stx1/stx2* and *invA* genes, respectively.

Results: Increasing active sampling time (5, 10, and 20 min) decreased coliforms (<0.77, <0.19, and <-0.47 log CFU/m³, respectively) and generic *E. coli* counts (<0.74, <0.24, and <-0.44 log CFU/m³, respectively). *Salmonella* and STEC were not detected. Higher bacterial populations were seen at 12 vs 24 h during passive sampling: total counts (0.53 vs 0.24 log CFU/cheesecloth/h), coliforms (0.27 vs 0.13 log CFU/cheesecloth/h), and *E. coli* (<0.23 vs <0.10 log CFU/cheesecloth/h). Out of 6 passive 12h samples, 4 tested positive for *Salmonella* in at least one enrichment/media combination. STEC was not detected.

Significance: Findings highlight the impact of sampling strategies on assessing potential risks of bioaerosol-mediated contamination in produce environments.

P2-152: SALMONELLA ISOLATED FROM FINISHING BEEF STEERS WITH AND WITHOUT LIVER ABSCESS: ANTIBIOTIC RESISTANCE PROFILE AND STRAIN TYPING

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Antimicrobial resistance (AMR) in *Salmonella* negatively impacts public health and significantly contributes to economic losses. Although strategies are employed to curb AMR, concerns persist across the industry.

Purpose: Observe the AMR *Salmonella* isolates status and conduct isolate clustering to establish strain correlations.

Methods: *Salmonella* isolates (n=516) were sourced from a study evaluating *Salmonella* associated with liver abscesses. Isolates were recovered from liver abscess, feces, nasal swabs, and rumen fluid. Resistance characterization was performed using the Sensititre™ system, and descriptive statistics were visualized using R Studio 4.4.2. Liver-abscess-positive steers (n=17) were subjected to *Salmonella* isolation (feces, nose swabs, rumen fluid, and liver abscess). These isolates underwent strain typing using Fourier Transform-infra-Red (FT-IR) spectroscopy system (IR Biotyper® Bruker). Data was analyzed using the manufacturer's software, and dendrograms were constructed using Euclidean distance. Unweighted pair group method with arithmetic mean was used for clustering.

Results: 7.17% of isolates exhibited AMR. Single AMR was frequent against tetracycline, ampicillin, chloramphenicol, colistin, ceftriaxone, and amoxicillin. One multi-resistant isolate was identified. Strain typing of 54 *Salmonella* isolates from liver abscesses revealed clusters demonstrating strain relatedness based on sample type. Strain typing within the same animal revealed that 13 animals presented a single *Salmonella* cluster of mainly liver abscess isolates, whereas 4 animals showed a single cluster of *Salmonella* from feces and nose swabs, and a multi-cluster from other sample types.

Significance: Low AMR prevalence to key antibiotics was observed, highlighting improvement in antibiotic stewardship. Common strain associations within animals identified through FT-IR spectroscopy indicate distinctions among liver abscesses and other sources. Clustering may indicate a single contamination source. Strain typing is a valuable tool for understanding relatedness and tracking transmission across animals and within the same animal.

Award Application: Developing Scientist Award

P2-153: TEMPORAL AND ENVIRONMENTAL FACTORS ASSOCIATED WITH THE SURVIVAL OF ESCHERICHIA COLI TVS 353, A SURROGATE FOR SALMONELLA ENTERICA, IN MANURE-AMENDED GEORGIA SOILS AND ITS FATE IN BULB ONION

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Georgia, one of the leading poultry-producing states in the US, generates approximately 2 million tons of poultry litter annually. It is often pelletized or composted as biological soil amendments of animal origin (BSAAOs) and applied to agricultural fields. Understanding how environmental factors and manure types affect pathogen survival is crucial for assessing the risk of foodborne illness associated with consuming fresh produce grown in manure-amended soils.

Purpose: To quantify the impact of amendment type and dynamic environmental conditions on the survival of *E. coli*.

Methods: A two-year field study was conducted in a completely randomized design in Georgia, which included Heat-Treated Poultry Pellets (HTPP), Composted Poultry Litter (PL), and Unamended (UN) plots inoculated with *E. coli* TVS 353 (surrogate of *Salmonella enterica*) and negative control. Bulb onions were transplanted onto selected HTPP plots. Soil samples were collected for up to 140 days and enumerated for *E. coli*, and population data was fitted to linear mixed

effect model (LME) using amendment type and weather variables (cumulative rainfall (cmrain2), air temperature (at6012), relative humidity (RH12), soil temperature (ast12), wind speed, (W1)) as predictors.

Results: The application of HTPP and PL, significantly increased the population of *E. coli* by 1.3 and 1.2 log CFU/g compared to UN (Year 1), respectively, and population significantly decreased in both years ($p < 0.05$), irrespective of soil amendment treatment. However, the decline was inconsistent, accounted by weather variables. LME for soil amendment treatment plots identified significant weather predictors ($p < 0.05$), RH12 (+0.35) in Year 1; W1 (-0.76), at6012 (-1.66), and ast12 (+1.57) in Year 2. in LME comparing HTPP plots with and without onions, W1 (+0.69) during Year 1; at6012 (-0.92), ast12 (+1.35), cmrain2 (+0.37), and W1 (-0.36) in Year 2 were significant ($p < 0.05$).

Significance: The LME model derived a mathematical relationship between environmental variables and amendment type in the *E. coli* population.

Award Application: Developing Scientist Award

P2-154: IMPACT OF VAM-S BACTERIOPHAGE SOLUTION ON THE ENVIRONMENTAL MICROBIOME IN POULTRY LITTER SYSTEMS FROM COMMERCIAL OPERATIONS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: The increasing demand of poultry products has led to an increase in the production of chickens. During chicken production, live birds are the primary source of contamination by food-borne pathogens, including Salmonella. Pre-harvest mitigation strategies can reduce the risk of contamination and improve food safety. As a result, there is a growing demand for alternatives in pre-harvest mitigation strategies for poultry production.

Purpose: This study aimed to determine the effect of the application of a bacteriophage treatment to commercial poultry litter at different concentrations on the microbiome of the poultry litter.

Methods: Three treatments of 2.5 mL each were prepared: Phosphate Buffered Solution (negative control), undiluted (1×10^{11} PFU/mL) bacteriophage solution VAM-S (treatment 1), and the diluted (1×10^9 PFU/mL) solution (treatment 2). Chicken litter samples (25 g each) were mist-inoculated, incubated at 23°C for 24 hours, and subjected to DNA extraction using QIAGEN kits. Only one replicate for each treatment was done since the sequencing would provide enough replications. DNA underwent 16S sequencing, and the microbiome analysis assessed microbial shifts.

Results: Results demonstrated a significant difference of treated samples compared to non-treated samples by decreasing sample richness by approximately 14% in treatment 1 and 9.6% in treatment 2, ultimately lowering the number of bacterial species. Treated samples also had a higher diversity since the species had a more even relative abundance. The treatment also reduced the pathogenic families Staphylococcaceae and Enterobacteriaceae and maintained beneficial families such as Lachnospiraceae and Bacteroidaceae. Furthermore, pathogenic strains of Salmonella, Clostridia, and Escherichia-Shigella were

significantly reduced or eliminated. The results demonstrated that the bacteriophage treatment has a significant effect on the microbial composition and diversity of poultry litter.

Significance: The modification in the microbiome offers a promising strategy to prevent the colonization of birds with pathogenic bacteria and subsequent contamination of food products.

P2-155: SURVIVAL AND PERSISTENCE OF ACID-ADAPTED *E. COLI* O157:H7 IN RAW MANURE UNDER ENVIRONMENTAL CONDITION

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: *Escherichia coli* O157:H7, a pathogen commonly found in cattle manure, exhibits remarkable persistence in various environmental conditions.

Purpose: This study investigated the survival of acid-adapted *E. coli* O157:H7 in raw manure under simulated Louisiana summer conditions.

Methods: *E. coli* O157:H7 was cultured at ~pH 7, adapted to pH 5 and 4, and inoculated into sterile raw manure (6.5 log CFU/g). Samples were incubated for 120 days in environmental chamber simulating Louisiana's summer (temperature, RH, light) in drying and slurry setups. Samples were analyzed on days 0, 1, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, and 120 for pH, water activity, moisture, and bacterial count by plating on CT-SMAC. Manure dried for 0, 30, and 90 days was also heat-treated at 55°C and 60°C for 0, 30, and 60s. Data were analyzed with ANOVA and Tukey's test ($p < 0.05$).

Results: Initial pH of all raw manure samples was ~7.3. In the drying setup, aW decreased significantly ($p < 0.05$) from 1 to 0.93 by Day 40, stabilizing at 0.74 from Day 90 to 120. Bacterial counts in drying setup decreased significantly ($p < 0.05$) by 3.84, 3.75, and 3 logs for *E. coli* O157:H7 adapted at pH 7, 5, and 4, respectively, with pH 4 adapted bacteria showing higher persistence. In slurry setup, bacterial growth at all pH levels decreased from ~6.5 to ~3.8 log CFU/g by Day 120 ($p < 0.05$). Heat treatment, on Day 0, resulted in >0.5 log reduction at both 55°C and 60°C. By Day 90, pH 5 adapted bacteria were undetectable immediately, while those at pH 7 and 4 showed significant reduction within 30s, and became undetectable after 60s at 55°C.

Significance: While prolonged environmental exposure significantly reduces *E. coli* O157:H7 in raw manure, adaptation to lower pH conditions may enhance bacterial persistence and survival under stress.

Award Application: Developing Scientist Award

P2-156: EVALUATION OF BOILING WATER AND STEAM TREATMENT FOR HYDROPONIC SUBSTRATE DECONTAMINATION FROM LISTERIA MONOCYTOGENES

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Rockwool, a popular substrate in hydroponic agriculture, requires effective sanitation due to its exposure to nutrient-rich water, which can pose potential food safety risks. Effective sanitization techniques such as hot water and steam treatment not only address food safety concerns but also support sustainability by enabling the reuse of rockwool, reducing both environmental impact and resource waste.

Purpose: This study investigated the effectiveness of boiling water and steam treatments in reducing *Listeria monocytogenes* on rockwools used in hydroponic systems.

Methods: Rockwool cubes (1 cubic inch) were spot inoculated with a cocktail of *Listeria monocytogenes* (101M, V7, LCDC, and Scott A) resulting in 7.87 ± 0.24 log CFU/g after inoculation. Contaminated cubes were exposed to boiling water or saturated steam (90°C) for 10, 20, 30, 40, 50, 60, 300, and 600 s. Post-treatment, samples were diluted in PBS and enumerated on Oxford agar after 24 h incubation at 37°C. Enrichment in tryptic soy broth was used to confirm *Listeria* presence/absence. Data were analyzed using ANOVA and Tukey's test ($P < 0.05$).

Results: Both treatments significantly ($p < 0.05$) reduced *L. monocytogenes* levels within 10 s, with reductions of 5.60 and 6.72 log CFU/g for boiling water and steam, respectively. Boiling water treatment showed no further reduction until after 5 min (> 6.70 log CFU/g reduction). Steam treatment reduced *Listeria* to below detectable limits (< 0.88 log CFU/g) after 40 s; however, after enrichment and subsequent plating, *Listeria* colonies were observed, indicating sublethally injured cells.

Significance: Boiling water and steam treatments are promising, sustainable interventions for rockwool safety in hydroponic systems. Further studies are needed to assess treatment's impact on rockwool integrity and determine the minimum treatment time for complete pathogen inactivation.

Award Application: Developing Scientist Award

P2-157: MINIMUM INHIBITORY CONCENTRATIONS OF COMMERCIAL COPPER PESTICIDES ARE DEPENDENT ON SALMONELLA SEROVAR AND PREPARATION METHOD

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Copper (Cu)-based pesticides play a vital role in controlling bacterial and fungal diseases in produce crops.

Purpose: This study aimed to quantify the minimum inhibitory concentration (MIC) of two commercial copper pesticides against eight *Salmonella* serovars.

Methods: MIC assays with two Cu pesticides—copper octanoate ($C_{16}H_{30}CuO_4$) and cuprous oxide (Cu_2O)—were performed on eight rifampicin (R)-resistant *Salmonella* serovars using serial dilutions and multiple replicates in a 48-well plate; eight plates per combination of serovar, pesticide, and method were used to determine MIC. Two methods, dilution- and vortex-based, were compared for preparing $C_{16}H_{30}CuO_4$. Cu pesticides were either diluted in sterile DI water or added directly to *Salmonella* cultures (9.05 ± 0.17 log CFU/mL) at the maximum recommended application rate for tomato. After incubation at 35°C for 24 h, aliquots from wells were streaked onto Tryptic Soy Agar-R, then incubated at 35°C for another 24 h. MIC was defined as the lowest Cu concentration that inhibited growth across all eight blocks. Linear models were used to assess differences in MIC values by preparation method, serovar, and Cu pesticide.

Results: For the dilution method, $C_{16}H_{30}CuO_4$ had a MIC of 0.86 ± 0.00 mg/mL Cu across all serovars. Cu_2O had a MIC of 113.9 ± 35.8 mg/mL Cu, ranging from 101.25 to 202.5 mg/mL, with *S. Newport* having the highest MIC. The MIC for Cu_2O using the dilution method was 114.2 mg/mL Cu (95% CI = 83.1, 145.3),

significantly higher than $C_{16}H_{30}CuO_4$. MIC values for $C_{16}H_{30}CuO_4$ did not differ significantly between serovars ($p > 0.05$), but the vortex method resulted in an approximately 0.59 mg/mL Cu lower MIC (95% CI = -0.65, -0.53) compared to the dilution method.

Significance: MIC values for the two Cu pesticides against *Salmonella* serovars differed significantly. These results highlight the variability in antimicrobial efficacy between Cu formulations and suggest that preparation method can influence MIC outcomes.

Award Application: Developing Scientist Award

P2-158: CHARACTERIZATION OF SALMONELLA NEWPORT OVER TIME AND SPACE IN PRODUCE PREHARVEST WATER FROM THE EASTERN SHORE OF VIRGINIA

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: *Salmonella enterica* serotype Newport has been associated with outbreaks linked to produce from the Eastern Shore of Virginia (ESVA). A prior study on microbial water quality on the ESVA isolated *S. Newport* from water samples collected from 20 ponds over two growing seasons.

Purpose: Characterize genetic relatedness among *S. Newport* isolates over time and space using whole genome sequence (WGS) data.

Methods: *S. Newport* isolates (n=39) from water samples were sequenced and downloaded from the NCBI Pathogen Detection platform. *in silico* serotyping was done with SeqSero and SISTR available on the Enterobase platform (v1.2.0). To identify clusters, core genome multilocus sequence typing (cgMLST) and hierarchical clustering (HierCC) were performed on Enterobase. Using the CSI phylogeny 1.4 platform, single nucleotide polymorphism (SNP) analysis was performed to determine the relation among the isolates within the same hierarchical cluster (HC). The threshold to determine clones was ≤ 5 SNPs.

Results: The 39 *S. Newport* isolates represented a total of 36 sequence types (ST) and were clustered into six hierarchical clusters [HC_120 (SNPs:0-30), HC_122 (SNPs:0-58), HC_632 (SNPs:11-29), HC_945 (SNPs:2), HC_6304 (SNPs:9), HC_18726 (SNPs:0)]. in HC_120, two isolates (0 SNP) were isolated at the same location (Pond 672) in the same growing season but different sampling days (8/8/2016 and 8/29/2016). in HC_122, two isolates (0 SNP) were isolated at different locations and growing seasons (Pond 543 on 9/14/2015 and Pond 681 on 6/20/2016) whereas two others (1 SNP) were isolated at different locations but the same growing season (Pond 284 on 8/3/2015 and Pond 326 on 7/20/2015). in HC_945, two isolates (2 SNPs) were isolated from the same location (Pond 369) over the two growing seasons (7/6/2015 and 6/6/2016).

Significance: Findings highlight *S. Newport*'s persistence across pond locations and seasons, offering insights into pathogen transmission, informing targeted interventions, and enhancing food safety strategies.

Award Application: Developing Scientist Award

P2-159: ENVIRONMENTAL SOURCE OF SALMONELLA SEROVARS AFFECTS INFECTIVITY IN HCT-8 CELLS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: *Salmonella enterica* is an invasive enteric bacterium; however, there is potential variability in virulence and invasion properties by specific serovar and associated with environmental conditions.

Purpose: This study uses a human cell culture infectivity model to assess differences in *Salmonella* serovar invasion.

Methods: infectivity of 13 different serovars was evaluated in human HCT-8 cells, including outbreak isolates and 3 serovars isolated from Delaware farm soils (Newport, Javiana, Norwich). Confluent HCT-8 monolayers were established in 24-well plates and *Salmonella* cultures were sub-cultured individually in Luria-Bertani (LB) broth (starting inoculum 6-7 log CFU/mL). *Salmonella* cultures were serially diluted in triplicate and added to HCT-8 cells with one dilution per well (1mL) in RPMI-1640 medium, along with the negative control heat-killed *Salmonella* culture. Cells were incubated for 2hr, washed with Hanks Balanced Salt Solution (HBSS), and incubated for 2hr with RPMI plus 100µg/mL gentamicin. Wells were washed with HBSS, shaken with 0.1% Triton X-100 in pHosphate Buffered Saline (PBS), and *Salmonella* were enumerated in duplicate on XLT-4 media. log counts were analyzed by Tukey analysis (JMP).

Results: Serovars Typhimurium LT-2 (lab), Javiana (soil), Newport (soil), Norwich (soil) and infantis (poultry) were highly invasive with greater than 6.5 log CFU/mL recovered, but were not significantly different from each other. Fewer invasive cells, average of 5.8 log CFU/mL, were detected for serovars Newport (tomato), Enteritidis (poultry), and Anatum (clinical) ($p=0.0021$). Even fewer invasive cells, average of 5.2 log CFU/mL, were detected for serovars Braenderup (tomato) and Javiana (tomato). The fewest invasive cells were detected for Montevideo (clinical, 4.2 log CFU/mL), Agona (alfalfa, 4.1 log CFU/mL) and Kentucky (poultry, 2.3 log CFU/mL). The least invasive serovars were significantly different from all other serovars ($p<0.0001$).

Significance: Cell culture invasion was influenced by *Salmonella* serovar, with

those isolated from soils and poultry more invasive than those isolated from tomatoes or sprouts. The role of environmental isolation on virulence and infectivity deserves more attention.

Award Application: Developing Scientist Award

P2-161: EFFICACY OF THERMAL TREATMENTS IN INACTIVATING *E. COLI* AND *E. COLI* O157:H7 IN RAW MANURE

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: The application of raw manure provides essential nutrients that enhance soil fertility; however, it also poses significant public health risks due to the presence of pathogenic bacteria. While composting can effectively reduce pathogen levels, it often requires several months, highlighting the need for alternative methods, such as thermal treatments, to quickly inactivate pathogens in raw manure

Purpose: This study evaluated the effectiveness of thermal treatments at various time-temperature combinations in inactivating generic *E. coli* and *E. coli* O157:H7 in raw manure

Methods: Samples of raw manure were weighed into sterile aluminum trays and inoculated with *E. coli* O157:H7 (ampicillin resistant) and *E. coli* (nalidixic acid resistant) to achieve ~8 log CFU/g. The samples were heated at 50°C, 55°C, 60°C, and 65°C in a preheated oven for 3, 4, and 5 hours. Manure samples were analyzed before and after heat treatment by plating onto TSA with ampicillin or nalidixic acid. Data were analyzed using ANOVA and Tukey's test ($P < 0.05$)

Results: The initial moisture content and pH of the raw manure were $76.95 \pm 0.36\%$ and 7.43 ± 0.08 , respectively. At 50°C and 55°C, *E. coli* O157:H7 counts significantly reduced by one log (1.52–1.54 log CFU/g) after 5 hours. However, at higher temperatures (60°C and 65°C), with similar treatment time, *E. coli* O157:H7 levels were reduced by 4.44–4.49 log CFU/g. Generic *E. coli* showed similar trends, with 1.58–1.6 log CFU/g reduction at 50°C and 55°C, and 3.86–4.92 log CFU/g reduction at 60°C and 65°C

Significance: Direct heat treatment of raw manure is an effective method for inactivating bacterial pathogens, presenting a viable alternative to the traditional composting process, which can take several months to significantly reduce pathogen levels

P2-162: COMPARING THE PATHOGEN REDUCTION POTENTIAL OF UV WATER TREATMENT WITH COMPETITION FROM BENEFICIAL BACTERIA

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Biocontrol products are often used in hydroponic systems, relying on microbial competition to reduce threats from plant pathogens in the recirculating nutrient water. There has been a recent surge in commercially available “beneficial bacteria” products marketed with claims of enhancing crop performance when added to hydroponic systems.

Purpose: This study explores the efficacy of employing a beneficial bacteria product as a biocontrol agent for a human pathogen (*Escheria coli* #25922) in the nutrient water of hydroponic systems growing kale microgreens, compared to a physical intervention (ultraviolet water treatment).

Methods: Commercially-available NFT (Nutrient Film Technique) hydroponic systems were used to grow microgreens of Red Russian kale (*Brassica oleracea*) in a nutrient solution containing Hydro-Gro Leafy (8.87%) and calcium nitrate (6.96%); the electrical conductivity was maintained between 1.2 and 1.6 mS/cm. The nutrient solution in three of the nine systems was double-treated with a UV-C device (MiniPure MIN-1; 500ml capacity) emitting peak irradiance at 254nm at a flow rate of 6 L/min. Three received a dose of a commercially available plant growth-promoting bacteria product applied in accordance with manufacturer recommendations, and three served as controls. Samples were collected before and after each treatment on experiment day one, and then twice weekly until harvest along with plant height, pH, and EC measurements. Following harvest, each system’s fresh weight and representative SPAD value were measured. The experiment will be repeated a total of three times.

Results: Preliminary results show that the UV treatment resulted in an immediate 1.87 average log reduction of *Escherichia coli*. Four days after the treatment, the average reduction from the initial pre-treatment log CFU/ml was 2.95 in the systems treated with UV, 1.94 in the systems treated with beneficial bacteria, and 1.18 in the control systems.

Significance: The potential to use beneficial bacteria for biocontrol of human

pathogens could be synergistic for growers, potentially improving food safety as well as crop performance.

Award Application: Developing Scientist Award

P2-163: DEPOSITION OF SHIGATOXIGENIC *E. COLI* ON ROMAINE LETTUCE PLOTS NEAR A CAFO

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Cattle are natural reservoirs for Shigatoxigenic *E. coli* (STEC). While leafy greens have been grown in proximity to cattle feedlots for decades, the aerial deposition of STEC through fugitive dust has not been thoroughly investigated.

Purpose: To evaluate the natural contamination of romaine lettuce and associated matrices, research plots located ~2 miles from a >80,000 head cattle CAFO and compost operation were surveyed and samples analyzed for STEC during the fall (2023) and spring (2024) growing seasons.

Methods: Romaine lettuce 5-acre research plots were established and maintained following industry best practices in the Southwestern US. Soil, drag swabs, cheesecloth windbreaks (passive air), irrigation canal water (100L dead-end ultrafiltration), canal sediment, and lettuce tissue were collected roughly weekly for approximately 8 weeks during each growing season and enriched using the FDA BAM4a method. All enrichment qPCR positive samples were plated onto selective agars for isolate recovery. Whole genome sequencing confirmed STEC identifications.

Results: In total, 1,419 samples were tested for STEC, with 1.9% soil (n=324), 4.6% drag swabs (n=324), 11.9% cheesecloth windbreaks (n=84), 71.4% water (n=35), 37.5% canal sediment (n=16), 7.9% lettuce leaf composites (n=216), and 2.1% whole lettuce heads (n=420) qPCR positive. Both seasons yielded positive

samples in every matrix except for soil. Isolates were recovered from 34 samples yielding 32 genetically unrelated STEC strains representing 16 different STEC serotypes, with O168:H8 occurring most frequently. Cheesecloth windbreak samples yielded viable STEC representing five distinct strains. For one planting, identical O55:H12 strains were first recovered from 2 lettuce heads and in subsequent weeks from irrigation water and soil.

Significance: These findings indicate that STEC can survive in the air and that dust can potentially act as a transfer mechanism from a nearby land activity like a cattle CAFO. This highlights the importance of considering airborne transmission of pathogens when developing risk assessments and best management practices.

P2-164: CONTROL OF ESCHERICHIA COLI TVS 353 IN CERTIFIED ORGANIC SOILS USING A BIOFUMIGANT MUSTARD COVER CROP

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Biofumigant cover crops are reported to control plant pathogens in a variety of production systems. Their impact on foodborne pathogens has not been extensively studied. Mustard (*Brassica juncea*), a commonly used biofumigant because of its high glucosinolate concentrations, may potentially increase ecosystem benefits to farmers while subsequently decreasing produce safety risks.

Purpose: This study seeks to understand the impact of biofumigant mustard cover crops with and without plastic mulch on soil inoculated with the foodborne pathogen surrogate *Escherichia coli* TVS 353.

Methods: The mustard variety Pacific Gold was grown as a cover crop in certified organic fields, with eight plots planted with Pacific Gold and four plots left fallow as a positive control (n=12). After flowering, one 1 m × 6 m section of each plot was inoculated with two liters of a prepared Rifampicin-resistant *E. coli* TVS 353 culture (8 log CFU/mL). Plots were then flail mowed, tilled, and plastic mulch was applied to four of the mustard plots. Soil samples (10 g) from each plot were collected, diluted in 90 mL BPW, and rinsate was serially diluted and plated onto Tryptic Soy Agar on Days 0, 1, 5, 9, 14, and 21.

Results: At day 0, *E. coli* populations were 5.45 log CFU/g in control plots, 5.92 log CFU/g in mustard plots, and 5.57 log CFU/g in mustard with plastic plots. A mixed model analysis showed a significant difference ($p < 0.05$) in average *E. coli* TVS 353 reductions 21 days after inoculation between mustard plots (0.77 log CFU/g) compared to the positive control (1.32 log CFU/g) and those with mustard and plastic (1.57 log CFU/g).

Significance: The use of Pacific Gold mustard as a biofumigant, with or without

plasticulture, does not appear to be an effective solution to control *E. coli* TVS 353 in production soils.

P2-165: HEPATITIS A VIRUS AND FELINE CALICIVIRUS PERSISTENCE IN HYDROPONIC SOLUTION

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Controlled environment agriculture (CEA) enables year-round growth of produce to meet its growing demands. Besides maintaining the nutritional and sensory attributes of fresh produce, prevention of contamination from foodborne pathogens in CEA needs to be considered.

Purpose: The goal of this research was to determine the persistence of foodborne viruses in hydroponic solutions (used in CEA) over 21 days at room temperature (produce cultivation) and refrigeration (produce storage) to determine cross-contamination risk.

Methods: Two-hundred μL of hepatitis A virus (HAV) or feline calicivirus (FCV, a human norovirus surrogate) at 4 to 5 log PFU/mL were inoculated into 1.8 mL of a commercial hydroponic solution (pH of 5.8) within sterile beakers and stored at 25°C and 4°C for 21 days. Plaque assays were used to enumerate the recovered viruses. Each experiment was carried out in triplicate. Data were statistically analyzed using ANOVA with Tukey's adjustments on JMP v17.

Results: At 25°C, recovered HAV titers were 5.62 ± 0.09 , 5.61 ± 0.21 , 5.54 ± 0.25 , 5.07 ± 0.06 , 4.92 ± 0.04 , 4.83 ± 0.14 and 4.73 ± 0.40 log PFU/mL, while recovered FCV titers were 4.53 ± 0.23 , 4.46 ± 0.32 , 4.05 ± 0.25 , 3.67 ± 0.21 , 3.34 ± 0.29 , 2.26 ± 0.25 and 1.3 ± 0.00 log PFU/mL at days 0, 1, 3, 5, 7, 14, and 21 (D-values of 22.39 ± 9.87 and 6.39 ± 0.70 days, respectively). At 4°C, recovered HAV titers were 5.59 ± 0.12 , 5.65 ± 0.22 , 5.71 ± 0.41 , 5.60 ± 0.43 , 5.39 ± 0.39 , 4.9 ± 0.71 , and 5.37 ± 0.05 log PFU/mL, while recovered FCV titers were 4.45 ± 0.11 , 4.26 ± 0.15 , 4.07 ± 0.23 , 4.15 ± 0.39 , 3.80 ± 0.43 , 4.10 ± 0.16 and 3.31 ± 0.21 log PFU/mL at days 0, 1, 3, 5, 7, 14, and 21 (D-values of 41.34 ± 4.06 and 24.36 ± 3.07 days, respectively).

Significance: This study indicates that HAV (as expected) can persist longer than FCV in CEA hydroponic solutions. Thus, appropriate measures should be taken to prevent contamination of produce in CEA to decrease foodborne viral illness risk. Furthermore, this study shows the need to prevent cross-contamination at refrigerated storage by contaminated hydroponic solutions or hydroponically-

grown produce.

Award Application: Developing Scientist Award

P2-166: ULTRAVIOLET LIGHT FOR HEPATITIS A AND AICHI VIRUS INACTIVATION ON SURFACES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Ultraviolet-light emitting diode (UV-C LED) systems (at 254 and 279 nm) are being researched for surface decontamination. Hepatitis A virus (HAV) and Aichi virus (AiV) can be transmitted via contaminated food-contact surfaces, including polypropylene surfaces, leading to cross-contamination and outbreaks.

Purpose: The objective of this research was to determine the UV-C LED doses for the inactivation of HAV and AiV on polypropylene discs.

Methods: HAV or AiV (50 μ l of ~ 6.5 -7 log PFU/mL) was surface-spread and air-dried on sterile propylene discs and treated with either 254-nm (dose up to 14.5 mJ/cm²) or 279-nm UV-C LED (dose up to 13.5 mJ/cm²). Viral infectivity of recovered viruses was determined using plaque assays. Data from triplicate trials were statistically analyzed using mixed model analysis of variance with Tukey's adjustment ($p \leq 0.05$). The dose (D10-value) required to decrease HAV or AiV infectivity by one-log was calculated using the linear model.

Results: HAV and AiV treated with 254-nm UV-C LED at a dose of 10 mJ/cm² showed 1.41 ± 0.42 and 2.51 ± 0.85 log reduction, with 2.73 ± 0.30 and 4.87 ± 0.17 log PFU reduction at a dose of 14.5 mJ/cm², respectively. HAV treated with 279-nm UV-C LED at 5.4 and 13.5 mJ/cm² showed 1.02 ± 0.15 and 2.09 ± 0.07 log PFU reduction, while AiV showed 1.14 ± 0.28 and 2.41 ± 0.16 log PFU reduction with 2.4 and 6 mJ/cm², respectively. Linear model 254-nm UV-C LED D10-value for HAV and AiV were 5.33 ± 0.14 and 3.74 ± 0.37 mJ/cm², respectively, while with 279-nm UV-C LED was higher for HAV at 6.11 ± 0.40 mJ/cm² than AiV at 2.60 ± 0.37 mJ/cm² ($p \leq 0.05$).

Significance: These data show that both 254-nm and 279-nm UV-C LED systems can inactivate HAV and AiV on polypropylene surfaces. However, HAV with higher D10-values than AiV was more resistant than AiV to treatments with both UV-C LED systems. These doses are useful to design optimal dose delivery systems for viral inactivation on surfaces.

Award Application: Developing Scientist Award

P2-167: MICROBIOME ANALYSIS OF SELECTED RETAIL-DERIVED PRODUCTS

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Presentation type/Format: Technical

Category: Retail and Food Service Safety

Abstract:

Introduction: Understanding the microbiome of retail food products is crucial for addressing food safety and quality concerns.

Purpose: This study analyzed the surface microbiome of various retail food products, including peaches, pears, onions, cucumbers, nectarines, queso fresco cheese, pre-sliced hard salami, and pre-sliced oven-roasted turkey breast.

Methods: Collection of the microbes on the surface of the products was performed by rinsing each sample, per product type, with 250 mL of 1X Tris-EDTA buffer containing 2% Tween 80 using a sonicator. The rinse solution was centrifuged three times to obtain a concentrated pellet, which was used for DNA extraction. The extracted DNA was subjected to 16S rRNA sequencing on the Illumina MiSeq platform. A total of 96 samples, with 12 per product type, were analyzed. Taxonomic classification of sequences was performed using the DADA2 package in RStudio, with the SILVA database as reference database. Alpha diversity metrics, including Shannon and Simpson indices, were calculated for each product type and compared using one-way ANOVA, followed by a Tukey HSD test. Beta diversity was assessed using Bray-Curtis dissimilarity.

Results: Alpha diversity (Shannon and Simpson indices) was significantly higher ($P < 0.001$ for both) in fresh produce (2.4 to 4.1, 0.82 to 0.95) compared to queso fresco cheese (0.70, 0.38) and deli meats (1.36 to 1.68, 0.67 to 0.69), indicating a more diverse microbial ecosystem in minimally processed foods. Beta diversity analyses showed distinct microbial community compositions across product types. Cheese, hard salami, and turkey breast were dominated by fermentation-associated genera, including *Lacticaseibacillus*, *Latilactobacillus*, *Pediococcus*, and *Lactococcus*. In contrast, fresh produce harbored genera commonly associated with food spoilage or plant diseases, such as *Pseudomonas*, *Pantoea*, *Psychrobacter*, *Serratia*, and *Erwinia*. Additionally, species such as *Pseudomonas* and *Serratia* support the survival of *Listeria monocytogenes* against sanitizers within their mixed biofilms, which could pose a significant food safety issue in retail settings.

Significance: This study provides insights into microbial diversity in retail foods, with implications for food safety and quality control.

Award Application: Developing Scientist Award

P2-168: INVESTIGATION OF THE INACTIVATE EFFICIENCY OF COMMON FOODBORNE PATHOGENS IN DARK LEAFY GREEN VEGETABLE JUICE (DLGVJS) BY USING PULSED LIGHT SYSTEM

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Dark leafy green vegetable juices (DLGVJs) have garnered significant attention due to their nutritional benefits, high production volume, and economic value. However, their association with foodborne pathogens presents a critical public health concern. Pulsed light systems (PLS), a proven non-thermal technology in the food industry, remain underexplored for enhancing the microbial safety of DLGVJs.

Purpose: This study aimed to evaluate the inactivation efficiency of common foodborne pathogens in DLGVJs using a pulsed light system.

Methods: DLGVJs (kale, chard, collard green) were inoculated with rifampicin-resistant cocktails of *Salmonella* spp. and *Escherichia coli* O157:H7 (approx. 7.0 log CFU/mL) and dispensed into standard petri dishes (3 mm depth). A laboratory-scale PLS system (SteriPule-XL® 3000, Xenon Corp., Wilmington, MA) was used for treatment. Samples were positioned 5 cm from the lamp with an intensity of 2.86 W/cm². Culture-dependent methods were used to evaluate the inactivation effects of PLS across varying treatment durations and distances. Physicochemical properties (pH, color) and aerobic plate counts (APC) in uninoculated DLGVJs were also assessed post-treatment.

Results: The pH and color of DLGVJs did not change significantly following treatment ($P > 0.05$). Diluted juice demonstrated enhanced inactivation efficiency due to lower absorbance. APC was reduced by approximately 2.0 log CFU/mL after 30 seconds of treatment. For inoculated pathogens, reductions of ~2.0 log CFU/mL were observed after 120 seconds. Inactivation efficiency was influenced by treatment duration and lamp distance.

Significance: PLS effectively inactivated foodborne pathogens in DLGVJs while maintaining their physicochemical properties. This technology offers a promising non-thermal solution for improving the safety of DLGVJs and supporting market

growth.

Award Application: Developing Scientist Award

P2-169: DOES PHYSICAL STRUCTURE DRIVE HYGIENE BEHAVIOURS IN SCHOOL FOOD HANDLERS? THE BROKEN WINDOW THEORY APPROACH

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Yiannas (2015) draws attention to the importance of physical structure by discussing the criminological “Broken Windows Theory (BWT)” in food safety contexts, implying that food handlers working in a structurally flawed environment are inclined to engage in poorer practices. However, the applicability of BWT to food safety environments remains underexplored.

Purpose: This study aimed to investigate the applicability of the Broken Windows Theory within food safety contexts by analyzing the relationship between physical structure and food safety behavior in public schools in Brazil.

Methods: Food safety compliance was assessed using a risk-based checklist applied in 321 public schools. The BWT hypotheses were tested using Partial Least Squares Structural Equation Modelling. Checklist items with over 25% non-applicable responses were removed, and categories were reorganized. Behavioral factors—including personal hygiene, food hygiene, ingredients storage and management, and environmental hygiene—were treated as dependent variables. Physical structure was modeled as the independent variable. Food safety documents and training were included as control variables.

Results: Training had a significant positive effect only on the ingredients storage and management category ($\beta = 0.11$; $p = 0.03$). Food safety documents were positively related to physical structure ($\beta = 0.31$; $p = 0.047$). Physical structure also significantly influenced all dependent variables: personal hygiene ($\beta = 0.36$; $p < 0.001$), food hygiene ($\beta = 0.23$; $p = 0.001$), ingredients storage ($\beta = 0.09$; $p = 0.01$), and environmental hygiene ($\beta = 0.49$; $p < 0.001$).

Significance: These findings demonstrate the potential applicability of the Broken Windows Theory to food safety. A well-maintained physical environment plays a critical role in promoting positive food safety behaviors, influencing food handlers

at all stages of food preparation and service, and ultimately reducing the risk of unsafe food handling practices.

P2-170: AVOIDING ALARM FATIGUE: A RISK-BASED PROFILE FOR PRODUCT ALERT TIMING

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Alarm fatigue in foodservice settings, caused by excessive ambient air temperature alerts, can delay responses to critical deviations and compromise food safety.

Purpose: This study examined the relationship between ambient air, simulated, and actual product temperatures using wireless sensors in a reach-in refrigerator to develop a risk-based approach to product alert timing.

Methods: Diced tomatoes and shredded lettuce were stored in 1/6 size pans with sensor probes measuring actual product temperatures, while six additional sensors tracked ambient air and simulated product temperatures at various locations within the refrigerator. Data were collected under normal and stress conditions including power outages (4- and 12-hours) and open-door scenarios (frequently opened and door left ajar). Predictive microbiology models were used to assess the potential for growth of *Salmonella*, pathogenic *E. coli*, and *Listeria monocytogenes* during the stress scenarios.

Results: Ambient air temperatures poorly predicted product temperatures under all conditions. During normal conditions, ambient readings were consistently higher than product temperatures. However, simulated product temperatures closely matched actual product temperatures, with deviations under 2°F. Stress tests showed ambient temperatures rising faster and higher than product temperatures, which stayed below 41°F significantly longer. Simulated product temperatures remained highly accurate, averaging deviations of 0.52°F (tomatoes) and 0.98°F (lettuce). Microbial modeling suggests minimal growth for *Salmonella* and *E. coli* (<0.5 log) during a 12-hour power outage, though *Listeria monocytogenes* posed a slightly elevated risk (approaching 1.0 log growth).

Delaying ambient temperature alerts until deviations exceed 2 hours could reduce alarm fatigue without compromising food safety.

Significance: This research proposes a risk-based alarm management strategy, leveraging simulated product temperature data to align food safety practices with regulatory standards while minimizing unnecessary alerts.

P2-171: AN ASSESSMENT OF THREE RETAIL FOOD SAFETY TRAINING PROGRAMS FOR FOOD SERVICE EMPLOYEES IN PENNSYLVANIA

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: According to the US Centers for Disease Control, food safety is an important public health issue, with foodborne illnesses affecting roughly 9.4 million people each year in the United States. A 2018 Public Health Report indicated that the economic burden of a single outbreak to a restaurant can range between ~\$4000 to \$2.2 million. Therefore, preventing foodborne illnesses in Pennsylvania food service facilities could bring a total economic savings of \$2.6 million to \$1.1 billion per year.

Purpose: This project evaluated three different training modalities (in-person ServSafe™, online TAP Series, and Penn State Extension's Virtual Food Safety Manager [VFSM] training) and determined their impact on learning outcomes, such as food safety behavior changes immediately after training (short-term) and 3–6 months later (long-term), as well as participants' final ServSafe™ test scores.

Methods: Participants (n=90) were recruited by county-based Extension educators and assigned to one of three different training modalities: ServSafe™, TAP Series, or VFSM. Penn State Extension educators provided each of the trainings or access (TAP Series) to participants, evaluated learning outcomes (e.g., changes in food safety behaviors), and assessed final ServSafe™ test scores.

Results: Fifty participants completed an assigned training and the ServSafe™ exam. The ServSafe™ training had the lowest pass rate among the three training modalities at 76%, while both VFSM and TAP Series achieved 100% pass rates. Mean test scores were highest for VFSM (84.90%), followed by TAP Series (81.13%), and ServSafe™ (76.32%). Despite differences in pass rates and scores, statistical analysis showed no significant differences in mean exam scores across the three modalities.

Significance: These findings suggest that virtual and online asynchronous food

safety courses such as VFSM or TAP Series may be as effective as traditional face-to-face training, potentially offering greater flexibility and accessibility without compromising learning outcomes.

Award Application: Undergraduate Student Award

P2-172: FOOD SAFETY CULTURE ASSESSMENT IN THE SUPERMARKET: INCLUDING DIFFERENT SECTORS

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Category: Retail and Food Service Safety

Abstract:

Introduction: Supermarkets represent a critical point for food safety as they offer a wide variety of products and sectors, including fresh and processed foods, and non-food items. Food Safety (FS) culture has been investigated in food businesses as a risk factor; however, it was not previously reported in food businesses with multiple sectors.

Purpose: To assess FS-culture in a supermarket, testing an assessment method based on food services.

Methods: This case study was conducted in a supermarket in Brazil, including different sectors (bakery, confectionery, pastry, and distribution). The FS-culture was assessed using a mixed-method and data triangulation approach validated for institutional food services. The method encompassed eight elements (leadership, communication, commitment, knowledge, work pressure and social norms, work environment, risk perception, and management system, styles, and process) assessed using questionnaires for food handlers and managers, checklists (infrastructure and sanitary risk), and participant observations. The data were scored from reactive (1) to proactive (3) based on specific scales for each method and triangulated using an interpretation grid. The Brazilian Ethics Committee approved the study (2.040.338).

Results: Sixty-one food handlers with an average age of 31 years ($SD = 8.49$) and nine managers with an average age of 33.5 years ($SD = 8.32$) participated in the research. Most food handlers (83%) and managers (89%) participated in food safety training. The prevailing FS-culture was reactive (score 2), with the lowest scores (1–2) assigned to leadership, knowledge, risk perception, and management system, styles, and process. The highest score (3) was assigned to work pressure and normative beliefs.

Significance: The FS-culture was typified as reactive in the supermarket, highlighting opportunities for improvement in this food business with multiple sectors. The study demonstrated the feasibility of implementing a mixed-method

approach and data triangulation to assess FS-culture in a complex food retail environment.

P2-173: EVOLVING FOOD SAFETY CULTURE THROUGH THEORY OF PLANNED BEHAVIOR-BASED INTERVENTIONS: A CASE STUDY IN A BRAZILIAN AIR FORCE FOOD SERVICE

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Presentation type/Format: Technical

Category: Retail and Food Service Safety

Abstract:

Introduction: The Theory of Planned Behavior (TPB) is widely used to study human behavior and can guide interventions to improve food safety (FS) culture.

Purpose: To assess the effectiveness of TPB-based interventions in proactively evolving FS culture in a Brazilian Air Force foodservice setting.

Methods: This quasi-experimental study used a mixed-method design including questionnaires, sanitary risk and physical structure checklists, and participant observation. FS culture was scored on a 1–3 scale (reactive to proactive), and TPB constructs (attitude, perceived behavioral control, subjective norm, intention) were evaluated with a validated 31-item questionnaire. Interventions targeted constructs with significant effects ($p < 0.05$) on intention and the lowest-scoring FS culture elements. Pre- and post-intervention scores were compared using Student's t-test. Ethics approval: Brazil (0276/2022).

Results: 120 food handlers and 5 managers participated. Most handlers were male (96%) with an average age of 31 (SD = 11.91). FS culture began at an “active” level (score = 2), with risk perception rated most reactive (score = 1–2). Perceived behavioral control positively influenced intention to follow safe food handling [$\beta = 0.19$; $p = 0.006$; $R^2 = 0.09$]. Four interventions focused on identifying unsafe practices, understanding foodborne risk, and improving risk-reducing decisions. Post-intervention, FS culture improved (score = 2–3), with gains in knowledge and communication (score increases from 2 to 2–3). While TPB constructs didn't significantly change post-intervention, their high baseline scores reflected existing favorable intent toward FS behavior.

Significance: The intervention was effective in evolving FS culture from active to proactive. Perceived behavioral control was key to driving this shift, supporting better food safety conditions.

P2-174: EVALUATING THE EFFECTIVENESS OF MICROLEARNING IN ENHANCING FOOD SAFETY KNOWLEDGE AND BEHAVIORAL CONSTRUCTS OF FOOD HANDLERS

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Presentation type/Format: Technical

Category: Retail and Food Service Safety

Abstract:

Introduction: Microlearning has emerged as a promising educational strategy, leveraging short and focused modules.

Purpose: To evaluate the effectiveness of a food safety (FS) microlearning course in improving FS knowledge and Theory of Planned Behavior (TPB) constructs among food handlers in institutional catering.

Methods: A quasi-experimental study was conducted in a hospital catering operation in the Netherlands. A 24-item questionnaire based on Ajzen (1991) assessed FS knowledge and TPB constructs (attitude, subjective norm, perceived behavioral control, and intention) both pre- and post-intervention. Knowledge questions required "Yes," "No," or "I don't know" responses, with one point awarded for correct answers and zero for incorrect/uncertain answers. TPB constructs were measured using a 5-point Likert scale, with scores calculated by averaging the responses to related items. The microlearning intervention consisted of seven modules covering eight key hygiene practices, incorporating slides and real-life catering scenarios. Videos were divided into four parts: importance of the practice, correct execution, appropriate timing, and consequences of non-adherence. Each video lasted approximately four minutes. A Student's t-test for repeated measures was used to evaluate the impact of the intervention. The study was approved by the Ethics Committee in the Netherlands.

Results: Of 100 invited food handlers, 41 completed all study steps. Among participants, 68.2% were female and 33.3% were aged between 18 and 24 years. Statistically significant improvements were observed in perceived behavioral control ($p = 0.026$) and attitude ($p = 0.030$). No statistically significant changes were found in knowledge, subjective norm, or intention, which had already high pre-assessment scores.

Significance: The microlearning intervention was effective in enhancing confidence and hygiene performance. While food handlers already had sufficient knowledge and intention to follow hygiene practices, the training increased their self-efficacy and improved practice execution in the workplace.

P2-175: STAKEHOLDER PERSPECTIVES ON MANAGEMENT RESPONSIBILITY IN UK FOOD-SERVICE: INSIGHTS FROM ENVIRONMENTAL HEALTH OFFICERS AND SMALL AND MEDIUM-SIZED ESTABLISHMENTS (SMES)

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Presentation type/Format: Technical

Category: Retail and Food Service Safety

Abstract:

Introduction. in the UK, poor regulatory compliance in the food-service sector is often attributed to the characteristics of SMEs, including an ineffective food safety management system (FSMS). Effective management practices and regulatory inspections play a vital role in ensuring the safety of food served to consumers and minimising the risk of foodborne disease (FBD).

Purpose. This study explored the perspectives of Environmental Health officers (EHOs) and managers of SME food-service establishments on management responsibility for food safety compliance and the impact on food safety culture (FSC).

Methods. A mixed

Methods approach was used, including focus groups (n=2) with EHOs (n = 11) from rural and urban local authorities (LA), in-depth, semi-structured interviews with food-service managers (n=10) and a quantitative online FSC questionnaire targeting food-service managers (n=45). Thematic content analyses of the transcribed interviews using NVivo and quantitative analysis of the questionnaire using SPSS.

Results EHOs perceived managers have a legal obligation to implement a FSMS, be competent, and aware of the legislation and proactive “anticipating problems before they get them”. Recognising and completing actions including training, controlling hazards, being present and approachable and “everybody’s got to know their role”. Most managers emphasised food safety “not serving food that would endanger your customer “and 95% recognised the importance of management involvement in daily food service activities to ensure adherence to good food safety practices. However, discrepancies exist; for instance, receiving

legislative updates “When you get a visit from EHO”. Although, 91% perceived themselves to be ‘nearly’/‘totally’ responsible for developing food safety procedures, 80% believed staff shared this responsibility.

Significance. Engaging stakeholders provides valuable information on management responsibility and practices impacting food safety compliance and culture, highlighting areas for improvement. Understanding these perspectives is crucial for the development of tailored interventions to improve food safety outcomes in the sector.

P2-176: MANAGEMENT ATTITUDES AND PERCEPTIONS RELATED TO COMMON FOOD SAFETY NONCOMPLIANCE: IMPLICATIONS FOR FOOD SAFETY CULTURE IN UK MICRO/ SMALL AND MEDIUM-SIZED FOOD SERVICE ESTABLISHMENTS

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Presentation type/Format: Technical

Category: Retail and Food Service Safety

Abstract:

Introduction. Management commitment has been linked to improved food safety performance and the establishment and maintenance of a positive food safety culture (FSC). Furthermore, failure to meet regulatory standards is assumed to increase the risk of FBD.

Purpose. This study aims to examine how management attitudes and perceptions influence common hygiene inspection nonconformities in micro, small and medium-sized food service establishments (SMEs).

Methods. A mixed methods approach was employed, including archive retrieval and evaluation of food hygiene inspection reports (n=299). in-depth, recorded semi-structured interviews with food service managers (n=10) and a quantitative online FSC questionnaire targeting SME food-service managers (n=45).

Quantitative analyses of inspection reports and questionnaire using SPSS and thematic analysis of transcribed interviews using NVivo.

Results. Analysis of the inspection reports revealed that the most common non-compliance issues included poor cleaning (70%), damaged or unsuitable structure, cross-contamination (24%), stock rotation (22%) and poor temperature control (16%). Additionally, issues related to the food safety management system (FSMS), were prevalent including monitoring (49%) and inadequate training (30%). Ninety-seven percent of surveyed managers believed they understood the food-safety risks and the unlikelihood of consumers getting food poisoning. Cross-contamination was frequently cited as a significant risk, with one manager noting "Obviously cross-contamination is a huge one... that's probably one of the most key aspects of working in a restaurant, so we're very careful with that". However, 45% admitted difficulty following food-safety procedures during busy periods and 16% reported not reprimanding staff for poor hygiene practices. Affordability was

considered a key factor in carrying out structural repairs by 83% of managers, while 69% believed that the structure did not impact food-safety. Some managers (28%) considered keeping records unnecessary.

Significance. Findings provide valuable insight into the management of food safety and highlight the management behaviours that influence compliance and the development of a positive FSC.

P2-177: INVESTIGATING HAND HYGIENE AND CROSS-CONTAMINATION RISKS IN THE FOOD SERVICE SECTOR: A SYSTEMATIC REVIEW AND OBSERVATIONAL CASE STUDY

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Food service food handlers must ensure food safety, especially when handling ready-to-eat (RTE) products. Hands can transfer pathogens and allergens, making it essential to understand hand hygiene behaviours in food service environments to assess cross-contamination risks and prevent foodborne illness.

Purpose: To investigate food handler bare hand contact with items in food service environments, hand hygiene performance, and the potential for cross-contamination of RTE products.

Methods: A systematic review of 118 studies explored inclusion of observational data on food handler behaviours. An observational case study was carried out in a multi-site SME food service establishment specializing in bakery products. CCTV footage was captured over six hours at two sites, involving seven staff members. Hand contact occasions were coded and analysed using Observer XT Behaviour Analysis Software to quantify and sequence hand hygiene events and contact with RTE products.

Results: The systematic review identified a lack of observational data on food handler hand hygiene and cross-contamination risks, demonstrating the relevance of the present study. Over six hours, 1414 hand contact occasions were observed (site 1: 916; site 2: 498). Food handlers touched raw ingredients (22% site 1; 2% site 2) and RTE foods (6% site 1; 15% site 2). only six compliant handwashing attempts were recorded (site 1: 4; site 2: 2). instances of touching objects like ovens (20% site 1), tills (23% site 2), clothes (13% site 2), and faces (11% site 2) without subsequent handwashing before handling RTE products were common.

Significance: The study revealed a lack of compliant handwashing before handling RTE foods, emphasizing the need to raise food handler awareness of cross-contamination risks. Findings can inform recommendations for the food service sector on the importance of proper hand hygiene, regular cleaning of hand contact surfaces, and the identification of surfaces for environmental and microbiological testing.

P2-179: UV-C LED INACTIVATION OF AEROSOLIZED HEPATITIS A VIRUS DROPLETS ON SURFACES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Foodborne viruses are known to be transmitted via the fecal-oral route and through aerosolized droplets and contaminated surfaces. Novel methods to control their spread in the food environment including ultraviolet light (UV-C) technologies continue to be researched to determine optimal inactivation doses.

Purpose: The objectives were to determine the doses for the inactivation of bio-aerosolized deposits of hepatitis A virus (HAV) on stainless-steel and glass discs.

Methods: Viruses were aerosolized using a 6-jet-nebulizer within a modified chamber, deposited onto stainless-steel or glass discs (0.17 mL on 0.5x0.5 cm²) and immediately treated with 255-nm or 279-nm UV-C light emitting diodes (LED) (surface dose=0-76.5 mJ/cm² with 255-nm UV-C=0-8.1 mJ/cm² with 279-nm UV-C LED). Each triplicate treatment was assayed in duplicate, and data were statistically analyzed.

Results: Linear D10 values (UV-C dose required to achieve a 1-log reduction of aerosolized viral deposits) for HAV by 255-nm UV-C LED on stainless-steel and glass discs were 47.39 ± 7.40 and 40.0 ± 2.94 mJ/cm² ($R^2=0.94$ and 0.91), and by the Weibull model were 29.69 ± 5.49 and 35.25 ± 15.01 mJ/cm² ($R^2=0.99$ and 0.92), respectively. However, linear D10 values using 279-nm UV-C LED on stainless-steel and glass discs were 6.60 ± 0.27 and 5.57 ± 0.74 mJ/cm² ($R^2=0.98$ and 0.94), and with the Weibull model were 6.67 ± 0.63 and 5.21 ± 1.25 mJ/cm² ($R^2=0.98$ and 0.95), respectively. Treatments with 279-nm UV-C LED at 8.1 mJ/cm² showed 1.14 ± 0.09 and 1.29 ± 0.09 log PFU reduction, and 76.5 mJ/cm² 255-nm UV-C LED showed 1.76 ± 0.05 and 1.75 ± 0.07 log PFU reduction on stainless-steel and glass discs, respectively.

Significance: Thus, 279-nm UV-C LED showed higher inactivation efficiency for HAV inactivation than 255-nm UV-C LED. The linear model with higher R^2 values had a better fit for 279-nm UV-C LED than 255-nm UV-C LED, that showed tailing effects. The two UV-C LED systems could inactivate bio-aerosolized hepatitis A

viral deposits on model food contact surfaces to prevent foodborne viral transmission and outbreaks.

Award Application: Developing Scientist Award

P2-180: INHIBITION OF P. AERUGINOSA BIOFILMS BY TEA TREE EO IN VAPOR PHASE

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: *Pseudomonas aeruginosa* is a bacterium that's important in the food industry because it's a major food spoiler and a leading cause of food infections, and has the ability to form biofilms in different surfaces like stainless steel that is common in food industry. One alternative to inhibit biofilm formation is to use essential oils (EO`s) in vapor phase. Vapor phase could be effective at relatively lower concentrations than when applied in liquid phase, thereby causing less effect on sensory attributes.

Purpose: The aim of this work was to evaluate the inactivation of *Pseudomonas aeruginosa* (ATCC 27843) biofilms on stainless steel surfaces by tea tree (*Melaleuca alternifolia*) essential oil when exposed in vapor phase.

Methods: *P. aeruginosa* was incubated on BHI broth at 37°C for 24 h until exponential phase was observed. Sterile stainless steel plates (4.0 × 4.5 cm) were placed horizontally into jars. Then 100 µl of bacteria was inoculated to obtain an initial bacterial population of about 10⁴ CFU/ml. Jars were incubated at 24°C during 120 h. Tea tree (*Melaleuca alternifolia*) EO was added in the top of the jar to achieve final concentrations of 0, 250, 500, 750 and 1,000 ml/L of air. *P. aeruginosa* cells were collected and enumerated. Every test was performed in triplicate.

Results: Tea tree EO vapor contact showed that higher concentrations reaches its highest log reduction in *P. aeruginosa* biofilms. A 1.5 cycles reduction was reached at 1,000 ml/L of air concentration, showed significant differences (p<0.05) with respect to control.

Significance: Tea tree EO demonstrated the ability to affect the microbial reduction of *P. aeruginosa* biofilms. It can be used as an alternative in cleaning stainless steel surfaces in the food industry.

Award Application: Undergraduate Student Award

P2-181: ENGINEERED INTERVENTION TO REMOVE AND SANITIZE SALMONELLA IN EGG BIOFILMS

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Egg biofilms harbor the growth of *Salmonella* spp., and their effective removal and sanitization from food processing surfaces represents a challenge.

Purpose: This research studied the combination of an engineered enzyme with peracetic acid (PAA) in food processing surfaces as an intervention for egg biofilm removal and cell inactivation.

Methods: Three food processing surfaces (stainless steel, silicone, nylon) were tested. *Salmonella enterica* serovar Typhimurium ATCC53647 was inoculated in pasteurized liquid whole egg, and the biofilm was grown (37°C, 24 h, static conditions) in coupons (4.04 cm²). An engineered glycosyl hydrolase expressed and purified called CAase (0.150 mg/ml) was used to disrupt the biofilm. Afterward, sanitation of the coupons was conducted with PAA (90 and 120 ppm, 1- and 3-min). Scanning Electron Microscopy (SEM) was used to observe the effect of CAase and PAA in the biofilm structure and cells. Experiments were conducted by duplicate and ANOVA one-way was used to find significant differences ($\alpha = 0.05$).

Results: Microbial counts showed a mature biofilm (~ 7 log) in the three tested materials ($p > 0.05$). CAase alone allowed some cell inactivation (~ 2 log). However, using CAase followed by PAA (90 ppm) for 1 and 3 min decreased *Salmonella* counts in the three materials, although there were still a few cell survivors after the longest contact time. Meanwhile, the use of CAase and PAA (120 ppm) exhibited complete cell inactivation during the first minute of contact (Limit of detection < 1 log CFU/cm²). SEM images showed the thick biofilm with the proliferation of *Salmonella* cells. CAase opened the biofilm and exposed the

cells to PAA. The effect of PAA was observed as a clean surface with damaged cells.

Significance: This research presents a sustainable green option for effectively removing egg biofilms and inactivating *Salmonella* cells on common food-contact surfaces.

P2-182: CONCEALED OBSERVATIONS OF SANITATION VARIABILITY IN FOOD SERVICE SETTINGS

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: Human noroviruses are a frequent cause of acute nonbacterial gastroenteritis outbreaks. Over half of norovirus outbreaks (60%) can be traced to an infected person or carrier of norovirus who did not use Good Manufacturing Practices during the preparation and serving of food. The virus has a low infectious dose and is shed in large amounts by infected individuals, which proves to be a significant issue in food service settings that serve Ready-To-Eat (RTE) foods.

Purpose: This work analyzes the variability among food retail workers' sanitation behaviors under concealed conditions.

Methods: Four retail establishments in the Northeast United States were chosen as sites for this study. All four sites are similar in size and scale and produce/serve a variety of cooked meals, RTE foods, and beverages. To mitigate bias in sanitation practices and obtain true reflections, the research team was concealed at the time of data recordings. The researcher collecting data was identified as an "intern". The intern role entailed comprehensive on-the-job experiences to familiarize [themselves] with the department's operations, policies, and procedures. This allowed the researcher to roam each site freely and record observations. After each site visit, observations were qualitatively coded for themes and variances. Each site was sampled 30 times over six months to sample the variance in observed sanitation practices and behaviors.

Results: After 30 visits per site, qualitative coding analysis determined top variability of behavior in hand-washing procedures (71.5% of visits indicated hand-washing nonconformances, which was statistically significant; hand-washing times ranged from 5 to 12 seconds), sanitizer concentration verification (67% of visits indicated lack of daily sanitizer concentration verification, which was also significant), sanitizer contact time was rarely, if ever, adequate, and there was a significant language barrier that negatively affected food safety behaviors. All sites spray and wipe immediately when using sanitizers on surfaces, rendering them partially ineffective.

Significance: Results imply that norovirus, if present in the sampled food retail settings, can survive and spread when treated with disinfectants at shorter than EPA-recommended contact times. Hand-washing times below the recommended 20 seconds can also lead to the spread of norovirus.

Award Application: Developing Scientist Award

P2-183: A SURVEY ON THE CLEANING AND SANITIZATION PRACTICES FOR BLUEBERRY HARVEST CONTAINERS AND MECHANICAL HARVESTERS IN THE UNITED STATES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Currently, cleaning and sanitation of harvesting tools and equipment are not mandatory, although certain practices are encouraged by blueberry grower associations, cooperative extension services, and regulatory bodies in the United States. Thus, fresh blueberry producers may select their preferred cleaning and sanitization approaches.

Purpose: This study aimed to gain insights into the commonly used cleaning and sanitation practices by some blueberry producers in the United States by conducting an online survey.

Methods: A questionnaire consisting of 25 questions was used to collect information including demographic details, operation type, harvesting method, and cleaning and sanitization approach used by participating fresh blueberry establishments.

Results: A total of 71 responses were received in 2021. The vast majority of the respondents (90.4%) reported cleaning flats/lugs/crates at varying frequencies using cold or warm water, scrubbing, pressure, garden hose, or washing machine. Among these, 86.7% used detergents, surfactants, or unspecified products for cleaning. About 90.1% of respondents used quaternary ammonium, bleach, selectocide, or nonspecified products to sanitize containers, while 26.8% used sanitizing wipes. Fifty-nine respondents cleaned mechanical harvesters with cold or warm water and products like dish detergent or surfactants, and 55 of these also sanitized the equipment afterward.

Significance: The study suggests that while many producers employ appropriate sanitation practices, food safety education is needed to encourage broader adoption across all fresh blueberry operations.

Award Application: Developing Scientist Award

P2-184: DEVELOPMENT OF NONPOLAR LIQUID ANTIMICROBIALS TO ERADICATE SALMONELLA SPP. AND CRONOBACTER SPP. DESICCATED ON A STAINLESS-STEEL SURFACE

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: *Salmonella* spp. and *Cronobacter* spp. are pathogenic and opportunistically pathogenic organisms, respectively, with a history of contaminating low-moisture foods and their production environments. Remediation of such contamination often relies on non-water-based cleaning and sanitation methods.

Purpose: To evaluate the antimicrobial efficacy of nonpolar liquid delivery systems.

Methods: Cosmetic-grade cyclomethicone was supplemented with glacial acetic acid to provide antimicrobial activity (acidified cyclomethicone). A controlled water dispersion (3% v/v) was created within acidified cyclomethicone using siloxane-based emulsifiers and homogenized by shearing at 10,000 rpm, forming an acidified water-in-silicone (A-W/Si) emulsion. Lawn-based bacterial cultures (TSAYE) were inoculated onto stainless steel surfaces and equilibrated at either 75% or 33% equilibrium relative humidity (ERH) at 22 °C for 24 hours, to reach 7 log CFU/coupon. Antimicrobial assays were performed at 22 °C. Survivor presence was determined by enrichment in buffered TSB (37 °C, 24 h). Bacterial membrane viscosity was assessed using Fluorescence Lifetime Imaging Microscopy (FLIM) with a BODIPY-based fluorophore.

Results: Acidified cyclomethicone treatment (30 min, 500 mM acetic acid) eradicated *S. Enteritidis* (ATCC BAA-1045) and *C. sakazakii* (FSL F6-024) desiccated at 75% ERH (0/15 positive enrichments). However, at 33% ERH, enrichment tests were positive in most cases. The creation of a W/Si emulsion significantly enhanced efficacy. Cocktails of *Salmonella* (5 strains) and *Cronobacter* (6 strains) desiccated at 33% ERH were reduced to undetectable levels after A-W/Si emulsion treatment (10 min, 200 mM acetic acid). The mechanism may involve cyclomethicone's localization into bacterial membranes and subsequent viscosity reduction, as observed via FLIM-BODIPY.

Significance: To ensure robust efficacy across varying levels of bacterial dryness, dry sanitization strategies may need to incorporate controlled moisture levels.

P2-185: AERATED NANOBUBBLE WATER: A NOVEL STRATEGY TO ENHANCE SANITIZER EFFICACY AGAINST LISTERIA MONOCYTOGENES IN DAIRY PROCESSING ENVIRONMENTS

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: *Listeria monocytogenes* (LM), a major foodborne pathogen, presents challenges in dairy processing by forming persistent biofilms on food contact surfaces, leading to contamination and illness. Aerated water (AW) generated by nanobubble technology offers a promising solution, using fine oxygen bubbles to disrupt biofilms and inactivate bacteria through oxidative stress.

Purpose: To determine the anti-biofilm effect of aerated water, alone and in combination with commercial sanitizers, against LM biofilms in simulated dairy processing environments.

Methods: Three dairy isolates of LM were employed in this study. AW was generated with dissolved oxygen level of ~30 mg/dl and mean bubble size of $\sim 79.2 \pm 16.1$ nm, using a Moleaer nanobubble generator. Test solutions included control (tap water), chlorine (100 & 200 ppm), peroxyacetic acid (2 & 5%), and quaternary ammonium compounds (200 & 500 ppm), alone or combined with AW, tested on stainless steel and polystyrene surfaces. Anti-adhesion and anti-biofilm properties were evaluated on coupons treated with test solutions in the presence and absence of organic contamination (10% UHT), with *Listeria* enumeration following serial dilutions. The experiments were completely randomized, and data analyzed using R with significance tested at $p < 0.05$.

Results: Aerated water significantly reduced *Listeria* planktonic cells, inhibited adhesion, and inactivated established biofilms on polystyrene and stainless-steel surfaces when compared to the control in the presence and absence of organic contamination ($p < 0.05$). Further, sanitizers prepared in AW were significantly more effective against LM planktonic and biofilm cells than when prepared in tap water. Overall, 5% PAA in AW was found to be most effective in completely inactivating planktonic cells and reducing LM biofilm irrespective of the isolate tested.

Significance: AW and commercial sanitizers in AW offer a potential strategy to

control *Listeria* biofilms in dairy environments thereby reducing risk of contamination and improving food safety.

Award Application: Developing Scientist Award

P2-186: SANITATION STRATEGIES FOR MITIGATING LISTERIA MONOCYTOGENES ON FOOD CONTACT SURFACES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: In the fresh produce industry, increased concerns of cross-contamination from food contact surfaces (FCS) regularly encountered during postharvest handling have led many to scrutinize their sanitation regimes. Toward this goal, developing efficient, low-cost, and appropriate sanitation techniques while considering the nature of the FCS material needs to be broadly studied.

Purpose: The objective of this research was to determine the efficacy of various sanitization techniques and sanitizing agents to reduce the population of *Listeria monocytogenes* (LM) on commonly encountered FCS during postharvest fruit and vegetable handling.

Methods: Three commonly used FCS materials were selected: interlocking belt (IB) made of polypropylene, polyvinyl chloride (PVC) rollers, and closed-cell foam (CF). Coupons were spot inoculated with ~8 log CFU of an LM 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 with a detergent applied as a liquid with manual brushing or as a foam, followed by rinsing and sanitization with free chlorine (350 ppm), peroxyacetic acid (350 ppm), or no sanitizer. A control group received no treatment. The 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 35 °C and enumerated after 48 h. Nine samples were evaluated per treatment combination with a mixed model used to determine significant differences using ANOVA.

Results: For IB and CF, all treatments performed significantly better than the untreated control ($p < 0.001$), achieving log reductions of 1.83–3.01 and 3.28–4.57 CFU/coupon, respectively. For PVC, greater reductions were observed when a sanitizer was applied after detergent treatment ($p < 0.05$), achieving at least a 3.37 log CFU/coupon reduction.

Significance: Results indicate that FCSs influence the efficacy of cleaning and

sanitization approaches for materials commonly encountered in fresh produce postharvest handling.

Award Application: Developing Scientist Award

P2-187: EXPLORING THE SYNERGISTIC EFFICACY OF LACTIC ACID OR PERACETIC ACID, AND UV-C IN THE INACTIVATION OF SALMONELLA ON SOILED FOOD CONTACT SURFACE MATERIALS

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: *Salmonella* remains a pathogen of concern for fresh produce and effective cleaning and sanitization strategies of food contact surfaces (FCS) are necessary to prevent cross-contamination. The organic sector, often constrained by limited sanitizer options, seeks alternative methods that are effective and organic-compliant.

Purpose: This study assessed the inactivation of *Salmonella* on soiled food contact surface materials following exposure to lactic acid (LA; 2% v/v), Peracetic Acid (PAA; 120 ppm and 375 ppm), and/or ultraviolet light (UV-C; 5 mW/cm²).

Methods: Five commonly used FCS were selected: stainless steel, polyvinyl chloride, high-density polyethylene, polytetrafluoroethylene, and nylon. Coupons (7 cm²) were immersed into a mixture of soil, water, and blueberries and air-dried overnight. Coupons were then spot inoculated with ~8 log CFU *Salmonella* cocktail and air-dried for 1 h. Antimicrobial treatments were applied for 1 or 5 min using a chamber equipped with a 254 nm UV-C lamp and a disinfectant spray system. Three runs with two technical replicates (N=48) of seven experimental treatments, including LA, PAA, and UV, and combinations of LA-UV, PAA120-UV, and PAA375-UV, were investigated alongside positive (water-only) control. Coupons were neutralized in 30 mL Dey/Engley broth and 0.2% Tween 80,

vortexed, and spiral plated onto Tryptic Soy Agar and Xylose Lysine Deoxycholate. Plates were incubated at 37 °C and enumerated after 24 h.

Results: The effects of sanitizer, material, and their interactions, significantly impacted *Salmonella* reduction ($p < 0.05$) in a two-way model. The highest reductions were observed in PAA-based treatments, 6.1–6.3 log reduction/coupon across materials, followed by LA-based treatments (5.3–5.9 log reduction/coupon), significantly higher than control and UV-only treatments ($p < 0.05$). No significant differences were found between PAA and PAA-UV or LA and LA-UV ($p > 0.05$). UV achieved the lowest reduction, averaging 1.6–2.1 log CFU/coupon.

Significance: These results can help design targeted interventions that optimize sanitation protocols in organic produce.

Award Application: Developing Scientist Award

P2-188: ANTIMICROBIAL NANO-MIST PRETREATMENT FOR ENHANCING SUPERHEATED STEAM EFFICACY IN INACTIVATING ENTEROCOCCUS FAECIUM NRRL B-2354 ON DRY FOOD PROCESSING SURFACES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Superheated steam (SHS) is an emerging sanitation method for dry food processing environments. However, few studies have evaluated the synergistic effects of combining antimicrobial compounds with SHS.

Purpose: To evaluate the efficacy of antimicrobial nano-mist pretreatment in combination with SHS for inactivating microorganisms on a model dry food processing surface. **Methods:** Stainless-steel coupons inoculated with *E. faecium* (11.1 ± 0.4 log CFU/cm²) were conditioned (0.2 aw) and treated with nano-mist (water, 70% ethanol, 0.05% peracetic-acid) sprayed at 1 mL/min or acidified oil emulsion (100 μ L) at two different time intervals (10/20 s). Pretreated coupons were exposed to SHS at 150°C for 10 s. SHS experiments were conducted using bench-scale (100% steam content) and pilot scale equipment (5% steam, 95% hot air). Distance between SHS nozzle and coupons was maintained as 3 cm. The experiments were performed in triplicates and survivors enumerated by plate count (detection limit 0.08 log CFU/cm²).

Results: The come-up time to reach 150°C after various nano-mist pretreatment for lab-scale SHS was high (>200 s) compared to pilot-scale SHS (<200 s) due to transient evaporation of condensed steam in lab-scale SHS (100% steam) whereas, pilot-scale SHS (5% steam and 95% hot air) did not have any steam condensation. The microbial log reduction with lab-scale SHS regardless of nano-mist pretreatment was $>6.4 \pm 0.3$ log CFU/cm² whereas, pilot-scale SHS treatment resulted in a microbial reduction of $<2.8 \pm 0.2$ log CFU/cm² ($p < 0.05$). Subsequently, increasing pilot-scale SHS equipment treatment temperature (195 °C) with extended treatment time (30 s) resulted in higher microbial inactivation (3.3 ± 0.6 log CFU/cm²).

Significance: The research demonstrated antimicrobial pretreatments of surfaces improved SHS treatment efficacy. In addition, the study demonstrated the critical role of higher steam content in improving the sanitation efficacy.

P2-189: THERMAL DISPERSAL DURING DRY STEAM TREATMENTS INFLUENCES MICROBIAL REDUCTION IN 'DIFFICULT-TO-CLEAN' EQUIPMENT NICHES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Although superheated steam is effective for microbial inactivation on surfaces, the structural dimensions of “difficult-to-clean” sites of equipment may limit surface sanitization.

Purpose: To characterize the surface temperature profiles and inactivation of *Enterococcus faecium* NRRL B-2354 within “difficult-to-clean” sites by superheated steam as a function of depth and width of recess opening.

Methods: Three large test cells with opening widths of 1 cm² and depths of 4, 2, 1 cm, and one small test cell with an opening of 0.1 cm² and depth of 0.4 cm were fabricated out of stainless steel (304, 2B). Superheated steam (400°C) generated by a commercial unit (Bayzi Corporation, Cincinnati, OH) was directed at the geometric center of the opening from varying distances (3, 2, 1 cm). Surface temperatures were measured with K-type thermocouples at the (i) the exterior surface (1 cm from the opening), (ii) rim of the recess, and (iii) the bottom of the recess. *E. faecium* was dried down spot-inoculated at the geometric center of the bottom of each recess and exposed to superheated steam (3 cm distance) for predetermined times. An ANOVA and Tukey tests were performed.

Results: Depth of recess and nozzle distance significantly decreased surface temperatures within “difficult-to-clean” sites (10 s exposure) ($p < 0.001$), with significant differences between each depth and distance ($p < 0.05$). Temperatures were hottest at the bottom of the recess, except at the outer rim of the small cell which was approximately 20°C hotter. The maximum temperature of $334.7 \pm 3.8^\circ\text{C}$ (1cm depth, 1 cm distance) decreased 163°C and 99.6°C when increasing niche depth to 4 cm depth and nozzle distance to 3 cm, respectively. All depths (4, 2, and 1 cm) achieved >5 log CFU/cm² microbial reduction (420 s, 3 cm distance) at the bottom of the recess.

Significance: Equipment modifications could optimize thermal dispersal within “difficult-to-clean” sites and improve surface sanitization.

Award Application: Developing Scientist Award

P2-190: ULTRAVIOLET INACTIVATION (UV-C) OF VIRAL AEROSOLS IN AIR AND SURFACES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: The presence of viral aerosols in food industrial environments, including air and food-contact surfaces, poses a serious health risk to both consumers and workers.

Purpose: This study evaluates the effectiveness of UV-C treatment in inactivating viral aerosols both in the air and on food-contact surfaces.

Methods: Bacteriophages Q β , T1UV, and MS2, commonly used as viral surrogates, were used in the experiments. Viral aerosols were generated using a 4-jet Blaustein Atomizing Module (BLAM) atomizer. Air disinfection was performed using a combination of UV LED and low-pressure mercury lamps, while surface aerosolization treatments used UV-C LED at 279 nm. Stainless steel (316L) coupons (0.5-inch diameter) were used as food contact surfaces for aerosol deposition.

Results: Following aerosolization, the recovered concentrations of Q β and T1UV were 6.79 log (PFU/mL) and 6.08 log (PFU/mL), respectively, indicating reductions of 4.7 log and 3.92 log from their initial titers of 11.49 log (PFU/mL) and 10 log (PFU/mL). UV-C treatment reduced the microbial load by 0.51 log (PFU/mL) for Q β and 0.85 log (PFU/mL) for T1UV. Surface aerosolization of Q β on stainless steel coupons showed up to a 2 log (PFU/mL) reduction with UV treatment. In the case of MS2, up to a 1 log (PFU/mL) reduction was observed at a fluence of 128 mJ/cm². Drying alone contributed to a 1 log (PFU/mL) reduction for Q β and a 1.52 log (PFU/mL) reduction for MS2, indicating that desiccation also affects viral viability.

Significance: This research can assist in addressing the growing demand for effective viral mitigation strategies by providing scalable UV-C solutions for viral aerosol disinfection in food environments, including protection for workers.

Award Application: Developing Scientist Award

P2-191: ULTRAVIOLET C INACTIVATION OF SALMONELLA ENTERITIDIS IN RAW CHICKEN JUICE ON FOOD CONTACT SURFACES USING THE CONTAMINATION SANITIZATION INSPECTION AND DISINFECTION (CSI-D+) DEVICE

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: *Salmonella* is the leading cause of bacterial foodborne illness in the United States. The Contamination Sanitization Inspection and Disinfection (CSI-D+) device is a handheld portable system equipped with fluorescence imaging and dual operations for detecting and disinfecting microorganisms on food contact surfaces (FCS).

Purpose: The aim of this research was to assess the effectiveness of the CSI-D+ device in inactivating bacteria in food residue on common FCS found in food processing environments.

Methods: Raw chicken juice was inoculated with *Salmonella enterica* serovar Enteritidis at a concentration of 108 CFU/mL and introduced to stainless steel coupons. These coupons were then exposed to UV-C radiation at 275 nm using the CSI-D+ device at a low intensity (5 mW/cm²) for 15 seconds. Levels of *S. Enteritidis*, *Escherichia coli* (*E. coli*)/coliforms, and aerobic plate count (APC) were compared to untreated controls across three trials. Each trial included three treated and one untreated coupon.

Results: At 5 mW/cm² for 15 seconds, reductions of 0.38, 0.40, and 0.45 log CFU/g were recorded for *S. Enteritidis*, *E. coli*/coliforms, and APC, respectively. This corresponds to reductions of 58.5%, 60%, and 64%, respectively. Additional tests will assess the device's efficacy on various other FCS under both low (5 mW/cm²) and high (10 mW/cm²) intensities for 15 and 30 seconds.

Significance: This study is the first to evaluate the disinfection function of the CSI-D+ on food contact surfaces. Its successful application could support commercialization of the device in food processing facilities to enhance hygiene and ensure food safety.

Award Application: Developing Scientist Award

P2-192: RELATIVE PERFORMANCE OF RAPID HYGIENE ASSAYS AGAINST ALLERGEN-LADEN SOILS FOR CLEANING VERIFICATION WITH DAIRY PROCESSING EQUIPMENT

Primary Author:

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Different rapid hygiene assay technologies vary in their fundamental technologies, yet few studies compare their relative effectiveness at detecting allergen-laden soils on processing equipment.

Purpose: This work compares responses of common different rapid assay technologies against peanut and milk allergen-laden soils on dairy manufacturing equipment subjected to a basic cleaning regimen.

Methods: We compared three technologies suitable for allergen-laden soil detection during the cleaning process, namely, ATP, total adenylate (ATP+ADP+AMP, denoted as A3), and general protein with lateral flow immunoassay (LFI) as a reference. Four interior grids each with 20, 100 cm² sampling sites were designated within a stainless steel processing tank. After baseline cleaning, peanut butter ice cream mix was uniformly applied to the tank inner surface. Using a spray-ball, the tank was cleaned in three stages: water (7°C), hot water (45°C), then hot (45°C) caustic solution. At each cleaning stage, uniform swabbing was completed using the designated sites within each grid. The experiment was replicated four times yielding a total of 80 data points for each assay type. Analysis of variance (alpha of < 5%) was used to compare assay and treatment effects with pairwise comparisons completed where appropriate.

Results: As the cleaning stages progressed, the level of surface cleanliness increased accordingly confirming cleaning stage effectiveness. After the cold water and warm water stages, peanut and milk allergens responses for LFI, protein and A3 remained similar with the A3 assay indicating soil presence about 4 times higher than the ATP assay. After washing with caustic soda, neither protein nor LFI assays detected the presence of allergen-laden soil and both ATP and A3 were at levels below predetermined thresholds.

Significance: This work demonstrates strong but distinct correlations between LFI-based testing, an industry standard for post-cleaning allergen residue assessment, and more general rapid soil assays, including those that detect general proteins

and adenylate-containing soils. This study highlights the need to carefully select the appropriate assay type when allergen-laden soil is present.

P2-193: NANOTEXTURED 316L STAINLESS STEEL ENHANCES ANTIFOULING AND ANTIBACTERIAL POTENTIALS AGAINST SALMONELLA NEWPORT

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Pathogenic biofilms on stainless steel threaten food processing safety. Traditional removal methods struggle with niches and require repetition. Electrochemical etching holds promise in forming mechano-bactericidal nanostructures that disrupt bacterial envelopes, effectively inactivating attached bacteria and enhancing surface hygiene.

Purpose: This study investigates how varying electrochemical etching voltages influence the nanotopography of stainless steel surfaces and the oxidative stress levels of bacteria attached to these surfaces.

Methods: Stainless steel (316L) coupons were cleaned, dried, and etched with 48% w/w nitric acid as the electrolyte. A 0.38 cm² area was etched at 2.0V, 2.2V, and 2.4V for 60 seconds at 5 °C. Surface topography was analyzed using SEM and AFM. For bactericidal assays, *Salmonella* Newport was incubated with as-received and nanotextured coupons for 30 minutes at 37 °C. ROS levels were quantified using CellROX green fluorescence under confocal microscopy, and total and dead cells were assessed with DAPI and PI staining. Five spots per sample were analyzed.

Results: SEM revealed that increasing voltage from 2.0V to 2.4V reduced roughness by decreasing grain boundaries and promoting nanotexture formation. AFM showed a monotonic rise in surface skewness (Ssk) from -0.8 (unetched) to 1.57 (SS-2.4V) and kurtosis from 0.17 to 3.45, indicating sharper peaks at higher voltages. Batch variation and areal heterogeneity remain challenges with the current etching setup. Confocal microscopy showed significantly higher ROS intensity on SS-2.4V $(2.05 \pm 0.99) \times 10^4$ vs. control $(6.57 \pm 6.70) \times 10^3$ ($p < 0.0001$). A strong correlation between PI and ROS intensities ($R^2=0.70$) supports a ROS-mediated antimicrobial mechanism. Surface-attached cells decreased by 56.1% (SS-2.4V), 51.4% (SS-2.2V), and 49.5% (SS-2.0V), indicating improved antifouling properties.

Significance: Nanotexturing of stainless steel by scalable electrochemical etching appears feasible, and voltage is critical for fine-tuning nanotopography sharpness.

Preliminary results indicate these nanotopographies may protect stainless steel against *S. Newport* through a dual antifouling-antimicrobial mechanism, potentially reducing microbial load in food processing environments.

Award Application: Developing Scientist Award

P2-194: REMOVAL AND INACTIVATION OF CORONAVIRUS SURROGATES ON ABIOTIC SURFACES USING PRE-SATURATED DISINFECTANT WIPES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Disinfectant wipes (DW) are designed to inactivate microorganisms on surfaces. It is unclear if viruses are chemically inactivated or simply removed during wiping process.

Purpose: To investigate the removal and inactivation of coronavirus surrogates on abiotic surfaces using pre-saturated blank polypropylene wipe (BW) and DW.

Methods: Glass and vinyl coupons were contaminated with 5.6-6.2 log TCID₅₀ bovine coronavirus or human coronavirus OC43 containing 5% soil load. The inoculum was air-dried, and immediately wiped using (1) BW, (2) H₂O₂-based, or (3) quaternary ammonium (QAC)-based DW by hand or Gardco® Gardner-scrub. The wiping process was standardized according to the EPA protocol of three times and six passes over the inoculated surface within 5 seconds. After 1 min, residual disinfectant on coupons and wipes was neutralized in a stomach bag followed by hand massaging for 10 s. The H₂O₂-wipe was neutralized in phosphate buffer saline containing 10% fetal bovine serum and 1300 U/mL catalase, whereas the QAC-wipe was neutralized using infection medium following centrifugation using Pierce detergent column. Both procedures required three washes in the Amicon Ultra4 unit. Viruses were then recovered by sonication and quantified by a TCID₅₀ assay using HRT-18G cells. Experiments were conducted in three biological replicates.

Results: After using BW, 4.2-4.6 log TCID₅₀ viruses were left on glass carriers, which was significantly lower than vinyl (4.8-5.1 log TCID₅₀). Virus transfer to BW was significantly higher from glass (5.6-5.7 log TCID₅₀) than vinyl (4.9-5.0 log TCID₅₀). Both DWs (contained H₂O₂ or QAC) achieved >3 log₁₀ TCID₅₀ for both viral surrogates on glass and vinyl, with both below the limit of detection on the used DW. Overall, there was no significant difference between hand and machine wiping.

Significance: Disinfectant wipes effectively remove and inactivate viral populations on glass and vinyl surfaces, reducing the risk of coronavirus

transmission.

P2-195: GROWTH AND SURVIVAL OF LISTERIA MONOCYTOGENES ON VARIOUS FOOD CONTACT SURFACES AND IMPACT OF ANTIMICROBIAL INTERVENTION

Primary Author:

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: *Listeria monocytogenes* (Lm) can adhere to and survive on food contact surfaces (FCS), increasing the contamination likelihood of ready-to-eat (RTE) foods. Antimicrobials are used to reduce Lm counts.

Purpose: Evaluate capacity of Lm to grow and survive on five FCS (with or without available nutrients) and the impact of antimicrobial application.

Methods: A five-strain Lm (serotypes 1/2a, 1/2b, 4b) cocktail was grown in subsequent overnight cultures (37°C) and used to inoculate sanitized FCS (acetal, Buna-N, HDPE, rubber, and stainless steel) coupons (1 x 1 cm; individually placed in test tubes with 10 mL tryptic soy broth (TSB)) and incubated (37°C; 72 h), with TSB refreshment every 24 h. TSB was decanted out, and coupons were subjected to antimicrobial treatment (representing nutrient-available growth) or were stored at 4.4°C for an additional 14 d (representing an absence of nutrient availability) and then subjected to antimicrobial treatment (tap water (positive control; PC), lactic acid (5%; LA), quaternary ammonium sanitizer (200 ppm; QUAT), and chlorine (200 ppm FAC; CL) by 10 sec submergence) or not treated (negative control). Coupons were transferred to a petri dish containing 5 mL neutralizing broth and scrubbed to removed adhered cells. Diluent was serially diluted and enumerated on Modified Oxford agar (37°C; 48 h).

Results: Following nutrient-available growth, LA achieved the greatest reductions ($p < 0.05$) for acetal, Buna-N, HDPE, and stainless steel (3.09, 2.57, 2.77, 3.87 log CFU/cm² reductions, respectively), while CHL was the most effective ($p < 0.05$) on rubber (4.54 log CFU/cm² reduction). Following nutrient-limiting growth, LA achieved the greatest reductions ($p < 0.05$) for acetal, HDPE, and stainless steel (4.25, 4.27, 5.04 log CFU/cm² reductions, respectively), while all antimicrobials were equally effective ($p < 0.05$) for Buna-N and rubber.

Significance: Lm displays varying growth capacity depending on FCS type and nutrient availability. Antimicrobials reduce Lm on FCS, with efficacy dependent on

the surface type × antimicrobial interaction.

Award Application: Developing Scientist Award

P2-196: EVALUATION OF FLORESCENT BIOMIMETIC PARTICLES AS A CLEANING VERIFICATION TOOL

Primary Author:

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: Food production industries face inspection and audit infractions due to inadequate sanitation practices and insufficient personnel training. To address this, more targeted sanitation training and tools are needed. This study investigates the application of Fluorescent Biomimetic Particles (fBMP) nanoemulsion as a tool to mimic bacterial properties and fluoresce under UV light.

Purpose: This project aims to study the physiochemical properties of fBMP nanoemulsion as a tool for sanitation training programs and provide comparisons against the commercial, melamine-based product Glogerm. By mimicking bacterial size and charge with curcumin and potato proteins, fBMPs can help improve food safety training and standardize cleaning practices in food production facilities.

Methods: A fBMP nanoemulsion is prepared by emulsifying curcumin in corn oil and potato proteins in phosphate buffer, then homogenized. A Glogerm solution was made by dispersing 10 g of Glogerm particles in 100 mL of phosphate buffer. Both emulsions' particle size and surface charge were analyzed over 10 days and compared to *Enterococcus faecium*. Emulsions were applied to different food contact surfaces and UV images were collected before and after cleaning to compare fluorescence.

Results: Comparing *E. faecium*'s charge of -32.44 mV, fBMP nanoemulsion's surface charge increased from -29 ± 0.26 mV to -26.1 ± 0.44 mV over 10 days, while Glogerm's charge was -12.74 ± 2.14 mV. fBMP's particle size increased from 0.809 ± 0.16 μm to 1.965 ± 0.035 μm over 10 days. Glogerm exhibited a larger particle size of 93.05 ± 11.38 μm . *E. faecium*'s $0.5\text{--}1.0$ μm range confirms the biomimetic nature of the fBMP emulsion.

Significance: The fBMP nanoemulsion mimics the charge and size of *E. faecium*, suggesting potential use as a visual tool in sanitation training programs.

P2-197: INVESTIGATING THE ANTIMICROBIAL EFFICACY OF COMMERCIALLY AVAILABLE ELECTROSTATIC SPRAYERS ON FOOD CONTACT SURFACES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: inefficient sanitization of food contact surfaces (FCS) can serve as a vehicle for cross-contamination of human pathogens such as *Listeria monocytogenes*. Nonetheless, little is known about novel commercially available sprayers, such as electrostatic sprayers, as an alternative disinfection tool on FCS.

Purpose: The study evaluated the antimicrobial effectiveness of electrostatic spraying on FCS using common food-grade sanitizers: chlorine bleach (NaClO) and peracetic acid (PAA).

Methods: *Listeria innocua* ATCC 33090, a surrogate for *Listeria monocytogenes*, was spot inoculated (500 μ L) onto stainless steel or plastic surfaces (3 " x 3 ") and air-dried for about 90 mins to allow bacterial attachment. Commercial electrostatic sprayers—handheld (ES) and backpack models (E1S)—and a conventional garden sprayer (GS) were used to spray on the inoculated surfaces using 25 ppm of NaClO or PAA applied from a 25 cm distance from the sprayer nozzle for a contact time of 60 s followed by bacteria recovery and enumeration. Low PAA concentration was adopted after sanitization with 80 ppm, with all three sprayers showed bacteria count below the detection limit. **Result:** Except for the sanitizers ($p \geq 0.05$), there were significant differences between the levels of the contact surfaces and sprayers ($p < 0.05$). A two-way interaction effect ($p < 0.05$) was also observed among all three factors: contact surfaces, sprayers, and sanitizers. E1S had a significantly higher log reduction than ES and GS (2.84, 1.62, and 1.04 log₁₀ CFU/cm², respectively). In addition, the log reduction observed on the stainless steel (2.41 log₁₀ CFU/cm²) was higher than on plastic surfaces (1.26 log₁₀ CFU/cm²).

Significance: This study shows that electrostatic spraying could be a potential alternative to disinfecting food contact surfaces compared to a garden sprayer.

Award Application: Developing Scientist Award

P2-198: NOVEL SANITATION TECHNOLOGIES FOR IMPROVED CONTROL OF BACTERIAL COMMUNITIES IN MEAT PROCESSING FACILITIES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Bacterial communities residing in biofilms on non-product contact surfaces contaminate foods. Biofilm control through sanitation is impeded by the resistance of biofilm-embedded microbes to sanitation chemicals.

Purpose: This study aimed to evaluate the inactivation of bacteria in multi-species biofilms by ozone nano-bubbles.

Methods: Twelve bacterial biofilm communities, consisting of up to 19 different bacterial strains each (total of 130 strains of 19 genera) were reconstituted to match the composition at the site of isolation in a pork processing facility. Biofilms were grown for 6 days at 4 or 25°C on stainless steel (12 communities × 2 temperatures). *Pseudomonadaceae*, *Enterobacterales*, and lactic acid bacteria dominated the communities. Biofilms were treated with sodium hypochlorite (200 ppm), peracetic acid (40 ppm) for 1 min, or with ozone nano-bubbles in water (7 ppm) for 3 min. The composition of the biofilm communities was determined by sequencing of 16S rRNA amplicons and by differential cell counts taken before and after sanitation.

Results: The cell counts of biofilm communities ranged from 5.75 ± 0.96 to 8.45 ± 0.16 log CFU/cm² or higher. The biofilm biomass as judged by crystal violet staining ranged from 0.43 ± 0.05 to 2.22 ± 0.35 at 570 nm. The cell counts were lower in biofilms grown at 25°C compared to 4°C ($P < 0.05$). The bactericidal effect of ozone nano-bubbles, peracetic acid, or sodium hypochlorite in reducing cell counts ranged from 0.021 ± 0.44 to 1.30 ± 0.20 , 0.19 ± 0.29 to 6.37 ± 0.00 , and 2.02 ± 0.010 to 6.36 ± 0.00 [$\log(N_0/N)$], respectively. The bactericidal effect was specific to certain communities, and none of the communities resisted treatment with all three sanitizers (i.e., none had a cell count reduction of less than 1 log CFU/cm²). Principal component analysis revealed that the three sanitizers altered the biofilm community composition differently.

Significance: The composition of the biofilm community, rather than biofilm thickness, determined the efficacy of sanitizers. The use of different sanitizers within the same facility or on the same processing line may be advantageous.

Award Application: Developing Scientist Award

P2-199: DEVELOPING A UV-C DISINFECTION TUNNEL TO CONTROL LISTERIA IN ORGANIC PRODUCE INDUSTRY

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: The organic produce industry faces challenges in maintaining sanitation standards on equipment, creating environments conducive to listeria biofilm growth.

Purpose: This study aims to investigate the efficacy of UV-C light in controlling sessile cells and biofilms of *Listeria* through a uniquely designed UV-C disinfection tunnel.

Methods: *Listeria innocua* (ATCC #33660) strain was used. The UV-C disinfection tunnel providing 3600 exposure was used for microbial inactivation. Stainless steel (SS) and high-density polyethylene (HDPE) coupons of 3''×3'' in size were selected. Sessile cells were treated with six different positions (T1-Right, T2-Left, T3-Entry, T4-Exit, T5-Upper, T6-Bottom) based on their location inside the disinfection tunnel. For biofilms, we selected three different positions: T1-(Right/Left), T2-(Entry/Exit), and T3-(Upper/Bottom). Treated coupons were collected, immersed in 0.1% BPW, plated on MOX, and recorded as log CFU/cm².

Results: Experiments were conducted three times with both forms of bacteria. The sessile form of bacteria was recorded below the detectable levels (1.23 log CFU/cm²) with a single light system activated at different positions on both HDPE and SS surfaces. The biofilms were treated with single and four light systems. For both SS coupons and HDPE coupons, a significant difference was observed within light systems ($p < 0.05$) and across various positions ($p < 0.05$). On SS coupons, log population decreased significantly (2–3 log CFU/cm²) across all treatments under both the single-light and four-light systems compared to the control (5.30 log CFU/cm²). On HDPE, biofilm populations showed significant reductions under treatments (T1, T2, T3) compared to the control (4.47 log CFU/cm²).

Significance: UV-C lighting can serve as an effective intervention controlling

Listeria sessile and biofilm forms on food contact surfaces.

P2-200: ISOLATION AND CHARACTERIZATION OF A NOVEL PHAGE ENDOLYSIN (LYSLM3) WITH BROAD-SPECTRUM ANTIMICROBIAL ACTIVITY

Primary Author:

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: The increasing outbreaks of *Listeria monocytogenes* underscore the potential of bacteriophage-encoded endolysins, which lyse the peptidoglycan of bacterial cell walls, as effective biocontrol agents.

Purpose: This study aimed to identify and characterize a novel phage-derived endolysin, LysLM3, through phage genome analysis for its antimicrobial potential against *L. monocytogenes*.

Methods: Phage vB_LmoA_KFSLM3, specific to *L. monocytogenes*, was isolated from soil, purified, and sequenced using an Illumina MiSeq platform. Genome annotation was performed using RAST and BLASTP. ORF12, identified as an endolysin (LysLM3), was cloned into the pET28a(+) vector, expressed in *Escherichia coli* BL21 (DE3) via IPTG induction, and purified with a Ni-NTA superflow column. Domain and structural characterization were performed using NCBI CDD and DALI. Host range analysis included 57 foodborne pathogen strains, including 16 *L. monocytogenes* strains. Lytic activity was optimized using a turbidity reduction assay under varying pH (2–12), temperature (–20 to 100°C), and NaCl concentrations (0–500 mM).

Results: The genome of vB_LmoA_KFSLM3 was 39,955 bp with 64 ORFs and a G+C content of 37.1%. ORF12 encoded an N-acetylmuramoyl-L-alanine amidase with a predicted molecular weight of 33 kDa. LysLM3 featured two cell wall-binding domains and secondary structure consisting of 18% α -helix, 30% β -strand, and 52% coils. It exhibited specificity toward ten *L. monocytogenes* strains. Optimal lytic activity occurred at pH 9.0–11.0, –20 to 60°C, and 100–300 mM NaCl.

Significance: LysLM3 demonstrated novel and robust antimicrobial activity against pathogenic *L. monocytogenes*, supporting its potential application in food safety and biocontrol strategies within the food industry.

Award Application: Developing Scientist Award

P2-201: EVALUATION OF THE EFFECTIVENESS OF COMMONLY USED SANITATION STRATEGIES AGAINST DUAL-SPECIES BIOFILMS OF LISTERIA MONOCYTOGENES AND PSEUDOMONAS FLUORESCENS IN THE FOOD INDUSTRY

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Multispecies biofilms exhibit enhanced resistance and tolerance against external stresses such as cleaning and disinfection, due to complex interspecies interactions including nutrient sharing, metabolic cooperation, and robust extracellular polymeric substances (EPS). These biofilms are more challenging to manage than single-species biofilms in food processing environments.

Purpose: To evaluate the efficacy of commercially available sanitizers in controlling growth and formation of dual-species biofilms on surfaces representative of food processing environments.

Methods: Dual-species biofilms were developed over 96 hours at $25 \pm 2^\circ\text{C}$ using a CDC biofilm reactor on stainless steel, PVC, Teflon, and HDPE. Coupons were treated with one of four sanitizers for 5 minutes: chlorine (500 ppm), peracetic acid (120 ppm), lactic acid (4%), or silver dihydrogen citrate (5%). Treated coupons were neutralized, and biofilms dislodged via sonication and vortexing prior to enumeration on Modified Oxford Agar, Cetrimide Agar, and Tryptic Soy Agar. Confocal microscopy was used to visualize microbial distribution and interspecies interactions. Experiments were conducted in triplicate. Statistical analysis included two-way ANOVA and Tukey's HSD.

Results: The highest *L. monocytogenes* counts were observed in control samples on PVC and Teflon (8.30 and 8.54 log CFU/coupon, respectively; $P < 0.05$). Chlorine was the most effective sanitizer ($P < 0.05$), reducing *L. monocytogenes* to 5.18 log CFU/coupon and *P. fluorescens* to 4.87 log CFU/coupon on stainless steel. Peracetic acid and lactic acid achieved moderate reductions of 2–3 log CFU/coupon across surfaces. Silver dihydrogen citrate was the least effective ($P > 0.05$). Sanitizer efficacy was higher on stainless steel and HDPE compared to PVC and Teflon ($P < 0.05$). Confocal microscopy showed surface type influenced microbial spatial arrangement in biofilms.

Significance: This research provides initial insights into the growth behavior and sanitizer tolerance of dual-species biofilms, supporting the need for surface-specific interventions to reduce food safety risks.

Award Application: Undergraduate Student Award

P2-202: CONFIRMATION OF STAPHYLOCOCCUS AUREUS BIOFILM INHIBITION AND REMOVAL EFFECTS BY DISINFECTANTS AND CLEANING METHODS

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Since biofilm is difficult to eliminate using conventional cleaning methods and leads to hygiene issues such as foodborne illness, it is essential to remove biofilm to ensure hygienic surfaces.

Purpose: This study aimed to evaluate the reduction effects of chemical disinfection and physical cleaning methods for biofilm, both individually and in combination, on biofilms formed on various materials used in food processing tools and manufacturing facilities. Additionally, the study sought to determine the most effective method for maximizing the removal of cell lysates remaining on surfaces after sterilization.

Methods: Chemical disinfection and physical cleaning methods were individually tested for their ability to remove biofilm from glass, plastic, silicon, and stainless steel coupons. The antibacterial effectiveness of sodium hypochlorite, chlorine dioxide, citric acid, and malic acid at various concentrations was evaluated as chemical disinfectants. Physical cleaning methods included four types of scrubbers (steel, acrylic, sponge, and polyester) tested with and without

detergents. The most effective method from each category was then combined to assess biofilm inhibition and the removal of residual biofilm.

Results: Sodium hypochlorite and chlorine dioxide at concentrations above 100 ppm, as well as citric acid and malic acid at concentrations above 15%, exhibited significant reduction effects on biofilm formation. However, residual biofilm due to cell lysis was observed. Among physical cleaning methods, the use of detergents improved biofilm removal, although some residues persisted. The combined chemical disinfection and physical cleaning method was more effective in reducing biofilm and eliminating residual biofilm than individual methods.

Significance: Despite the use of disinfectants, residual biofilm can remain on surfaces, rendering them hygienically inadequate. Therefore, incorporating an additional cleaning process using detergents and sponges is necessary to ensure complete biofilm removal and maintain surface hygiene.

P2-203: INFLUENCE OF THE EVALUATION METHOD ON THE ASSESSMENT OF THE SUSCEPTIBILITY OF *LISTERIA MONOCYTOGENES* TOWARDS QUATERNARY AMMONIUM COMPOUNDS

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: The quaternary ammonium compounds (QACs) are commonly used as disinfectants for the control of *Listeria monocytogenes* in food processing environments. Unfortunately, their increasing resistance is of great concern to food industry.

Purpose: To evaluate and compare the susceptibility of *L. monocytogenes* isolates from food processing environments to QACs by means of agar diffusion methods, suspension, and surface challenge tests.

Methods: Strains of *L. monocytogenes* isolated from frozen vegetables (n=23), broccoli (1), cheese (n=3), and avocado (n=1) processing environments, and *L. monocytogenes* ATTC 19115 were used. The susceptibility to QACs (50, 200, 500, 1000 ppm) was evaluated by agar diffusion (disk and well)

Methods. Growth inhibition zones were measured, and strains were categorized into resistant, intermediate, and sensitive to QACs (500 ppm) based on K-means clustering. In addition, strains were inoculated (10 log CFU/mL) in QACs solution (200 ppm/30 s) and on stainless-steel (8.4 log CFU/cm²) and exposed to QACs (200 and 500 ppm/30 s and 2 min); surviving populations were quantified. A Pearson correlation analysis was conducted between agar diffusion

Methods and stainless-steel challenge testing.

Results: Based on the agar well diffusion method

Results strains were classified as sensitive (≥ 21 mm), intermediate ($16 \text{ mm} \leq \text{zone} < 21 \text{ mm}$), and resistant ($< 16 \text{ mm}$); agar disk diffusion method showed

similar values (sensitive: ≥ 17 mm; intermediate: $12 \text{ mm} \leq \text{zone} < 17$ mm; resistant: < 12 mm). The agar well diffusion method showed a major sensibility than the agar disk diffusion method ($p > 0.05$). Regardless the strain, the suspension method achieved a complete inactivation of the microbial population (> 8.3 log CFU/mL), while on stainless-steel bacterial reductions oscillated from 0.9 to 4.8 log CFU/cm². A negative correlation ($\rho = -0.4$) between the agar diffusion

Methods and the stainless-steel surface assay was observed.

Significance: Agar diffusion

Methods can be used as a rapid and reliable screening tool to assess bacterial susceptibility to QACs.

P2-204: REMOVAL OF CLINICALLY RELEVANT COW'S MILK ALLERGENIC PROTEINS FROM FOOD GRADE STAINLESS STEEL SURFACES: ULTRA-MICROFIBRE VS MULTI-PURPOSE WIPES

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: Clinically relevant cow's milk allergenic proteins include Bos d 5 (β -lactoglobulin) and Bos d 11 (β -casein). ingestion of these proteins by sensitised individuals can result in severe allergic reaction and even death. The food industry must therefore ensure that food contact surfaces are cleaned sufficiently well to minimise the risk of allergenic milk protein cross-contact and consequently reduce the risk of harm to susceptible consumers.

Purpose: To compare the efficacy of ultra-microfibre cloths with multi-purpose wipes in the removal of allergenic milk proteins from food grade stainless steel surfaces.

Methods: Dry milk powder, liquid milk, and dried-on milk were applied to the surface of food grade (304) stainless steel trays (n=3/soil type). Dry or damp ultra-microfibre cloths (Vikan) and multi-purpose wipes (J-cloths) were used to wipe the milk from the surfaces until visibly clean. Surfaces were then swabbed for any remaining milk allergens. The swabs were extracted in phosphate buffered saline containing tween, and the extracts were analysed for β -lactoglobulin and β -casein using a proprietary ELISA method (inBio).

Results: For powdered milk, dry and damp ultra-microfibre cloths removed between 4- and 7-fold more allergen than dry and damp multi-purpose wipes. For liquid milk, dry and damp ultra-microfibre cloths removed significantly more β -lactoglobulin and β -casein than dry and damp multi-purpose wipes ($p<0.01$ and $p<0.001$ respectively), with up to 30-fold more allergen removed. For dried-on milk, damp ultra-microfibre cloths removed significantly more β -lactoglobulin and β -casein than damp multi-purpose wipes ($p<0.01$ and $p<0.0001$, respectively), with ~10-fold more allergen removed (dry cloths not tested).

Significance: Ultra-microfibre clothes demonstrate significant efficacy for the removal of clinically relevant allergenic milk proteins from food grade stainless steel contaminated with varying forms of milk. This greatly reduces the risk of surface to food product cross-contact and ultimately reduces the potential exposure of consumers to these allergenic proteins.

P2-205: UTILISATION OF A NOVEL, BUSINESS-BESPOKE OBSERVATIONAL APPROACH TO DETERMINE OPERATIVE CLEANING AND SANITATION BEHAVIOURS

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: The importance of direct observational methods has been cited to assess adherence to food safety protocols, enabling identification of non-conformances and have been increasingly employed in the food industry, offering a more accurate representation of food-handlers' actual practices.

Purpose: This study aimed to utilise an approach to record observed food-handler behaviours related to cleaning and sanitation compliance and identify areas for improvement.

Methods: An overt direct observation method was employed using a bespoke digital interface to capture both qualitative and quantitative data. This novel approach enabled the timestamping of data points, providing detailed insights into behaviours related to key cleaning and sanitation practices. Observations were conducted in the high-risk area of a ready-to-cook food manufacturer.

Results: Observations of the cleaning and sanitation practices were successfully conducted over a period of 22 hours, resulting in the capture of n=4517 actions and notes related to the cleaning and sanitation practices established in company cleaning protocol. Mean duration of the observation session was n=1 hour. The findings suggest an overall lack of compliance with the observed cleaning procedures. It was determined that in the case of the product cooling trays the observed compliance was 8%. The low compliance observed was due to lack of implementation of the rinsing of the detergent cleaning step (92%). Identified common barriers towards compliant implementation of the cleaning procedures were related to the equipment and factory layout. The layout of the product cooling tray washing facilities was determined to be inconducive to the linear flow of the cleaning procedure. To overcome the infrastructure-related barriers it was determined that additional time and effort is required by the food-handlers.

Significance: The study demonstrates the successful use of a bespoke direct overt observational approach to collect quantitative and qualitative data on cleaning

and sanitation practices in a food manufacturing company.

P2-206: EFFECTIVENESS OF DRY SANITATION METHODS FOR THE REMOVAL OF ALMOND BUTTER RESIDUE FROM A NUT BUTTER MILL AND A STAINLESS-STEEL PIPE

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Allergen-related recalls for nut and seed butters highlight the risk of cross-contact due to shared equipment. Purpose: This study evaluated the impact of dry sanitation methods at removing almond butter residue from a nut butter mill and piping. Methods: Milling experiments used a commercial nut butter mill to process ~220 g roasted almonds into almond butter. The mill received the following treatments: no cleaning, flushing with vegetable oil (500 mL), cleaning inner surfaces with a brush/scrapper, and cleaning inner surfaces with a brush/scrapper followed by use of an alcohol-based sanitizer. After sanitation, ~6.5 kg of roasted peanuts were milled into peanut butter and samples were collected every 60 s. For the pipe experiments, interior surfaces of a 3.8 cm diameter, 30.5 cm long stainless-steel pipe were coated with heated (55°C) almond butter and then connected to a pump via a butterfly valve. The pipe was either left uncleaned or flushed with 18.9 L vegetable oil (55°C). Subsequently, 31.8 kg peanut butter (55°C) was pumped through the pipe and samples were collected every 10 s. Sanitation trials were conducted in triplicate. Almond protein levels in peanut butter samples were measured with an almond-specific ELISA kit. Results: When the mill was not cleaned, 343 ± 371 ppm almond protein was detected in peanut butter after 1.3 kg peanuts were processed in the mill. Flushing the mill with oil, using a brushing/scraping treatment, and using brushing/scraping and an alcohol-based sanitizer resulted in 118 ± 35.1 , 29.1 ± 11.1 and 0.31 ± 0.29 ppm almond protein, respectively in peanut butter after 1.3 kg peanuts were processed. Almond protein levels in peanut butter were ≤ 2.41 ppm when ~12 and ~2.9 kg of peanut butter was pumped through the almond butter-contaminated pipe after no cleaning and after an oil flush, respectively. Significance: Dry sanitation methods differ in their ability to remove nut butter residues from equipment surfaces.

P2-207: EVALUATING THE EFFICACY OF CITRIC ACID TO ENHANCE THE FOOD SAFETY OF FISH SKIN SNACKS

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Presentation type/Format: Poster

Category: Seafood

Abstract:

Introduction: Fish skin byproducts are rich in nutrients and have significant potential for sustainable value-added snacks. Organic acids such as citric acid are proven to reduce microbial load; when combined with thermal processing and water activity (A_w) control, they form a robust multi-hurdle strategy to enhance food safety and shelf stability.

Purpose: The effectiveness of citric acid as a pretreatment for microbial reduction in fish skins was evaluated in combination with thermal processing and A_w control to ensure the safety of fish skin snacks.

Methods: Fish skins from sea bass were treated with four NaCl/Citric Acid solutions: a) 0.5%/0.5%, b) 1.0%/1.0%, c) 1.0%/0.5%, and d) 0.5%/1.0%. Microbial analyses were conducted for Aerobic Plate Counts (APC) and generic *E. coli*. The most effective treatment ("a") was applied to fish skins, which were then processed via freeze-drying, air frying, baking, deep frying, and dehydration. A_w was measured immediately after processing and again during storage on days 0, 15, and 30. Data were analyzed using ANOVA and Tukey's HSD (SAS 9.4).

Results: The 0.5% NaCl/0.5% citric acid solution achieved the greatest microbial reduction for APC and *E. coli* counts, reducing 2.5 and 2.8 log CFU/g, respectively ($p < 0.05$). In contrast, the least effective treatment was 1.0% NaCl/0.5% citric acid, which reduced 0.9 and 1.1 log CFU/g for APC and *E. coli*, respectively. Baking at 170°C and air frying at 180°C resulted in A_w values below 0.38, effectively inhibiting microbial growth for up to 30 days. Freeze-drying maintained stability with A_w values of 0.52–0.70, while dehydration ($A_w = 0.38$ –0.40) and deep frying ($A_w = 0.40$ –0.50) were also effective.

Significance: Citric acid as an initial treatment for microbial reduction, followed by technologies such as freeze-drying and dehydration, resulted in safe and high-

quality fish skin snacks. This approach supports food safety and promotes the sustainable valorization of seafood by-products.

Award Application: Undergraduate Student Award

P2-208: THE ROLE OF AQUACULTURE PRACTICES, PHYSICOCHEMICAL PARAMETERS, AND WEATHER ON POPULATION DYNAMICS OF *VIBRIO PARAHAEMOLYTICUS* AND *VIBRIO VULNIFICUS* IN OYSTERS AND SEAWATER

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Presentation type/Format: Technical

Category: Seafood

Abstract:

Introduction: *Vibrio parahaemolyticus* (Vp) and *Vibrio vulnificus* (Vv) are significant foodborne pathogens responsible for vibriosis in humans and are primarily transmitted through the consumption of raw oysters. Rises in vibriosis cases and the geographic spread of *Vibrio* spp. are linked to anthropogenic climate change, reinforcing a need to understand the effects of aquaculture practices (AP), physicochemical parameters (PP), and weather on *Vibrio* abundances.

Purpose: The objective of this study was to investigate the effects of AP, PP, and weather on total and pathogenic Vp and Vv in oysters and seawater.

Methods: Six oysters and 1L of water were collected biweekly from two different aquaculture practices (floating and bottom) in three sites in the Chesapeake and Delaware inland Bays from April to October 2022–2023. Weather conditions and PP were recorded during each collection. Abundance of Vp (tlh+, tdh+, and trh+) and Vv genes (vvhA+ and vcgC+) from oyster and water samples were quantified using MPN-qPCR.

Results: The seasonality of *Vibrio* was demonstrated with gene abundances

increasing in warmer months and decreasing in cooler months. Relatively high averages of total Vp and Vv were seen in October (1.97 and 1.21 log MPN, respectively), suggesting an increased seasonal spread of the species. Gene levels did not significantly differ with AP ($p > 0.05$). However, detection levels of pathogenicity genes were significantly different among sites ($p < 0.05$), suggesting site-specific factors are the major drivers of variation. Vp was positively correlated with temperature and salinity, while Vv showed correlations with temperature and total algae phycoerythrin. Levels of Vp and Vv did not reveal clear trends in response to weather.

Significance: This study reveals the extended seasonality of Vp and Vv, site-specific variations with pathogenic *Vibrio* spp., novel correlations with PP, and updated data for risk assessments to assist the seafood industry.

P2-209: A DNA-METABARCODING APPROACH FOR SEAFOOD AUTHENTICATION AND SPECIES DATABASE CONSTRUCTION

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Presentation type/Format: Poster

Category: Seafood

Abstract:

Introduction: Ensuring seafood authenticity has become increasingly challenging as supply chains grow more complex, making reliable species identification critical for consumer protection.

Purpose: This study developed a DNA metabarcoding method using 16S mitochondrial DNA to identify seafood species and build a comprehensive animal food reference database.

Methods: We tested a universal 16S primer set against 11 common food animals including fish, shellfish, poultry, and mammals. After optimizing PCR conditions through a gradient PCR (53°C proved optimal), gel imaging and Sanger sequencing were applied to confirm amplification success. We built a reference database by querying NCBI database using the target primer set and filtering animal species against FDA and FAO lists of edible species. To validate our method and survey local seafood diversity, we tested this primer amplification of 60 local market samples including fresh, frozen, and canned products, covering fish, crustaceans, mollusks. and the Sanger sequence

Results were BLAST-searched in NCBI and our custom database.

Results: For the 11 testing animal species, we got excellent matches (97-99% identity) to known sequences. Our reference database included about 108,000 sequences from nearly 64,000 species, with sequence fragments ranging from 200-250 bp. This database covered 84 commonly consumed fish, but initially lacking many major invertebrates. Among the 60 commercial seafood samples, this 16S primer set successfully amplified most fresh and frozen products, giving clear DNA bands. However, some canned fish products showed fainter amplification bands, indicating potential DNA degradation in canned products. The sequences from two canned fish products didn't match their labels (tuna and

sardine). Along the way, we added 25 new invertebrate species to the database, filling some important gaps in shellfish coverage.

Significance: This straightforward approach to seafood testing gives regulators and industry a practical tool for identifying seafood sources and catching mislabeled products.

P2-210: SHORT-TERM PERSISTENCE OF CYCLOSPORA CAYETANENSIS OOCYSTS IN ARID SOIL

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: *Cyclospora cayetanensis* causes foodborne outbreaks affecting thousands of people in the U.S. *Cyclospora* oocysts are expected to be highly resistant in the environment. However, the persistence of oocysts in soil and herbs is unknown.

Purpose: Persistence of *C. cayetanensis* in soil and herbs contaminated with the parasite in CONVIRON™ growth chambers was evaluated in low rainfall/arid and wet/humid conditions at moderate and hot temperatures.

Methods: Four experiments were performed (dry and wet, high and moderate temperatures). In each experiment, soil was inoculated with 400 *C. cayetanensis* oocysts (n=8 spots) and individual leaves of cilantro, parsley, and basil were inoculated with 100 oocysts (n=8 leaves/herb). Samples of soil and leaves were collected at 7, 14, 21, 28, 35, 42, 49, and 56-days inoculation (dpi). The presence of the parasite in soil was confirmed by concentration via flotation in high density sucrose solutions, DNA extraction, and real-time PCR specific for the parasite. The parasite was detected in herb leaves following the Bacteriological Analytical Manual (BAM) chapter 19b method.

Results: Under arid/dry conditions, independent of the temperature (moderate/hot), the parasite showed short-lived persistence in soil, with samples only positive for *C. cayetanensis* between 7 dpi and 14 dpi. Under wet/high water content conditions positive detection was observed in the soil at each sampling collection. Positive detection results were observed in herbs along the study, in both arid and wet conditions. No statistically significant differences in CT values were observed among samplings ($p>0.05$).

Significance: Soil may play an important role in food contamination. Short-term persistence of oocysts in soil occurs when herbs are grown in dry conditions,

independent of moderate or high temperatures, whereas they survive longer under wet conditions. These findings will allow FDA to better understand the transmission mechanisms of *C. cayetanensis* and establish control strategies to disrupt the cycle.

Award Application: Undergraduate Student Award

P2-211: SURVIVAL OF THE NOROVIRUS SURROGATE MS2 IN LEAFY GREENS DURING STORAGE

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: Norovirus (NoV) contamination of ready to eat foods remains a public health concern and can cause health and economic impacts worldwide. Fresh leafy greens can be contaminated with NoV at various points in the production chain. The survival of NoV on leafy vegetables during storage remains relatively understudied.

Purpose: The purpose of this study was to evaluate the survival of NoV surrogate MS2 bacteriophage on leaves of fresh cress (*Nasturtium officinale*), mint (*Mentha spicata*), and basil (*Ocimum basilicum* L.) during refrigerated storage.

Methods: The propagation and enumeration of MS2 was carried out using the double-layer technique with *Escherichia coli* C3000 as a host. Viral titers were determined after incubation for 18 h at 37°C. Samples (~3 g) of leaves were spot inoculated with MS2 (100µl; final counts 8 log PFU/g) and leaves were stored in polypropylene boxes with perforated lids at 5 °C. MS2 survival was measured 30 min after inoculation and then every 48 h of storage over 10 days. All experiments were conducted three times in triplicate. The results were evaluated by ANOVA and Tukey's test ($p < 0.05$).

Results: The survival of MS2 varied by leafy green tested. MS2 titer did not decrease significantly in watercress leaves over the time evaluated ($p > 0.05$). A reduction of 0.8 PFU/g in mint leaves was recorded after 5 days of storage ($p < 0.05$) without further changes. MS2 titer declined by 1 log PFU/g in basil after 10 days of storage ($p < 0.05$).

Significance: MS2 survived to different degrees on different leafy greens under refrigerated conditions suggesting that norovirus might behave similarly. Generally good survival of MS2 on leafy green highlights the importance of preventing NoV contamination of these foods.

P2-212: FIRST REPORT OF THE GI.5 [P4] RARE NOROVIRUS RECOMBINATION VARIANT IN MEXICO

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Norovirus (NoV) is the main cause of acute gastroenteritis outbreaks and exhibits high genetic variability, driven by recombination events at the ORF1-ORF2 junction.

Purpose: This study aimed to detect, sequence, and analyze norovirus genogroups GI and GII in fecal samples from patients with gastroenteritis in Monterrey, Mexico, to determine their prevalence, identify circulating variants, and assess viral recombination events.

Methods: A total of 386 stool samples were subjected to RNA extraction and viral detection by qPCR for NoV GI and GII. Positive isolates were sequenced using Sanger sequencing. Phylogenetic and recombination analyses were conducted using MEGA11 and RDP4 software, respectively.

Results: A rare norovirus GI.5 [P4] variant in Northeastern Mexico was identified for the first time in a single positive isolate (MTY0115; GenBank: PQ369661). Norovirus genogroup GII was not detected. The prevalence of GI genogroup was 0.25% (1/386). Phylogenetic analysis of the partial RdRp/VP1 region revealed clustering with global GI.5 [P4] sequences. Recombination analysis showed 96% identity with a GI.5 [P5] isolate from Moscow, Russia (FJ383818) as the major parent, and 94.8% similarity with a GI.4 [P4] isolate from France (MK956177) as the minor parent.

Significance: The identification of recombinant norovirus variants and their prevalence rates is crucial for public health and the food industry, as it provides critical data for developing preventive strategies, early detection systems, and effective outbreak response plans.

Award Application: Undergraduate Student Award

P2-213: EXAMINING THE SURVIVAL OF HUMAN INFLUENZA VIRUS H1N1 AND MURINE NOROVIRUS IN RAW-MILK CHEESES

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: The spillover of H5N1 high pathogenic influenza (HPAI) virus to dairy cattle, and its excretion in milk in high titers, has created a new interface for human exposure and raised food safety concerns. While it has been shown that pasteurization is effective in inactivation of influenza viruses in milk, not much is known about viral survival in raw milk cheeses. Since influenza viruses were not considered as foodborne, no standard method exists for their extraction from food commodities, including dairy products.

Purpose: We aim to develop a method for extraction of infectious viruses from raw-milk cheeses and examine their survival in dairy products.

Methods: We examined the efficacy of multiple methods for extraction of infectious H1N1 virus (as a surrogate for H5N1 viruses) and murine norovirus (MNV) from different types of raw milk cheeses including cream cheese, brie, cheddar, and a firm cheese. We then employed the best performing method to examine whether H1N1 and MNV can survive the cheese making process by making cream cheese and feta cheese from artificially inoculated raw milk in the lab.

Results: We did not detect any infectious H1N1 virus in the whey; however, infectious viral particles were detected in the curd of both types of cheeses. To study the effect of aging, salt, pH, and microbiome in different cheeses on viral survival, we artificially inoculated different types of cream cheese, brie, cheddar, and a firm cheese—all made with raw milk—and examined viral survival for 8 weeks. H1N1 viruses survived for 8 weeks with a gradual decline in infectivity in harder cheeses, while the virus demonstrated a more rapid decline in infectivity in soft cheeses.

Significance: The method developed here could be used by health authorities for

surveillance of foodborne viruses in dairy products. The data provided could help risk assessment for the safety of dairy products.

P2-214: HYBRID PAPER/PDMS MICROFLUIDIC DEVICE INTEGRATED WITH RNA EXTRACTION AND RECOMBINASE POLYMERASE AMPLIFICATION FOR DETECTION OF NOROVIRUS IN FOODS

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human norovirus (HuNoV) is recognized as one of the leading causes of acute gastroenteritis in fresh produce globally. Due to the absence of an in vitro mammalian cell culture model for HuNoV, murine norovirus 1 (*MNV-1*) serves as a commonly used surrogate. Conventional detection methods involve tissue culture procedures, RNA isolation, and two-step RT-qPCR, which are impractical for either on-site or in resource-limited settings. Recombinase polymerase amplification (RPA), a robust isothermal amplification method, offers a rapid and efficient alternative for nucleic acid detection.

Purpose: A hybrid paper/polydimethylsiloxane (PDMS)-based microfluidic device integrating RNA extraction, RPA reaction, and lateral flow assay (LFA) was developed to achieve rapid detection of *MNV-1*.

Methods: For specificity test, two *MNV-1* strains and four other foodborne gastroenterol viruses were investigated. Cellulose paper strip and RPA reaction were separately used to extract viral nucleic acids and amplify RNA extraction at 40°C for 20 min. When the sample containing the targeted analyte moved via the lateral flow strip, either positive or negative result could be evaluated by the naked eyes.

Results: RPA reaction demonstrated 100% specificity to *MNV-1*. The on-chip detection of *MNV-1* was completed within 35 min, demonstrating 100% specificity to *MNV-1* in our settings. The detection limit of this microfluidic device for *MNV-1* was 200 PFU/mL, comparable to the in-tube RPA reaction. It also successfully detected *MNV-1* in lettuce and raspberries at concentrations of 170 PFU/g and 230 PFU/g, respectively, without requiring extra concentration steps.

Significance: We developed a cost-effective, rapid, and reliable approach for detecting a HuNoV surrogate using a microfluidic device based on RPA and LFA. This device shows great potential for foodborne virus detection in agri-food products, particularly in remote and resource-constrained settings.

P2-215: UTILIZATION OF ENGINEERED BACTERIA FOR THE CONCENTRATION OF NOROVIRUSES PRIOR TO DETECTION

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human noroviruses are the leading cause of non-bacterial gastroenteritis globally. A significant challenge in managing human noroviruses is the need to concentrate viruses from food and environmental samples before detection. Existing concentration methods each have some limitations, which can include high cost, scalability issues, tendency to co-concentrate inhibitors, and generally low-capture efficiency.

Purpose: This study aims to develop a novel norovirus concentration reagent by engineering nonpathogenic *Escherichia coli* (*E. coli*) to express norovirus-affinity peptides on the bacterial surface to capture virus.

Methods: Human norovirus affinity peptides were cloned at the C-terminus of ice nucleation protein(INP) and expressed in *E. coli* BL21. The engineered *E. coli*'s ability to capture norovirus GI.1 Norwalk and GII.4 Sydney were assessed through a suspension assay using stool containing infectious norovirus quantified by RT-qPCR. Additionally, the interaction between the bacteria and Virus-Like Particles was visualized with TEM.

Results: Capture efficiency results for GII.4 Sydney ranged from 66% (SD±4.0) to 81% (SD±0.24) for 7 different engineered *E. coli* strains with different peptides; with the highest capture efficiencies, 81.34% (SD±0.2) and 76.55% (SD±1.0) being higher than that observed for *E. cloacae* (76%, SD ±3.0), as well as the no-peptide scaffold control. For GI.1 Norwalk, capture efficiency results ranged from 33%(SD±15.1) and 73%(SD±17.3), which was lower compared to GII.4 Sydney. The engineered *E. coli* with the highest capture efficiencies for the GI.1, 60.2% (SD ±0.1) and 72.6% (SD±17.3), were higher than those observed with *E. cloacae* (56.8%, SD ±18.6), control. Norovirus particles were also confirmed to interact with bacterial cells by visualization with TEM, with the binding occurring on the surface.

Significance: Due to its simplicity in production, cost-effectiveness, potential for scalability, and the promising capture efficiency reported here, this report describes a reagent that shows promise for concentrating norovirus from food, clinical, and environmental samples.

Award Application: Developing Scientist Award

P2-216: VIRUCIDAL EFFICACY OF A PERACETIC ACID-BASED FORMULATION AGAINST TULANE VIRUS, 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 foodborne illnesses in the U.S. Effectively being able to inactivate noroviruses is crucial for their control. Peracetic acid (PAA)-based disinfectants are commonly used in food production and preparation settings to prevent pathogen transmission; however, despite the extensive research done on their efficacy against other norovirus surrogates, a limited amount of work on PAA-based agents against Tulane virus exists. This is important as norovirus surrogates' susceptibility to different inactivation agents can differ, and comprehensive understanding of the efficacy of an agent against as many surrogates as possible better informs what can be used against human noroviruses.

Purpose: This study investigates the efficacy of a commercial EPA-registered PAA-based disinfectant against Tulane virus, a human norovirus surrogate, in suspension.

Methods: Different concentrations of a commercial PAA-based disinfectant were prepared (150, 50, 20 ppm). Virus inactivation was evaluated with disinfectant over a range of times (1-10 min), neutralized, and quantified using plaque assay. Measurements were made in triplicate and analyzed using Two-way ANOVA with the Tukey's post hoc test ($p < 0.05$).

Results: At the highest concentration of 150 ppm PAA, 2.59 log reduction was observed with 30 seconds of contact time, while at longer contact times, > 4 log reduction was observed. At 50ppm, 1.59 and 2.56 log reductions were observed with 1 and 5min, respectively. With 20ppm, at least 10 min contact time was required to reach 2.56 log reduction.

Significance: Evaluating how effective PAA-based disinfectants are against Tulane virus provides a better understanding of their potential application, as well as provides more information of the efficacy of PAA against a widely used human norovirus surrogate that can better provide context with other surrogates about its efficacy against human noroviruses.

Award Application: Developing Scientist Award

P2-217: CORRELATION OF VIRAL INFECTIVITY OF MURINE NOROVIRUS (MNV-1) PRE-EXPOSED AT DIFFERENT TEMPERATURES BY TCID₅₀, RT-QPCR AND CRISPR-CAS13A BASED-DETECTION, AFTER RNASE PRE-TREATMENT

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: Reliable norovirus detection in food is hindered by the inability of standard methods to correlate genome copies with infectivity, highlighting the need for accurate values between standard and new detection methods in combination with capsid integrity assays.

Purpose: To correlate murine norovirus (MNV-1) quantification by TCID₅₀, RT-qPCR, and CRISPR-Cas13a using RNase pretreatment in viral samples pre-exposed to different temperatures.

Methods: Individual viral suspensions ranging from 6.9 to 0.9 log₁₀ gc/rx were pre-exposed to 80, 37, 20, and 5°C. Suspensions were treated with 20 U of RNase ONE™ and quantified by TCID₅₀ in RAW 264.7 cells, RT-qPCR, and CRISPR-Cas13a-based detection. Suspensions without RNase served as controls. Statistical analyses were conducted using ANOVA ($p > 0.05$), Tukey's post hoc test, and Pearson correlation (using R programming).

Results: Viral suspensions pre-exposed at 80°C showed a reduction of 0.184 log₁₀ TCID₅₀/mL, compared to 0.014 log₁₀ TCID₅₀/mL at 5°C (titers of 4.9 log₁₀ gc/rx). RT-qPCR at 80°C showed a reduction of 1.08 log₁₀ gc/mL compared to 0.40 log₁₀ gc/mL at 5°C. A strong positive correlation ($r = 0.98$) was observed between viral titers using RNase pre-treatment at 37°C (RT-qPCR) and those without RNase (TCID₅₀). At the lowest viral load (0.9 log₁₀ gc/rx) pre-exposed at 37°C, a ratio of 1:10.89 (with RNase) and 1:3.85 (without RNase) was observed for TCID₅₀:RT-qPCR. CRISPR-Cas13a showed positive qualitative results at all viral levels with and without RNase pretreatment.

Significance: These findings demonstrate that RNase pretreatment combined with RT-qPCR and CRISPR-Cas13a aligns well with infectivity measured by TCID50, providing accurate molecular assay values. This enhances norovirus detection for food safety applications, including recall decisions in a risk-based manner.

Award Application: Developing Scientist Award

P2-218: EFFICACY OF SANITIZERS IN PREVENTING CROSS-CONTAMINATION OF FOODBORNE VIRUSES IN WASH WATER

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Foodborne virus contamination can occur across the farm-to-fork continuum. Cross-contamination by viruses is a risk in post-harvest environments. This research focuses on the addition of antimicrobials in wash water systems, where viral cross-contamination risks are not well understood. Chemical oxygen demand (COD) levels, type of antimicrobial, and level of antimicrobials will be tested as these affect sanitizer efficacy

Purpose: This study presents data highlighting cross-contamination issues in a postharvest washing environment and strategies for assessing data gaps regarding enteric viruses

Methods: Cherry tomatoes (n=3/treatment) were inoculated with hepatitis A virus (HAV) or Tulane virus (TV), placed in a 1 L wash water system with uninoculated cherry tomatoes (n=9/treatment), and treated with 80 ppm peracetic acid (PAA), measured using PAA test strips, in water of varying COD levels (40–600 ppm), representative of soil buildup in wash water systems. Tomatoes were swirled in this wash system for 1 minute before removal for virus quantification. Sanitizer was neutralized with sodium thiosulfate. Water samples (100 mL) were collected. Control tomatoes received virus inoculum without treatment. Infectivity was determined by TCID₅₀ and RNA presence by qPCR

Results: TV and HAV were detected from control tomatoes with 0.2% and 0.5% recovery efficiencies, respectively. Both qPCR and TCID₅₀ infectivity showed there is a significantly larger transfer of virus into the water than onto the uninoculated tomatoes ($p < 0.05$) for both HAV and TV. PAA at 80 ppm resulted in a 4-log reduction of HAV from inoculated tomatoes and a 5-log reduction of TV from inoculated tomatoes. By qPCR, 2–3 log RNA was detected as transferred onto uninoculated tomatoes in all systems. Interpretation of TCID₅₀ infectivity is difficult due to a high cytotoxic effect in the cells caused by PAA, but viable virus was detected in some samples

Significance: Interactions between enteric viruses, soils, and sanitizers are

complex and continued optimization is necessary to further elucidate cross-contamination potential

Award Application: Developing Scientist Award

P2-219: DEVELOPMENT OF A STRATEGY FOR SEQUENCE CONFIRMATION OF CYCLOSPORA CAYETANENSIS IN CONTAMINATED FOOD AND WATER SAMPLES

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: *Cyclospora cayetanensis*, a parasite causing diarrheal illness, is a worldwide public health concern due to its association with the consumption of contaminated fresh produce and water.

Purpose: To establish a sequencing-based method for *C. cayetanensis* that is complementary to the FDA-validated qPCR detection target, Mit1C, by comparing two mitochondrial gene regions: Mit3 and Mit4cox1.

Methods: Samples of lettuce and raspberries (n=52) and agricultural water (n=19) were seeded with either high (200) or low (five for produce, six for water) levels of *C. cayetanensis* oocysts. Clinical fecal specimen controls (n=35) were also tested. Samples were first tested using the FDA-validated qPCR assay and grouped based on Ct values. Touchdown conventional PCR was used to amplify Mit3 and Mit4cox1, followed by visualization, purification, and concentration assessment of the amplicons. Purified amplicons underwent Sanger sequencing, assembly using SeqMan Ultra 17, and phylogenetic analysis against reference sequences from *C. cayetanensis* and related parasites.

Results: All samples tested positive by qPCR with Ct values ranging from 28 to 38. Sequence confirmation was successful using either Mit3, Mit4cox1, or both in samples with Ct ≤ 35. Samples with Ct > 35 did not always yield confirmatory sequences, which is expected due to low template concentrations. Phylogenetic analysis confirmed that sequences were specific to *C. cayetanensis*. Mit4cox1 showed greater specificity, distinguishing *C. cayetanensis* from *Eimeria* and *Isospora* with 7-22 (median = 16) SNPs, compared to only 1-8 (median = 3) SNPs for Mit3.

Significance: Sequence confirmation of *C. cayetanensis* is essential for supporting positive qPCR detections, especially in cases requiring regulatory action. Mit4cox1 provides a complementary locus to Mit1C and enhances confidence in detection through greater sequence specificity.

P2-220: THERMAL INACTIVATION OF NOROVIRUS AND DISTINGUISHING RESIDUAL INFECTIOUS VIRAL PARTICLES USING TULANE VIRUS AS A SURROGATE

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Outbreaks of norovirus in cooked food products have raised concerns about the cooking time-temperature combinations being insufficient to fully inactivate virus particles, potentially posing significant health risks to consumers.

Purpose: To investigate the thermal inactivation of norovirus and the presence of residual virus particles.

Methods: Phosphate buffered saline (PBS) was inoculated to a final titer of 7 log₁₀ plaque forming units (PFU) per ml of Tulane virus, a surrogate for norovirus. Virus inactivation was assessed following heat treatment in a circulating water bath at 50°C to 60°C for various durations commencing once the sample reached the desired temperature. Infectious virus particles were quantified using an overlay plaque assay and reverse-transcriptase quantitative PCR with an RNase pre-treatment (RNase RT-qPCR). Fully inactivated samples underwent subsequent blind passages to determine if residual virus particles remained.

Results: Virus particles quantified using RNase RT-qPCR exhibited minor reduction in viral titer over time that were not significant at any temperature compared to the control sample (21°C) ($p > 0.05$). Comparatively, plaque assay results showed a greater viral reduction over time for all temperatures despite not all time-temperature combinations significant in viral reduction ($p > 0.05$). Heat treated samples at 60°C reached the limit of detection (LOD) of 1.35 log₁₀ PFU per ml in less than 20 seconds after the come-up period. Samples heat treated at 60°C showed signs of infectivity after one blind passage and subsequent passages despite reaching the LOD. RNase RT-qPCR only showed a 0.15 log₁₀ genomic copies per ml decrease after 20 seconds when heat treated at 60°C.

Significance: The identification of residual virus particles by the blind passage indicates the potential for RNase RT-qPCR to detect these residual infectious virus particles. This is essential for food processors and risk-assessments as norovirus is

highly infectious and residual virus particles can pose significant public health concerns.

Award Application: Developing Scientist Award

P2-221: IMPACT OF A SIMULATED GASTROINTESTINAL TRACT ON THE PERSISTENCE OF FOODBORNE HEPATITIS VIRUSES

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Hepatitis A virus (HAV) and hepatitis E virus (HEV) are major causes of foodborne viral infections worldwide.

Purpose: These viruses resist the physiological conditions of the small intestine to reach the large intestine and subsequently the liver, but the mechanism underlying their resistance through human digestion remains unknown, highlighting the importance of studying HAV and HEV.

Methods: in this project, these hepatic viruses were studied using INFOGEST 2.0, a static in vitro model, and TIM-1, a dynamic in vitro model, to replicate the physiological conditions of the stomach and small intestine during human digestion. The impact of the food matrix on virus persistence during digestion was studied in triplicate for INFOGEST and in duplicate for TIM-1, using samples of berries, oysters, lettuce (HAV) and pork liver pâté (HEV) artificially contaminated with 1×10^7 PFU/sample and 1×10^7 genome copies/sample for HAV and HEV respectively. Results were compared with a control consisting of water without food matrix. Viral quantification was carried out by lysis plaque assay (HAV) and RT-qPCR combined with a PMAxx pretreatment (HAV and HEV).

Results: Our results showed that in static digestion, the amount of HAV initially inoculated in food matrix and in water remained the same until the end of the digestion ($p > 0.05$). Similarly, in dynamic digestion, the amount of HAV remained consistent at every stage of the digestion ($p > 0.05$). For the HEV, in static digestion, results showed no significant reduction in water ($p = 0.0716$), while in liver pâté the quantity of HEV inoculated at the start remains the same at the end of digestion ($p = 0.7349$).

Significance: The results of this project provide evidence of the persistence of HAV and HEV in the gastrointestinal tract, while contributing to a better

assessment of the viral risk to consumers.

P2-222: CHOOSING A RELIABLE METHOD TO DETECT HEPATITIS A VIRUS IN FROZEN BERRIES: COMPARISON BETWEEN ULTRACENTRIFUGATION AND PRECIPITATION CONCENTRATION-BASED DETECTION METHODS

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: In recent years, several foodborne outbreaks have been linked to the presence of hepatitis A virus (HAV) in berries. However, the usually low concentration of this virus on contaminated berries makes the interpretation of HAV presence or absence in produce routinely tested extremely challenging.

Purpose: This study aims to compare the efficiency of two standard methods, based either on ultracentrifugation or PEG/NaCl precipitation concentration step to recover HAV on berries.

Methods: Frozen strawberries and raspberries (25g - 50g/sample) were inoculated with HAV at either 0 (uninoculated), 1×10^2 (low level) or 1×10^3 (high level) genome copies/sample. Recovery of HAV was carried out in compliance to the FDA Bacteriological Analytical Manual chapter 26 method using the ultracentrifugation concentration step (UC) or the ISO 15216-2:2019 method using the PEG/NaCl precipitation concentration step (PC) with small modification to ease the comparison of both detection methods. All experiments were replicated at least four times; uninoculated (n=5), low level (n=12) and high level (n=4).

Results: On frozen strawberries, recovery of HAV was obtained in 4 out of 12 samples at low-level and 3 out of 4 samples at a high-level of inoculation using the UC method. As for the PC method, all samples were positive at low-level (12/12) and high-level of inoculation (4/4). All uninoculated samples were negative for both methods. The same

Results for low-level were also obtained for the frozen raspberry samples. At low-level of inoculation, difference in recovery was observed ($P < 0.0001$, Chi-Square

test) for the two recovery methods on both frozen strawberries and raspberries. No difference ($P > 0.05$) between methods were observed at uninoculated and high-level of HAV.

Significance: This study provides essential information on the importance of choosing the right method for virus detection in berries, since the use of robust and sensitive methods are needed to prevent foodborne illnesses associated with HAV.

P2-223: PERSISTENCE OF ESCHERICHIA COLI AND EIMERIA TENELLA, A CYCLOSPORA CAYETANENSIS SURROGATE, IN IRRIGATION DISTRIBUTION SYSTEMS

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: In recent years, reported cases of infection to protozoan parasites as well as positive samples to *Cyclospora cayetanensis* associated with grown produce have increased in the U.S.

Purpose: As contamination events of irrigation water can lead to contamination of crops, the aim of this study was to assess the persistence of *Eimeria tenella* (*C. cayetanensis* surrogate) and *Escherichia coli* in drip tubes used for crop production.

Methods: Artificial ground water (AGW) was inoculated with either *E. coli* TVS355 (1.63×10^3 CFU/ml) or *E. tenella* (6×10^3 oocysts/ml), of which >90% were sporulated. Contaminated water (30ml) was then distributed in segments of polyethylene drip tube and incubated at 21°C for 5 days ($n=6$ for *E. coli* and $n=9$ for *E. tenella*). After 0, 3 and 5 days, samples were removed from incubation, water was purged, and an additional 30ml of uninoculated AGW was added to rinse each segment. *E. coli* survival was determined using 0.45µm membrane filter on MacConkey agar with rifampicin (LOD: 0.5 CFU/ml) while *E. tenella* persistence was determined by qPCR for purged and rinse water samples.

Results: At day zero, *E. coli* population was lower in rinsate at 0.92-log CFU/ml than in purge water 3.24-log CFU/ml ($P<0.0001$, Tukey's multiple comparison test). After three days, *E. coli* population decreased ($P<0.0001$) for both types of samples and were below the LOD after five days. As for *E. tenella*, the presence of oocysts' DNA was also lower ($P<0.0001$) between rinsing (1.64-log oocysts/ml) and purge (2.86-log oocysts/ml) water. However, it did not decrease ($P>0.05$) after 5 days of incubation for both types of samples.

Significance: This study established that protozoan parasites can be detected on a longer period than *E. coli* in AGW contained in drip tubes, emphasizing the need for better understanding its persistence in agricultural water and its adhesion to material used in crop irrigation.

P2-224: ATTACHMENT OF INFECTIOUS AND HEAT-TREATED HUNOV GI TO PORCINE GASTRIC MUCIN: DEVELOPMENT OF AN ELISA ATTACHMENT ASSAY

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human norovirus (HuNoV) is the leading cause of foodborne outbreaks across the world. Though some standard methods to detect HuNoV in food have been published, none of them assessed the infectivity of the viral particles found in contaminated samples.

Purpose: The goal of this study was to develop an ELISA assay using the attachment property of HuNoV to porcine gastric mucin (PGM) and to evaluate the effect of heat treatments on HuNoV to predict its infectivity.

Methods: ELISA plates were coated with 100µg/ml PGM then blocked with a caseinate buffer. HuNoV GI.1 (1×10^6 genome copies/sample [100µl]) was heat-treated for 2 minutes at 37°C (positive control), 56, 63, 72 and 90°C, then plated. Monoclonal anti-HuNoV GI IgG1 antibody was added followed by secondary antibody. Absorbance was read at 450nm, sample absorbances were divided by negative control wells to express the Positive/Negative ratio (P/N); values ≥ 2 were considered positive. All experiments were replicated three times.

Results: The concentration and incubation time of PGM, primary and secondary antibodies were optimized to improve the signal of positive samples. The composition and incubation time of the blocking buffer was also optimized to reduce background noise in wells. Once those parameters were selected, attachment of HuNoV was assessed with different heat treatments. At 37°C, P/N (6.18 ± 0.80) was significantly higher ($P < 0.0001$, Tukey's multiple comparison test) than all other heat treatments, indicating intact virus; significant difference ($P = 0.0005$) was also shown between 56°C (4.42 ± 1.50) and 63°C (2.87 ± 0.32) showing a partial attachment of HuNoV to PGM. Finally, 72°C (1.05 ± 0.03) and 90°C (1.00 ± 0.10) showed no difference and no attachment of HuNoV to PGM.

Significance: The presence of non-infectious virions is the main issue when developing detection methods for HuNoV in food. Methods to discriminate between infectious viruses and non-infectious viral particles are essential to avoid recalls associated with non-infectious viral particles and reducing food waste.

Award Application: Developing Scientist Award

P2-225: IDENTIFICATION AND QUANTIFICATION OF PHENOL COMPOUNDS AS INHIBITORS OF FOODBORNE VIRUS RT-QPCR DETECTION

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: The accuracy of RT-qPCR for detecting foodborne viruses can be compromised by inhibitory compounds, leading to false-negative results.

Purpose: This study aims to identify inhibitory molecules in berries and evaluate purification methods to minimize their impact.

Methods: Four phenolic compounds (ellagic, caffeic, hydroxybenzoic acids, and quercetin) were tested in RT-qPCR at concentrations close to their natural levels in raspberries. Standard curves (10^1 to 10^6 genome copies/ μ L) were generated using hepatitis A virus (HAV) plasmid both with and without the compounds, with a 3.3 Cq difference indicating inhibition. Inhibitory compounds were then tested using the ISO 15216-1:2017 method, spiked with HAV at 10^3 genome copies/sample. Three pretreatments were tested to eliminate inhibitors prior to amplification: Zymo oneStep PCR inhibitor Removal Kit (method A), MobiSpin S-400 column (method B), and RNA dilutions. The controls included a compound-free sample, a virus-free sample, negative extraction and RT-qPCR control, a positive control, and a standard curve with water as the reference. All experiments were performed in triplicate.

Results: The study showed that ellagic acid, at its natural concentration, completely inhibited the RT-qPCR reaction, preventing HAV detection using the ISO method ($p = 0.0026$). Neither a tenfold dilution nor method A ensured reliable detection, with 3/3 and 2/3 replicates undetected, respectively. In contrast, method B successfully detected the virus in all three replicates and achieved a significantly higher recovery rate (25.78%) compared to undiluted samples ($p = 0.050$). Hydroxybenzoic acid delayed RT-qPCR detection, while caffeic acid caused invalid amplification curves due to the absence of exponential amplification. Both acids did not significantly affect virus detection using the ISO method. Quercetin showed no inhibitory effects.

Significance: The study identifies compounds responsible for RT-qPCR inhibition

and provides a basis for developing strategies to mitigate their impact. These findings will enhance the reliability of foodborne virus detection, contributing to improved food safety.

Award Application: Developing Scientist Award

P3-01: EVALUATING LOT-TO-LOT VARIABILITY OF MICROBIAL INDICATORS AND SALMONELLA SPP. LOADS AND PREVALENCE TO DETERMINE MICROBIAL INDEPENDENCE OF CHICKEN PART RINSE SAMPLES USING DIFFERENT LOT DEFINITIONS IN A COMMERCIAL PROCESSING FACILITY

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: in 2024, the USDA-FSIS released a comprehensive restructuring package for regulatory pathogen control in poultry products. This package includes new performance standards for *Salmonella*.

Purpose: To evaluate the in-plant variability based on AC (Aerobic Counts) and *Salmonella* spp. loads, between different lot-to-lot definition alternatives to identify a statistically robust and commercially feasible lot definition for chicken part rinse samples.

Methods: Four-pound samples of chicken parts (tenders, wings, breasts, leg quarters, and whole legs) were randomly collected from a production line across six facilities using the Rinse part sample methodology 10 times per one-ton combo, with two plants sampled over three days. Each day had two parts sampled, totaling eight sampling days. Each replicate included four samplings per shift, accounting for flock, accumulation, and shift effects. Sampling consisted in one combo every two hours representing 25% of daily production. AC loads were analyzed using the Tempo® system, and *Salmonella* were assessed using GeneUp® protocols. Statistical analyses (ANOVA, Tukey's test, chi-square, Kruskal-Wallis, and pairwise Wilcoxon) were performed, with a 95% confidence level.

Results: For tenders, AC and *Salmonella* counts differed significantly between combos across three plants ($p < 0.05$), with higher *Salmonella* counts at the end of the second shift in two plants. For breasts, no significant differences were found in *Salmonella* counts or AC in two plants ($p = 0.68, 0.41$). For wings, no significant

differences in *Salmonella* loads or AC were observed between combos ($p = 0.3$ – 0.16). Whole legs and leg quarters showed no differences in *Salmonella* loads, but AC varied significantly ($p < 0.05$). *Salmonella* loads ranged from 0 to 3.66 logCFU/ml. While no overall differences were observed, significant variations were found when analyzing individual plants ($p < 0.05$).

Significance:

Results show significant variability in aerobic counts and *Salmonella* presence and loads across all samples and lot definitions. These findings highlight the need for further analysis to assess the independence of microbial loads between and within each combo. They also stress the importance of identifying the most representative lot definition for evaluating product compliance with regulatory standards in a commercially feasible way.

P3-02: MICROBIAL VARIABILITY OF SALMONELLA SPP. AND MICROBIAL INDICATOR LOADS IN POULTRY PARTS RINSE SAMPLES COLLECTED WITHIN A CHICKEN TENDER 1,800-POUND LOT IN A COMMERCIAL PROCESSING FACILITY

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Pathogen reduction initiatives require surveillance programs to verify the effectiveness of mitigation strategies during commercial processing. The U.S. Department of Agriculture's Food Safety and Inspection Service has released a comprehensive restructuring package for regulatory pathogen control in poultry products, with one component focused on addressing *Salmonella* as an adulterant.

Purpose: To evaluate the microbial population variability in chicken parts by exhaustively measuring indicators (total counts and Enterobacteriaceae) and pathogen loads and prevalence (*Salmonella*) within a 1,800lb pallet/combo with a robust sample size to assess the homogeneity of samples within a combo-size defined lot.

Methods: Chicken parts (tenders, wings, breasts, leg quarters, and whole legs) were randomly collected from production lines, mixed with 400 mL of BPW per rinse sample, and sampled 10 times per one-ton combo. Sampling occurred across six facilities, with two plants evaluated on three separate days, totaling eight sampling days. AC loads were analyzed using the Tempo® system, and *Salmonella* with GeneUp® protocols. To assess sample homogeneity within each combo, the differences between each pair of samples were calculated, and the average of these differences was compared to zero to determine homogeneity. A Chi-square analysis was used to compare *Salmonella* prevalence within each combo.

Results: The homogeneity of AC within samples in each pallet/combo showed that lot 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 averages differences greater than 0, which

indicates that differences between samples in the same pallet/combo were not homogeneous. Proportion of positives samples of 6 facilities indicates homogeneity within samples in each combo when *Salmonella* prevalence is low (<10%), with a significant difference between pallet/combos (Anova, $p < 0.05$).

Significance: The results showed significant differences in AC loads within samples in each pallet/combo and across lots. When *Salmonella* prevalence was low, *Salmonella*-positive samples were homogeneous within each pallet/combo, but different when prevalence >10%. These findings highlight the need for further analysis on sample homogeneity within lots and the importance of evaluating new sampling methods for accurate lot representation.

P3-03: RAPID AND RELIABLE DETECTION OF GUAIACOL-PRODUCING ALICYCLOBACILLUS IN FRUIT JUICES, FRUIT CONCENTRATES AND OTHER BEVERAGES BY REAL-TIME PCR

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: *Alicyclobacillus* spp. are Gram-positive, thermophilic, acidophilic, spore-forming bacteria known for their resistance to pasteurization and ability to cause off-flavors in acidic beverages, particularly fruit juices, by production of guaiacol. Their growth characteristics and the ubiquity of their spores, introduced via contaminated fruit, pose significant challenges to the beverage industry.

Purpose: This study evaluated the foodproof® *Alicyclobacillus* Detection LyoKit, a real-time PCR assay for rapid screening for *Alicyclobacillus* spp. and differentiation between guaiacol and non-guaiacol-producing strains, in enrichment cultures of juices and concentrates, and other beverages.

Methods: The PCR assay detects guaiacol-producing *Alicyclobacillus*, *Alicyclobacillus* spp., and an internal control in separate channels. 63 target-strains of 20 species, including guaiacol-producing strains like *A. acidoterrestris*, *A. herbarius*, *A. dauci*, *A. acidiphilus*, *A. fastidiosus*, and *A. suci*, as well as 46 relevant non-target strains of 20 species have been tested. A sensitivity of ≥ 3 CFU/mL was determined in post-enrichment spiked samples. DNA extraction from enrichment cultures using the foodproof® StarPrep Two Kit (manual) and the foodproof® Magnetic Preparation Kit VI (automated) was tested. A paired comparison study with the reference method IFU No. 12 (2019) was conducted.

Results: The assay demonstrated 100% specificity for *Alicyclobacillus* spp. and guaiacol-producing *Alicyclobacillus*, with no cross-reactivity among non-target species. A sensitivity of up to one cell per test portion can be reached even after short enrichment periods. Matrices like orange juice, concentrates (apple, orange, tomato), or raspberry syrup did not inhibit the PCR. The assay was successfully

tested on common PCR instruments. The comparison study showed 100% agreement.

Significance: The foodproof® *Alicyclobacillus* Detection LyoKit provides a rapid, sensitive, and specific method for detecting *Alicyclobacillus* spp. and guaiacol-producing strains in juice and juice concentrate samples. By detecting potential guaiacol production, the assay effectively supports spoilage prevention in the beverage industry, mitigating economic losses and ensuring product quality.

P3-04: AUTOMATED DNA ISOLATION COMBINED WITH REAL-TIME PCR ENABLES AN EASY-TO-HANDLE METHOD FOR THE SIMULTANEOUS DETECTION OF SALMONELLA AND CRONOBACTER ON SURFACES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Salmonella* and *Cronobacter* are responsible for a high lethality rate caused by the consumption of contaminated infant-, follow-on or medical-purposes formula. Due to this serious infection risk, it is essential to examine the environment of production sites with highly sensitive detection systems.

Purpose: This study analyzed the applicability and sensitivity of an automated DNA isolation system, the foodproof® Magnetic Preparation Kit I, in combination with the real-time PCR System, the foodproof® *Salmonella* plus *Cronobacter* Detection LyoKit, for surface examinations. Methods: Surfaces were spiked with low-level concentrations of *Cronobacter* or *Salmonella* with and without an excess of the respective other organism. Surfaces were dusted with probiotic milk powder to simulate the production site environment. Each surface was swabbed using Lethen broth hydrated sponges. The sample was enriched in 100 mL BPW for 16 hours at 36 °C. DNA extraction was conducted on the KingFisher™ Flex and analyzed with the PCR Kit (certified with manual extraction methods) on the LightCycler® 480. An amplification control and UNG treatment are included to avoid false-positive or false-negative results. For evaluation of the results, the study was conducted in parallel with ISO 6579-1:2017 and 22964:2017 methods.

Results: Low concentrations of *Salmonella* and *Cronobacter* on surfaces were detected successfully using an automated high-throughput method with the same sensitivity as the reference. Neither crosstalk between the detection channels nor high probiotic background flora influenced the correct detection. The results show 100% agreement between the alternative and the reference method.

Significance: The foodproof Magnetic Preparation Kit, in combination with the foodproof Real-time PCR System for simultaneous detection of *Salmonella* and *Cronobacter*, is a rapid method for surface examinations with the same sensitivity as the reference methods, significantly less hands-on time and, therefore, allows

for the processing of a large quantity of samples in parallel.

P3-05: CONTROLLING YEAST AND MOLD SPOILAGE IN ACIDIFIED SYRUPS USING NATURAL FLAVOR

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Yeast and mold spoilage in concentrated low pH/acidified syrups and flavors is a critical issue affecting product stability and safety. The acidic environment, while inhibitory to many bacteria, provides a niche for acid-tolerant yeasts and molds, necessitating effective control measures.

Purpose: To investigate the efficacy of natural flavor (Flavoset® 5400L) against yeast and mold isolated from various acidified syrups.

Methods: In-vitro screening of a natural flavor was conducted at different doses (0, 0.50, and 2.00%) in Potato Dextrose Broth (PDB) at pH 3.50 against yeast and mold strains isolated from low pH concentrated syrups. Broth solutions were aliquoted into 100-well plates, followed by inoculation with five strains of yeast and four strains of mold previously isolated from acidified syrups. Post-inoculation, plates were placed in a Bioscreen-C instrument and incubated at 30°C for 10–14 days. Optical density of each well was measured every 30 minutes at 600 nm to monitor growth. Growth curves were analyzed, and lag phase (days) and maximum growth rate ($\mu_{\max}$, h^{-1}) were estimated using the Gompertz model. Treatment performance was compared using one-way ANOVA ($p \leq 0.05$).

Results: Natural flavor at a dosage of 2.00% exhibited a fungicidal effect and completely inhibited all yeast and mold strains. At 0.50%, natural flavor significantly ($p \leq 0.05$) reduced growth rate and increased lag time for all yeast strains (0.39 h^{-1} , 2–4 days) compared to the control (1.89 h^{-1} ; 0.03–0.30 days). For all mold strains, a significant increase in lag time (3–5 days) was observed compared to the control (0.34–0.77 days).

Significance: Natural flavor, highly effective as a fungicidal agent, offers a potential clean-label solution for controlling spoilage in acidified syrups.

P3-06: CONTROL OF SPOILAGE IN COFFEE SYRUP SUPPLEMENTED WITH NATURAL FLAVORS

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Coffee syrup is highly susceptible to yeast spoilage due to its elevated sugar content (~55 Brix) and slightly acidic nature (pH ~5.00). Effective preservation strategies are essential to maintain product quality and extend shelf life. With the increasing consumer demand for clean-label products, there is growing interest in the use of natural antimicrobial solutions.

Purpose: This study evaluated the efficacy of a natural flavor-based system (Flavoset® 5400L) in suppressing yeast growth in coffee syrup over a 60-day storage period.

Methods: Coffee syrup formulations were prepared with two concentrations of Flavoset® 5400L, a smoke-based natural antimicrobial (0.60% and 0.75%), alongside a control formulation without any antimicrobial treatment. Samples were inoculated with a 5-strain yeast cocktail consisting of *Candida tropicalis*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Brettanomyces naardensis*, and *Dekkera bruxellensis* at an initial concentration of ~4 log CFU/mL. Following inoculation, samples were stored at 25°C and analyzed in triplicate for yeast growth on Potato Dextrose Agar (PDA) at regular intervals up to 60 days. Statistical differences among treatments were determined using one-way ANOVA ($p \leq 0.05$).

Results: The inclusion of smoke-based natural flavor significantly reduced yeast growth in treated coffee syrups compared to the untreated control ($p \leq 0.05$). The 0.60% and 0.75% natural flavor treatments achieved a reduction of 2.23 log CFU/mL by day 14 and maintained yeast levels below the spoilage threshold until day 58. Conversely, the untreated control reached ~5 log CFU/mL by day 58, indicating significant spoilage.

Significance: Smoke-based natural flavor antimicrobial system demonstrated effective control of yeast spoilage in coffee syrup, maintaining microbial stability

for up to 60 days under ambient storage conditions. This study underscores the potential of natural antimicrobial systems in supporting clean-label product development while ensuring extended shelf life.

P3-07: INACTIVATION OF THERMOPHILIC SPOREFORMERS IN DAIRY BASED HIGH PROTEIN BEVERAGES

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: High-protein dairy beverages have become increasingly popular because of their nutritional value and role in promoting health and fitness. However, their production poses significant microbiological challenges, particularly due to thermophilic spore-forming bacteria, which can survive heat treatments and compromise product quality and safety.

Purpose: The aim of the study was to provide thermal inactivation kinetics of *Geobacillus stearothermophilus* and *Geobacillus thermoleovorans* in dairy-based high-protein beverages.

Methods: Three model formulations containing 5%, 7%, and 10% milk protein isolate were evaluated for thermal treatment for low-acid shelf-stable beverage. The inactivation process was carried out using thermal death time (TDT) cells immersed in an oil bath at target temperatures of 115 °C, 121 °C, and 125 °C for various times. The Weibull model was applied to assess the inactivation kinetics under these conditions, with model parameters estimated at a reference temperature of 122 °C.

Results: The δ values increased with protein concentration, indicating enhanced heat resistance at higher protein levels for both the organisms. For *G. stearothermophilus*, δ values increased from 0.93 min at 5% to 1.84 min at 10%, with corresponding z-values rising from 7.66 °C to 8.60 °C. Similarly, *G. thermoleovorans* showed δ values ranging from 1.26 min at 5% to 1.71 min at 10%, while z-values remained relatively stable (8.10 °C to 8.41 °C).

Significance: Results indicate that both sporeformers exhibit increased heat resistance with higher protein concentrations, emphasizing the need to optimize thermal processes in high-protein dairy formulations to ensure effective microbial inactivation for commercial sterility of the shelf-stable product.

Award Application: Developing Scientist Award

P3-08: MICROBIAL INACTIVATION IN COLD-FILLED MAYONNAISE SAUCES

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Acid-resistant foodborne pathogens have caused numerous outbreaks in acid foods with pH values below 4.0, raising concerns regarding the safety of these products. A 5-log reduction in highly resistant *Listeria*, *Salmonella*, and *E. coli* species in shelf-stable foods with pH less than 4.6 is commonly achieved through hot-filling. However, cold-fill-hold processes present promising potential if microbial safety and stability are ensured.

Purpose: To determine the time required for a 5-log reduction of *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella enterica* in five formulations of commercially prepared mayonnaise sauces stored at a temperature of 60°F (15.5°C).

Methods: Commercially prepared sterile mayonnaise sauces (five formulations) were independently inoculated with a cocktail of five strains or serotypes each, of *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella enterica* and stored at a temperature of 60°F (15.5°C) to determine the holding times needed to achieve a 5-log reduction in the three highly resistant pathogens. Trials were performed in triplicate, followed by microbial count determination at sampling times. Samples were enumerated daily for up to four days followed by determination of inactivation curve.

Results: A cold-fill-hold process provided a 90% (1-log) reduction in pathogenic *L. monocytogenes*, *S. enterica* and *E. coli* in the samples at 15.5°C, with holding times in the order: *E. coli* > *L. monocytogenes* > *S. enterica*. Greater than 5-log reductions of pathogenic bacteria were estimated, on average, at 2.20, 2.03 and 1.58 days post inoculation, for *E. coli*, *L. monocytogenes* and *S. enterica* respectively. Moreover, among the samples analyzed, holding times were longest for the Ranch variant, while a faster 90% (1-log) reduction was observed for the Original sauce variant.

Significance: Future work will include a formal shelf-life analysis to evaluate the cold-fill-hold process as a potential alternative to thermal processing, building on the results of this study.

Award Application: Developing Scientist Award

P3-09: CLEAN-LABEL FOOD PRESERVATION SYSTEM AS A REPLACEMENT FOR POTASSIUM SORBATE AND EDTA TO EXTEND THE SHELF-LIFE OF RANCH DRESSING

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Ranch dressing is prone to spoilage due to microbial growth and oxidation, leading to undesirable off-tastes and odors. Despite low pH, it still requires a preservation system to maintain its shelf-life. With increasing consumer demand for clean-label ingredients, there is a shift away from synthetic preservatives like sorbates and EDTA.

Purpose: This study investigates the effectiveness of clean-label preservation systems as alternatives to synthetic preservatives in ranch.

Methods: Two clean-label preservation systems, FG1 (T1) and FG2 (T2), were incorporated into ranch samples and subjected to oxidation and microbial studies. The oxidation study was conducted by incubating one set of samples at 25°C for 60 days and another at 45°C for 30 days. The peroxide content (primary oxidation) and TBARS levels (secondary oxidation) were measured over time, comparing the clean-label treatments against a no-preservative control (T3) and an EDTA-sorbate positive control (T4). For the microbial study, the four treatments were inoculated with a mixture of *Lactobacillus plantarum*, *L. brevis*, and *L. fermentum* (LAB) and yeast (*Debaryomyces hansenii*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Pichia membranaefaciens*) cocktails at 2.5 log CFU/g. Uninoculated samples served as negative controls. The microbial growth was monitored over a 9-month period.

Results: Both T1 and T2 effectively prevented oxidation, maintaining peroxide and malonaldehyde levels below 10 meq/kg and 10 µg/g, respectively, at 25°C for 60 days. T2 also demonstrated comparable performance to the EDTA control at 45°C after 30 days, whereas the control samples exhibited oxidation within 14 days. For microbial preservation, both T1 and T2 reduced LAB and yeast counts to below 1 log CFU/g within 7 and 14 days, respectively, indicating cidal effects, while control samples showed significant microbial growth within 21 days.

Significance: Clean-label preservation systems were effective in extending the

shelf-life and maintaining the freshness of ranch dressing, meeting consumer demands for natural ingredients.

P3-10: EVALUATING THE PERSISTENCE OF SALMONELLA TENNESSEE AND LACTOBACILLUS BREVIS IN NON-ALCOHOLIC BEER UNDER LOW PASTEURIZATION

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Recent studies show standard beer pasteurization practices (1 min, 73.3–73.9°C) reduce spoilage organisms while causing significant changes in quality, generating interest in milder pasteurization methods; however, it is unknown how this impacts food safety risks for products like non-alcoholic beers (< 0.5% ABV)

Purpose: To evaluate the effect of pasteurization treatments and co-inoculation of a spoilage organism on the survival of *Salmonella* in non-alcoholic beers

Methods: Non-alcoholic beer products were brewed, filtered, and inoculated with *Salmonella* Tennessee (n=360), a subset of which were co-inoculated with *Lactobacillus brevis* (n=180) at approximately 8.0 log CFU/mL in one of three treatments (standard, low, and no pasteurization). Standard and low samples were pasteurized in a water bath at 79°C for 1 minute and 59°C for 1 minute, respectively, and immediately cooled for 10 minutes post-pasteurization. Samples were stored at 14°C and enumerated in duplicate on non-selective (tryptic soy) and selective (xylose lysine deoxycholate) media at 0, 1, 7, 14, 31, 93, 122, 150, and 183 days. Co-inoculation samples were additionally enumerated on universal beer agar with 0.1% cycloheximide. Data were analyzed in RStudio (v4.3.3) using the Kruskal-Wallis test and Dunn's Test ($p < 0.05$)

Results: Significant differences in *Salmonella* reductions were observed across pasteurization treatments, with 8.0 ± 0.0 , 2.18 ± 0.23 , and 0.0 ± 0.0 log reductions after standard, low, and no pasteurization, respectively. There were no significant differences in *Salmonella* log reduction between time points in the low pasteurized and non-pasteurized samples ($p > 0.05$). After 31 days, *Salmonella* log reduction in single inoculation samples was 1.2 ± 0.35 and 0.72 ± 0.59 for standard and low pasteurization, respectively. *Salmonella* log reduction did not

significantly differ under co-inoculation ($p > 0.05$)

Significance: Low pasteurization treatment of non-alcoholic beer at 59°C is insufficient to control *Salmonella*. Findings show *Salmonella* can survive in non-alcoholic beer for at least 31 days, even with co-inoculation

Award Application: Developing Scientist Award

P3-11: EFFECT OF FRUIT JUICE CONCENTRATE ON MYCELIAL GROWTH OF BYSSOCHLAMYS NIVEA

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Mold growth in juice not only affects sensory characteristics of finished products but poses a food safety risk with the potential production of mycotoxins. Additionally, the differing susceptibility of fruit juice concentrates to mold spoilage is not well categorized.

Purpose: In this study, red and white grapefruit, guava and watermelon concentrate were selected to evaluate potential inhibitory effects on the mycelial growth of *Byssoschlamys nivea*.

Methods: Fruit juice concentrates (60 brix°), were mixed with MEA to produce plates containing 10% red and white grapefruit, guava and watermelon concentrate. Media pH and water activity were adjusted to 3.0 ± 0.05 and 0.99, respectively. Mycelial agar plugs (7 mm in diameter) were cut from the actively growing mycelia of *B. nivea* and inoculated onto the fruit juice concentrate plates. Colony diameters were measured after 4 days at 28°C, and the inhibition rate was compared with a 0% concentrate control. Samples were plated in triplicate and the experiment was repeated twice.

Results: The red grapefruit concentrate was found to inhibit mycelial growth by $-15.4\% \pm 11.7\%$ (ANOVA: $P < 0.0001$, Tukey: $P < 0.05$). The guava juice was found to increase the growth rate of the mycelia by $16.5\% \pm 3.3\%$

P3-13: THE PREVALENCE, DISTRIBUTION, AND DIVERSITY OF SALMONELLA ISOLATED FROM PORK SLAUGHTERING PROCESSORS AND RETAIL OUTLETS IN THE SHANDONG PROVINCE OF CHINA

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: *Salmonella* is a foodborne pathogen that continues to occur in the meat industry and is primarily transmitted through the use of animal-based foods. Pork products are one of the most important routes of transmission, especially during the pork slaughtering process, which can easily spread *Salmonella* contamination, especially when hygiene measures are inadequate.

Purpose: This study aimed to investigate the prevalence, serotypes, sequence types, antibiotic resistance, and virulence genes of *Salmonella* isolates in the slaughter and distribution chains (outlets) in Shandong Province.

Methods: Samples were collected from 13 different sampling sources during the slaughter process at three slaughter plants (600 samples) and from 20 retail outlets supplied by these processors (45 samples).

Results: The prevalence was 18.9% amongst all the samples, with the highest prevalence observed in feces (40.0%), lairage pens (38.0%), and hides (34.0%). A total of 6 serotypes were identified, with *S. Rissen* (46.3%) and *S. Typhimurium* (32.0%) found to be the most prevalent serotypes. 86.8% of *Salmonella* isolates were multi-drug resistant, with the majority of strains resistant to erythromycin,

sulfisoxazole, and ampicillin. The multilocus sequence typing analysis revealed 6 STs were obtained from 45 isolates, with the dominant type ST469 accounting for 40.0% of the total, which suggested a high possibility of cross-contamination between the plant processing chain and retail outlets.

Significance: This study revealed the prevalence and correlation of *Salmonella* isolates between pork slaughterhouses and retail stores, and mapped the spatial distribution and mutation trends of the pathogen during the slaughter and sales process, providing an informative case study for effective prevention and control measures for *Salmonella* in the pork industry.

P3-14: STUDY ON THE TRANSFER OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI IN BEEF CATTLE INDUSTRY CHAIN AND QUANTITATIVE MICROBIOLOGICAL RISK ASSESSMENT

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: STEC has become a serious threat to public health safety, beef cattle are important hosts, evaluating its hazards is important for ensuring food safety.

Purpose: investigation of the cross contamination of Shiga-toxin producing *Escherichia coli* (STEC) during beef processing and conducting a quantitative microbiological risk assessment (QMRA) is essential for in-plant critical control points and food safety.

Methods; Identification to cross-contamination among different sampling sites was further conducted by combining multilocus sequence typing (MLST) with the distribution and characterization data. QMRA model of STEC for the beef steak supply chain in China was established, Monte-Carlo simulation method was used for separating variability and uncertainty of model's parameters, the stages of industrial processing, distribution, and consumption were considered in the exposure assessment.

Results: Hide plays a critical role in cross-contamination between feces, lairage pens and post-washing carcasses from a molecular perspective. The QMRA model predicted the mean probability of illness per serving was 3.17×10^{-7} , and a sensitivity analysis was performed, the cooking process and the initial level of STEC in the hide has a significant impact on controlling the risk of eventual

ingestion of STEC-contaminated steaks.

Significance; Combining the results of sensitivity analyses with those of traceability analyses, intervening measures revolves around de-hiding should be involved in the in-plant safety control Policy, and good cooking habits of consumers are also important for risk control. This study not only provides an assessment of STEC risk associated with beef steak, but also facilitates the identification of crucial targets for

P3-15: EVALUATING TEMPORAL CHANGES AND RISK EMERGENCE IN NON-TYPHOIDAL SALMONELLA ENTERICA SUBSPECIES ENTERICA SEROVARS AND VIRULENCE FACTOR GENES

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: Understanding temporal changes in *Salmonella* virulence is essential for timely and proactive control of *Salmonella* in foods; however, the genetic dynamics underlying *Salmonella* virulence changes over time remains unclear.

Purpose: We conducted a temporal analysis of *Salmonella* prevalence and virulence factor gene (VF) carriage in isolates collected from 2016 to 2023 to evaluate evolution of serovar virulence.

Methods: We used protein clustering to screen 64,284 *Salmonella* isolates from US (human (n=30,442), chicken (24,552), and beef (9,290)) for the presence of 33,007 VFs from eight categories: 'nutrition', 'effector delivery system', 'exotoxin', 'motility', 'regulation', 'stress response', 'antimicrobial resistance', and 'other'. 350 VFs were present in between 100 and 61,070 (95%) isolates, and thus informative in characterizing the *Salmonella* higher virulence (HV) genetic profile. We evaluated temporal changes in serovar prevalence, carriage of 23 individual VFs (including the 15 most informative), and carriage of VF categories from 2016–2023, using a Bayesian test of differences in rates and proportions. We also evaluated connections between changes in VFs over time and emerging virulence based on previous identification of HV serovars.

Results: Total VF carriage in all *Salmonella* increased by 4% (95% CrI: 4.1–4.4%) in 2020–2023 compared to 2016–2019, and by 9% (8.8–9.4%) in 2023. This trend is consistent for *Salmonella* sourced from chicken, beef, human clinical cases, and most serovars. However, changes differed across VF genes and categories; for example, 'exotoxin' carriage decreased by 1.8% (2.3–1.2%) while 'nutrition' carriage increased by 6.6% (6.3–6.9%). In contrast, we saw no significant changes in the top 20 most prevalent serovars in 2020–2023 vs 2016–2019.

Significance: While the prevalence of HV serovars has remained stable over the last eight years, total VF carriage has increased for most serovars, as has the carriage of many VFs and VF categories. Nuanced surveillance of temporal changes in VFs is necessary to detect and target virulence emergence.

P3-16: ENHANCING FOOD SECURITY THROUGH TERTIARY SOCIAL HEALTH INSURANCE PROGRAMME

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: The Tertiary Institutions Social Health Insurance Programme (TISHIP) is a social security system whereby the healthcare of students in tertiary institutions is paid for from funds pooled through the contributions of students. TISHIP caters for the healthcare needs of Nigerian students in tertiary institutions who, due to their studentship status, cannot benefit under other health insurance programmes.

Purpose: To determine the pattern and predictors of utilisation of the Tertiary Institution Social Insurance Programme among undergraduates of Osun State University and explore how such programmes can indirectly support food security.

Methods: A descriptive cross-sectional study design was used for this study. From Osun State University, a total number of 422 students were selected using a multi-stage sampling technique. A total number of 422 questionnaires were used to collect data from respondents in Osogbo and Ejigbo campuses. Data for this research was collected via a self-administered questionnaire that contained open and closed-ended questions. Analysis was done using SPSS Version 25.0. Confidence level was set at 95% with a p-value of <0.05 taken as statistically significant.

Results: The majority of the respondents were within the age 20–24 (62.3%). More than half of the respondents (55.5%) were from Osogbo campus, and the majority (35.5%) were in 400 level. Over half of the respondents (51.2%) had poor knowledge of TISHIP. However, more than half (53.8%) utilise TISHIP. Most of the respondents that had good knowledge of TISHIP were in 300 level, and the majority of those who utilise TISHIP were from Osogbo campus and mainly in 300 and 400 level.

Significance: Findings highlight the need to improve students' awareness of TISHIP to optimise utilisation. By reducing healthcare costs, students and their families can assign more resources to food and nutrition, strengthening resilience against food insecurity.

P3-17: ASSOCIATION BETWEEN SOCIODEMOGRAPHIC FACTORS AND ACUTE GASTROINTESTINAL ILLNESS IN A CANADIAN LINKED COHORT

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: Acute gastrointestinal illness (AGI) remains a significant public health issue and the risk of contracting it based on sociodemographic characteristics remains poorly defined.

Purpose: To identify the risk of contracting AGI based on various sociodemographic factors.

Methods: Canadian Community Health Survey respondents were followed from time of interview, to December 31, 2017, using the National Ambulatory Care Reporting System (NACRS) to capture emergency department (ED) visits and the Discharge Abstract Database (DAD) to capture hospitalizations due to AGI. A series of sociodemographic and health indicators were selected for assessment based on prior literature. Associations were assessed using Cox proportional hazards regression to estimate hazard ratios (HRs).

Results: 470,700 respondents were linked to the DAD and 190,700 were linked to the NACRS. Six percent ($n = 10800$) of respondents incurred ED-treated AGI visits and 2% ($n = 9400$) were hospitalized. Associations included being a male gender, young adults or seniors, having less than a secondary school education, low income, being a racial minority, being an immigrant to Canada, living alone, men who have sex with men (MSM), being under or overweight, cigarette smokers, alcohol drinkers, having a chronic condition, poor perceived health, and being compliant with national recommendations for produce consumption. of note, in unadjusted models MSM had a 3% risk of requiring an ED visit (HR 1.03 95% CI 0.97-1.10) and a 136% increased risk of requiring a hospitalization (HR 2.36 95% CI 2.23-2.49), and people with poor perceived health had an 81% increased risk of requiring an ED visit (HR 1.81 95% CI 1.73-1.89) and a 209% increased risk of requiring a hospitalization (HR 3.09 95% CI 2.96-3.22).

Significance: This study identified novel risk factors for requiring a hospital-based visit for AGI, which can be used to inform public health proGramming.

Award Application: Developing Scientist Award

P3-18: FOOD AND WATER SAFETY PRACTICES AND PREPAREDNESS OF ONTARIANS AT HOME DURING POWER OUTAGES AND OTHER EMERGENCIES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Proper food and water safety practices and preparedness during power outages is important to prevent food- and water-borne illnesses, as such emergencies can compromise drinking water systems and the ability to store food at safe temperatures at home.

Purpose: This study was conducted to understand the food and water safety preparedness of Ontarians during power outages and other emergencies at home.

Methods: A cross-sectional survey was delivered to an online panel of Ontario residents using the web-based Zoho Survey platform in June 2024. The survey was developed based on existing guidelines and prior research. It included questions about previous experiences with power outages, emergency preparedness and current food and water safety practices, behavioural intentions for future emergencies, and sociodemographic characteristics. Results were tabulated descriptively.

Results: A total of 752 participants completed the survey. Only 8% of respondents (n=62) felt that they were 'very prepared' for an extended power outage at home (7-point scale from not at all prepared to very prepared). To determine the safety of perishable food in refrigerators during a power outage, 68% of respondents (n=510) considered the amount of time in the refrigerator. Of these respondents, only 11% (n=54) would discard perishable food kept for more than 4 hours. Additionally, 53% of respondents (n=398) considered placing perishable food in cold weather to keep it safe for longer, and 59% (n=445) considered the smell and taste of water to determine if it was safe to drink during a power outage.

Significance: The findings highlight the preparedness levels and certain unsafe food and water handling behaviours during power outages. Findings can support the development of tailored public health messaging and outreach to protect Ontarians from food and water-borne illness and ensure they are well prepared for emergencies.

P3-19: BULK BIN BOTHER - MULTISTATE OUTBREAK OF ESCHERICHIA COLI O157:H7 INFECTIONS LINKED TO ORGANIC WALNUT CONSUMPTION

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: In 2024, an outbreak of Shiga toxin-producing *Escherichia coli* (STEC)O157:H7 infections was investigated; epidemiologic and traceback evidence indicated organic walnuts as the likely source.

Purpose: To prevent additional illnesses by identifying the potential source and possible contributing factors of contamination.

Methods: Illnesses were identified through PulseNet, CDC's laboratory network for foodborne disease surveillance, and ill people were interviewed with food history questionnaires. State and FDA laboratories used FDA BAM methods to attempt recovery of STEC from food and environmental samples. State and federal officials collected records to determine the source of implicated products.

Results: We identified 13 cases from California (7) and Washington (6) with illness onsets during February 1–April 4, 2024. of the 11 patients interviewed, all reported consumption of walnuts and either specified, or purchase records indicated, organic walnuts. Samples were collected by FDA and state partners from patients, the manufacturer, and supplying walnut orchards. Results from two samples could not rule out the presence of STEC, but the outbreak strain was not isolated from any product or environmental samples. Nine points of service (POS) were included in traceback; all cases traced reported purchasing bulk walnuts at grocery/health food stores. Traceback records for 8/9 POSs converged on a single manufacturing firm via two distributors. FDA and state partners from California

initiated two inspections at the manufacturer and the supplier, including multiple orchards, with no contamination source identified. The manufacturing firm, responsible for shelling and bulk packaging of the nuts, issued a recall of two lots of organic walnuts on April 25, 2024.

Significance: Traceback and recall processes were complicated by purchases from bulk bins at retail settings and distributors failing to document connections between lot numbers received and those shipped to customers, a common challenge in traceback investigations.

P3-20: MICROBIAL HAZARD ANALYSIS OF FRESH PRODUCE AVAILABLE AT ATHENS-CLARKE COUNTY, GA-SERVING RETAILERS

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: The safety of food in the retail food environment may influence disparities in foodborne illness burden; however, characterization of differential microbial hazard exposure through food at retail remains limited.

Purpose: Characterize fresh produce contamination in the context of socioeconomic and demographic profiles of communities the retailers serve.

Methods: *k*-means clustering using American Community Survey data revealed two communities in and around Athens, GA with distinct socioeconomic and demographic features. Cucumbers, lettuce, and tomatoes were sub-sampled from retailers in each community (432 samples yielding $n=144$ experimental units). 10-g samples were homogenized in 90 mL buffered peptone water and plated to EC Petrifilm plates. Samples were enriched for *Salmonella enterica* using Rappaport-Vassiliadis-Soya and Tetrathionate broths before isolation on XLT-4 and Hektoen Enteric agar. Colonies were presumptively identified via latex agglutination and confirmed using MALDI-TOF MS.

Results: Generic *Escherichia coli* load was below the countable range (15 colonies) for all samples. Mean coliform load (MCL) on cucumbers differed due to the interaction between community and store type ($p<0.01$). Groceries in the low-resource community had the lowest MCL, 1.92 ± 0.25 log CFU/g, across all treatments ($p<0.05$). Lettuce MCL also differed due to the community and store type interaction ($p<0.01$), with lettuce from high-resource community groceries showing greater MCL (2.09 ± 0.19 log CFU/g) than other treatments ($p<0.05$), except for supermarkets in the low-resource community. No significant interactions or main effects influenced tomato MCL. No *Salmonella* was detected.

However, 24% of 338 presumptive positive isolates were identified as *Klebsiella pneumoniae* and *Enterobacter* spp., members of the ESKAPE group of clinically significant antimicrobial-resistant organisms.

Significance: Differential MCL on fresh produce at Athens-serving retailers appears to be influenced by complex factors beyond community privilege. Additionally, a high prevalence of clinically relevant opportunistic pathogens was detected.

Award Application: Developing Scientist Award

P3-21: VERIFICATION OF HACCP IN POULTRY SLAUGHTERHOUSES IN JAPAN: A RESULT OF MICROBIOLOGICAL SURVEY OF FACILITIES IN JAPAN DURING 2020-2022

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: The establishment of a sanitation management system based on HACCP at slaughterhouses and poultry slaughterhouses plays an extremely important role in ensuring the microbiological safety of livestock products.

Purpose: The purpose of this study is to establish an evaluation method to verify the validity of the status of sanitary management based on HACCP in poultry slaughterhouses in Japan, based on the results of microbiological surveys conducted regularly by local governments throughout Japan and international trends.

Methods: We analyzed the trends of microbiological inspection data including total aerobic plate counts (APC), Enterobacteriaceae counts, and *Campylobacter jejuni* reported by local governments for poultry slaughterhouses in all over Japan during the three-year period from 2021 to 2023 and estimated the implementation status of appropriate sanitary management.

Results: The overall mean APC was 3.93 ± 0.90 log CFU/cm², with 22 (0.45%) samples exceeding +3 SD and 193 (3.9%) samples exceeding +2 SD. The overall mean Enterobacteriaceae count was 2.57 ± 0.92 log CFU/cm², with 8 (0.16%) samples exceeding +3 SD and 75 (2.3%) samples exceeding +2 SD. *C. jejuni* was detected in 28.7% (505/1756) of the samples with an overall mean (+SD) of 0.58 ± 0.98 log CFU/g and a maximum of 5.00 log CFU/g. The 49 samples (2.8%) exceeded the European target of 3.0 log CFU/g. No clear seasonal variation in *Campylobacter* counts was observed throughout the year.

Significance: Based on the results of the contamination survey, we can propose a standard value (annual mean +2 SD) for poultry meat as a target value for process control of APC and Enterobacteriaceae counts in poultry processing. Furthermore, the proposed process control target for *Campylobacter* counts is the annual mean + 2 SD (2.4 log CFU/g) or the European standard of 3.0 log CFU/g.

P3-22: CLIMATE-DRIVEN FOOD SAFETY CHALLENGES: THE IMPACT OF RISING TEMPERATURES, FSIS FACILITY DENSITY, AND SALMONELLA RATES IN THE USA

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: Climate change poses significant challenges to food safety, particularly in managing foodborne pathogens such as *Salmonella*. increased temperatures and regional distribution in Food Safety and infection Service (FSIS) facilities have not been fully examined with *Salmonella* pathogens in the U.S.A.

Purpose: We examined the impact of increased temperatures and FSIS facility density on regional *Salmonella* rates across the standard nine US Environmental Protection Agency (EPA) climate regions. in addition, we provided insights into managing foodborne risks under changing environmental conditions.

Methods: We used data on FSIS facilities from the US Department of Agriculture of Agriculture (USDA) FSIS, average temperatures ranked historically for 2023 from NOAA, and population-adjusted *Salmonella* rates from CDC PulseNet data. FSIS facilities were geospatially mapped for each EPA climate region and evaluated relative to the national total of approved facilities. We employed descriptive statistics and geospatial visualizations, including quantile classification to identify *Salmonella* trends.

Results: Regions with higher average temperatures, such as the Southeast & Caribbean (128°F) and Northeast (128°F), reported elevated *Salmonella* rates of 23.69% and 18.26%, respectively. The Midwest, with a similar facility density (19.9%), reported a *Salmonella* rate of 19.7%. in contrast, cooler regions, such as Alaska (28.4°F) and the Hawaii & Pacific Islands (76.6°F), had significantly lower *Salmonella* rates of 10.77% and 16.59%, respectively. FSIS facility density was highest in the Southeast & Caribbean and correlated with higher *Salmonella* rates.

Significance: This study highlights spatial patterns between climate change, food production infrastructure, and foodborne pathogen prevalence. Regions with

warmer climates and higher FSIS facility densities were associated with increased *Salmonella* rates, emphasizing the need for targeted interventions. This work demonstrates the need for public health stakeholders to consider the changing environmental conditions in mitigating *Salmonella* infections.

Award Application: Developing Scientist Award

P3-23: HYGIENA® RECEIVES AFNOR CERTIFICATION FOR FOODPROOF® SALMONELLA PLUS CRONOBACTER DETECTION LYOKIT FOR INFANT CEREALS, INFANT FORMULA WITH OR WITHOUT PROBIOTICS AND INGREDIENTS, AND PRODUCTION ENVIRONMENTAL SAMPLES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Worldwide, *Salmonella* and *Cronobacter* species are considered the most harmful microorganisms in infant formula. Because these food products are intended for the most vulnerable population, regulatory agencies require screening for these pathogens throughout the production process.

Purpose: To obtain AFNOR certification for the foodproof *Salmonella* plus *Cronobacter* Detection LyoKit, a collaborative study was carried out as the second part of the validation study according to ISO 164140-2.

Methods: For each test parameter of the PCR kit, one interlaboratory study was organized by ADRIA Développement. Sixteen sets of 24 blind-coded samples were prepared. Each set of test portions included 8 uninoculated, 8 low-level inoculated, and 8 high-level inoculated samples. For *Salmonella* spp. detection, 25 g test portions, and for *Cronobacter* spp. detection, 10 g test portions of powdered infant formula with probiotics were analyzed by 16 participants. Samples were enriched in buffered peptone water (1:10 dilution) with 10 mg/L vancomycin and incubated for 16–20 hours at 37 °C. Following incubation, DNA extraction was performed using the foodproof StarPrep® Three Kit or the BAX® Prep Gram-Negative Lysis Kit, followed by real-time PCR analysis using a BAX Q7 or a LightCycler 480 instrument. Confirmation of PCR results was performed according to the respective ISO reference method (ISO 6579-1 or ISO 22964).

Results: The method described successfully yielded results comparable to the reference methods. Based on the results of the entire ISO validation study, the assay met all requirements compared to ISO reference methodologies to receive AFNOR certification (QUA 18/12-12/24 and QUA 18/13-12/24).

Significance: Hygiena offers the first certified method for the simultaneous detection of *Salmonella* and *Cronobacter* for the infant formula industry on the market.

P3-24: DEVELOPMENT OF A MULTIPLEX REAL-TIME PCR ASSAY FOR THE DETECTION OF HIGHLY PATHOGENIC SALMONELLA (HPS) IN POULTRY, BEEF AND PORK

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The USDA and industry work to find a balance between continuing to offer wholesome, nutritious meat products and reducing the risk of illness from *Salmonella* for humans. The poultry and beef industries need an easy-to-use method to identify the risk of *Salmonella* in food samples based on virulence or pathogenicity instead of simply presence or absence.

Purpose: This study evaluated the positive predictive value and negative predictive value of a real-time PCR assay developed to detect *Salmonella* based on virulence by testing approximately 700 well-characterized and serotyped enrichment samples from the poultry, beef, and pork industries.

Method: This multiplex real-time PCR assay is designed with three HPS targets (HPS-B, HPS-A, HPS-X) associated with virulence, and combines with a *Salmonella* spp. target and internal control. The assay was tested on 696 *Salmonella*-positive enrichment samples stored as glycerol stocks. Prior to analysis with the RT-PCR assay, the enrichment samples were well characterized through isolation techniques and deep serotyping (DST). In the absence of a reference method in the MLG for pathogenicity, the characterization of these enrichment samples serves as a “reference” or “truth dataset.”

Results: Based on the samples tested, the study demonstrated that the real-time PCR assay has a PPV of 97% and a NPV of 93%. Further, there are data points that demonstrate the assay is quite sensitive and was able to identify virulence markers of more pathogenic serotypes, confirmed by DST as present, but were not isolated using the culture methods employed.

Significance: This approach to *Salmonella* testing can enable the industry to make more risk-based decisions about their products to reduce the number of illnesses caused by *Salmonella* while continuing to provide consumers with nutritious and affordable meat.

P3-25: EVALUATION OF THE ANTIMICROBIAL EFFICACY OF NANOBUBBLE WATER WITH PERACETIC ACID FOR MIXED-SPECIES DYNAMIC BIOFILM CONTROL IN GROUND BEEF PROCESSING

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Biofilms pose challenges in food processing due to resilience against disinfectants and their capacity to harbor pathogens.

Purpose: To evaluate the antimicrobial effects of air-based nanobubble water (NW) or tap water (TW) containing peracetic acid (PAA) on mixed-species biofilm development on stainless steel (SS) and Buna-N rubber (R) coupons under organic loading in a dynamic system.

Methods: CDC biofilm reactors were filled with NW or TW infused with 0, 200, or 400 ppm PAA and 10% (v/v) beef purge, and inoculated with STEC surrogates. Planktonic counts and biofilm development on coupons were evaluated over 5 days at 4°C. Reactor fluid (20%) was replenished daily. Aerobic plate counts (APC) and Enterobacteriaceae (EB) populations were quantified on days 0, 3, and 5 using 3M Petrifilm after neutralizing, sonicating, and vortexing samples. Reactor pH was also monitored. A mixed-model ANOVA with Fisher's protected LSD ($n = 378$, 3 replicates, $\alpha = 0.05$) was used to identify treatment effects.

Results: Biofilms increased to >5 log CFU/coupon by day 5 at 0 ppm PAA ($P < 0.05$); NW reduced this growth by 0.7 log ($P = 0.02$) compared to TW. PAA treatments at 200 and 400 ppm significantly reduced APC and EB biofilm development ($P < 0.0001$). At 400 ppm PAA, all counts were undetectable (LOD = 1.1 log CFU/coupon) on all days. By days 3 and 5, APC levels were 1.4 and 1.2 log higher at 200 ppm than 400 ppm PAA, respectively ($P < 0.05$). R coupons supported more biofilm development than SS ($P < 0.0001$). pH increased from 5.8 to 6.7 in 0 ppm PAA reactors, while remaining at 4.6 (200 ppm) and 4.4 (400 ppm) in treated systems.

Significance: PAA at ≥ 200 ppm effectively maintained biofilm control in meat

processing systems with moderate organic loading, supporting its application for extended sanitation cycles.

P3-26: EFFICACY OF PERACETIC ACID SOLUTIONS PREPARED USING NANOBUBBLE WATER FOR CONTROLLING STATICALLY GENERATED MIXED-SPECIES BIOFILMS ON STAINLESS STEEL AND RUBBER

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Category: Food Safety Systems

Abstract:

Introduction: Biofilms developed under static conditions can be problematic in meat processing, especially on surfaces such as seals and crevices.

Purpose: Evaluate the antimicrobial effects of air-based nanobubble (NW) or tap (TW) water containing peracetic acid (PAA) on mixed-species biofilm development on stainless steel (SS) and Buna-N rubber (R) in a chilled static system with organic loading.

Methods: Twelve treatment and 2 control solutions of 5 or 10% (v/v) beef purge with 0, 200, 400 ppm PAA in TW or NW were added to vessels containing SS and R coupons. Treatment vessels were inoculated with STEC surrogates and stored at 4°C statically for 10 days with 20% of volume replenished daily. Planktonic and biofilm populations were quantified on days 0, 3, 5 and 10. A mixed-model ANOVA was used to identify significant treatment effects with appropriate pairwise comparisons performed using Fisher's protected LSD (n=335, 3 replications, $\alpha=0.05$).

Results: No differences ($P>0.05$) in APC or EB counts were observed comparing NW and TW treatments within PAA concentration across days. At 5% purge, R and SS coupons showed no difference ($P=0.52$) in APC biofilm growth; at 10% purge, R supported greater biofilm growth than SS ($P=0.008$). Organic level affected biofilm development in 200 ppm PAA solutions ($P<0.0001$), reaching 4.5 log APC/coupon at 10% purge compared to 2.0 logs at 5% purge. At 400 ppm PAA, APC biofilm development was higher ($P=0.043$) in TW solutions, but similar ($P=0.16$) in NW solutions between organic levels. PAA at 200 ppm inhibited biofilm growth for 5 days but allowed significant development between days 5 and 10 ($P=0.008$).

Significance: PAA at ≥ 200 ppm inhibited biofilm development by native meat microflora on SS and R surfaces under static, extended duration, refrigerated conditions encountered in meat processing with a strong influence by the level of

organic loading present.

P3-27: ANTIMICROBIAL RESISTANCE & VIRULENCE GENES OF ENTEROCOCCUS FROM RETAIL PORK

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Antimicrobial resistance (AMR) poses a significant threat to public health. Enterococci are ubiquitous bacteria commonly found in the environment and the food supply chain, including retail meats, that can carry and transfer AMR genes.

Purpose: To assess the presence of AMR genes and virulence genes in *Enterococcus* spp. from retail pork.

Methods: Retail pork samples were collected monthly from randomly selected grocery stores in Hawaii in 2020 and 2022 by the FDA National Antimicrobial Resistance Monitoring System (NARMS). The NARMS Retail Meat Isolation Protocol was applied to isolate *Enterococcus* from samples. Confirmed isolates underwent whole-genome sequencing to identify AMR genes and virulence genes.

Results: A total of 186 samples were collected and 72% of tested samples were positive for *Enterococcus*. Eighty-four isolates were successfully sequenced, including 66 *Enterococcus faecalis*, 17 *Enterococcus faecium*, and 1 *Enterococcus hirae*. All isolates of the three species carried one or more AMR genes. All of the 66 *E. faecalis* isolates harbored virulence genes, whereas 16 of 17 *E. faecium* isolates harbored virulence genes. No virulence genes were detected in *E. hirae*.

All *E. faecalis* isolates carried the *dfrE* gene confer resistance to trimethoprim, efflux pumps genes (*efrA*, *efrB*, *emeA*) related to multidrug resistance, and *IsaA* gene confer resistance to clindamycin, quinupristin-dalfopristin and dalfopristin. Most *E. faecalis* isolates (55/66) carried *tetL*, *tetM*, and *tetS* genes confer resistance to tetracyclines. All *E. faecium* isolates carried *aac(6')*-II and *msr(C)* genes confer resistance to aminoglycosides and macrolides. The *E. hirae* isolate carried *tetL* confer resistance to tetracyclines and *IsaA* genes confer resistance to clindamycin, quinupristin-dalfopristin and dalfopristin. Major virulence genes detected were *cps*, *ebp*, and *fsr* in *E. faecalis*, and *acm*, *scm*, and *sgrA* in *E. faecium*.

Significance: Monitoring *Enterococcus* and its resistance and virulence genotypes in retailed pork are critical for tracking AMR trends in retail foods.

Award Application: Developing Scientist Award

P3-28: BPW AND UHT ENRICHMENT PROTOCOLS TO DETECT *SALMONELLA* IN CHOCOLATE

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Salmonella* can persist in chocolate matrices. Due to the presence of inhibitory substances in chocolate matrices, it could be difficult to detect *Salmonella* strains. This is a significant concern for food industry. To improve the detection of *Salmonella* in 375 g cocoa and chocolate products, Assurance® GDS for *Salmonella* Tq (GDS *Salmonella*) are provided two protocols with two different enrichment broths.

Purpose: To validate specific protocols to detect *Salmonella* in cocoa and chocolate products up to 375 g using BPW or UHT milk + Brilliant Green dye enrichment broths followed by Assurance® GDS for *Salmonella* Tq detection method.

Methods: Sensitivity and Relative Limit of Detection (RLOD) studies were conducted by an external expert laboratory following ISO 16140-2. Chocolate matrices (375 g test portion) were inoculated by *Salmonella* strains and enriched for 24-32 h at 34-38°C in BPW (1:10 dilution ratio) or UHT milk containing 0.018 g/L of Brilliant Green (UHT+BG) dye (1:10 dilution ratio). Before analysis, a dilution of enriched samples is performed in wash buffer (100µL/900 µL for BPW and 300 µL/700 µL for UHT+BG). Then, detection is performed using Assurance® GDS for *Salmonella* Tq method followed by a confirmation step. A comparison with the reference method (ISO 6579-1/A1) was conducted using both protocols on different chocolate types.

Results: For each protocol, 60 samples (20 raw materials, 20 cocoa powders and 20 finished products) were analyzed for the sensitivity study. For the RLOD study, cocoa powder was tested with the UHT+BG protocol and hazelnut chocolate bar for the BPW one. The sensitivity study and the RLOD study met the Acceptance Limits (respectively 3 and 2.5) of an unpaired study. No significant differences were observed between the ISO reference method and the GDS *Salmonella* alternative one.

Significance: These data demonstrated allowance of two different enrichment methods followed by Assurance® GDS for *Salmonella* Tq for the detection of

Salmonella in large test portions inhibitory matrices.

P3-29: EXPANDING THE HOST RANGE OF BACTERIOPHAGES FOR IMPROVED DETECTION AND CONTROL OF FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: The food industry faces significant challenges in detecting and controlling foodborne pathogens, which can lead to costly recalls, reputational damage, and compromised consumer safety. Bacteriophages, natural predators of bacteria, offer a promising solution. However, their narrow host range limits their effectiveness.

Purpose: This study aimed to modify the tail fibers of T4-like phages to broaden their host range to increase the detection and control of foodborne pathogens, ultimately reducing the risk of foodborne illnesses.

Methods: The T4 phage was genetically engineered to add the adhesin genes (g38) from either T2, T6, or both T2&T6. The engineered phages were tested for their ability to infect the 72 *E. coli* strains from the ECOR collection. The host range of each engineered phage was compared to that of the wild-type T2, T4 and T6 phage.

Results: The host range of wild-type T4 phages did not broaden by swapping their tail fibers with those from T2 and T6 phages. However, the engineered phages showed a broadened host range when compared to wild-type T2 and T6 phages. This modification enabled the engineered phages to infect a wider range of bacterial strains from the ECOR library, mirroring the host ranges of T4. Nevertheless, the introduction of multiple adhesin genes into the T4 phage genome resulted in T4 phage with a broader host range, but with reduced infection efficiency.

Significance: Swapping tail fibers addressed the limitation of narrow host range by modifying the tail fibers of T4-like phages to broaden their host range. This innovation enabled the engineered phages to detect and control a wider range of foodborne pathogens, including those from the ECOR library. Our approach has significant implications for enhancing food safety and reducing the risk of foodborne illnesses.

Award Application: Developing Scientist Award

P3-30: REDUCTION OF SALMONELLA ON THERMALLY TREATED GOLDENBERRIES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Salmonella* outbreaks and recalls have been linked to fresh and dried fruits, which are used in a variety of bakery and confectionery products including cookies, soft bake bars, muffins, and chocolates. In low water activity environments, *Salmonella* can become increasingly heat resistant, which is of special concern when applying a thermal treatment to products with low aw.

Purpose: In a laboratory setting, *Salmonella*-inoculated goldenberries were thermally treated by drying in an oven to assess pathogen reduction for commercial use.

Methods: Commercially available goldenberries were washed and dried prior to inoculation with high levels of a *Salmonella* cocktail (*S. Typhimurium*, *S. Mons*, and *S. Enteritidis*). The sample size of 5 inoculated berries (approximately 25–30 g) was dried on aluminum foil boats at 60°C in a convection oven with allocates plated at 0, 10, 14, and 18 hours. Samples (n=3) were enumerated on tryptic soy agar with xylose lysine deoxycholate agar overlays and incubated at 37°C for 24 hours.

Results: The average starting concentration of the inoculated berries was 7.27 ± 0.09 log CFU/g. At 10, 14, and 18 hours, the average concentration of *Salmonella* on the berries was 3.02 ± 0.29 , 2.19 ± 0.03 , and 1.26 ± 0.24 log CFU/g, respectively. The aw and pH of the berries post thermal treatment were 0.82 and 3.32, 0.37 and 3.55, and 0.28 and 3.25 for the 10-, 14-, and 18-hour timepoints, respectively.

Significance: The thermal treatment applied to the goldenberries produced >4, >5, and >6 log CFU/g reductions of *Salmonella* for the 10-, 14-, and 18-hour timepoints, respectively.

P3-31: IMPLICATIONS OF SURFACE TOPOGRAPHY, CHEMISTRY, AND MICROBIAL SYMBIOSIS ON *LISTERIA MONOCYTOGENES* BIOFILM ON FOOD CONTACT SUBSTANCES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *L. monocytogenes* forms persistent biofilms on food contact substances, posing significant food safety risks.

Purpose: To investigate how *L. monocytogenes* persistence in biofilm is affected by surface topography, chemistry, and symbiotic species in produce processing facilities, and how the symbiosis is impacted.

Methods: Cocktail biofilms were cultivated on plastic coupons (polyethylene, polyoxymethylene, polypropylene, and polyvinyl chloride), each with three types of surface topographies: native, microdots, and microlines. Cocktail biofilms included monospecies biofilm (*L. monocytogenes*), dual-species cocktail biofilm (*L. monocytogenes* + *Escherichia coli* O157:H7; *L. monocytogenes* + *Pseudomonas fluorescence*; *L. monocytogenes* + *Ralstonia insidiosa*), and four-species cocktail biofilm (*L. monocytogenes* + *E. coli* O157:H7 + *P. fluorescence* + *R. insidiosa*). *L. monocytogenes* cocktail biofilms were grown for 7 days at 4 °C in lettuce juice extract to simulate produce processing conditions.

Results: Monospecies *L. monocytogenes* biofilm formation decreased on surfaces with microdots (0.4 log CFU/cm² reduction) and microlines (0.7 log CFU/cm² reduction). In dual-species biofilms, *P. fluorescence* consistently enhanced *L. monocytogenes* biofilm formation ($p < 0.01$), while *E. coli* O157:H7 and *R. insidiosa* showed surface-dependent synergistic ($p < 0.05$) or antagonistic effects ($p < 0.05$). Four-species cocktail biofilms exhibited synergistic symbiosis with *L. monocytogenes* under all conditions. The results suggest that surface topography, chemistry, and symbiotic species significantly influence biofilm dynamics, and symbiosis can be affected by surface properties.

Significance: Symbiosis plays a critical role in *L. monocytogenes* biofilm formation with implications for food safety. Mitigating synergistic interactions and leveraging antagonistic symbiosis could indicate potential intervention strategies against *L. monocytogenes* biofilms.

P3-32: ENERGY AVAILABILITY EXPANDS ECOLOGICAL BOUNDARIES OF HORIZONTAL PLASMID TRANSFER

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introductions: Horizontal gene transfer (HGT) is a primary contributor to the dissemination of antibiotic resistance. The recipients of HGT in natural environment span multiply genres. However, the factors allowing HGT and selecting the recipients remain unclear.

Purpose: The study aimed to decipher the physiological factors that determine the HGT.

Methods: HGT dynamics from *Salmonella* Typhimurium carrying a streptomycin resistance plasmid in fecal microbiota was monitored using 16s amplicon sequencing. The HGT mechanism was investigated using transcriptomics as well as biochemical assays of energetic perturbation.

Results: Our finding reveals that antimicrobial resistance transfer occurs frequently from the donor of resistance to its closely related kin through conjugation. The transfer frequency varies among different recipients. The presence of selective pressure at sublethal levels of streptomycin extends ecological boundaries for resistance transfer and enhances the frequency of conjugation. This increase in HGT is possibly a collateral effect of the stress response triggered by antibiotic exposure. To meet the energy demands associated with this stress response, recipient cells upregulate energy metabolism, resulting in elevated intracellular ATP levels that coincidentally support the energetic costs required for plasmid acquisition and maintenance. Suppression of ATP synthesis markedly disrupts HGT.

Significance: These findings provide new insights into the determinants of HGT and indicate potential control strategies to prevent the transmission of antimicrobial resistance in food systems.

Award Application: Developing Scientist Award

P3-33: AUTOMATING SAMPLE PREPARATION AND PCR SETUP IMPROVES RESULT REPEATABILITY

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Operator performance and skill play an important role even with simple and streamlined workflows. These workflows often have repetitive tasks which increase the risk for human errors, even with experienced operators. Automation of sample preparation and PCR setup improves consistency and ensures repeatability of results which is beneficial to testing laboratories of all sizes.

Purpose: The Thermo Scientific™ SureTect™ Automation platform utilizes three different automation instrument configurations to serve laboratories with different sample throughputs. A comparison study between different automation configurations was completed to assess equivalency between configurations and improvement in result repeatability compared to manual workflows.

Methods: A paired comparison study design included sample enrichment and post-enrichment inoculation at the required contamination level using various food matrices known to be challenging to lysis and PCR. Serial dilution of the inoculated aliquot was prepared using enriched test portion broth to enable testing above the limit of detection (LOD), at LOD, and below LOD. Dilutions were run through the different automation configurations (8 replicates each) and analyzed by qPCR using Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument. All the currently available SureTect assays were evaluated.

Results analysis included difference in Ct averages and standard deviations.

Results: The average Ct difference between the automation configurations and the standard deviation of Ct values were analyzed demonstrating the automated workflow performed equivalently compared to the manual workflow regardless of the automation configuration used. The result repeatability improved when using the automated workflow.

Significance: The study shows that automation of pathogen testing workflows can benefit laboratories with different levels of sample throughput in the food industry. The SureTect Automation platform enables reliable detection and differentiation of the most common food pathogens from a wide variety of food samples. The platform's validated performance supports accurate and timely data-driven food safety decisions.

P3-34: MOISTURE-ACTIVATED OXYGEN SCAVENGER BASED ON ACACIA CATECHU FOR ACTIVE FOOD PACKAGING: A PLANT-BASED ALTERNATIVE

Primary Author:

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Many food products on the market shelves are susceptible to oxygen. Prolonged exposure to atmospheric oxygen can cause undesirable flavor and textural changes, reducing product quality and shelf life.

Purpose: This study aimed to develop a natural, plant-based oxygen scavenging system (OSS) using ball-milled *Acacia catechu* powder and sodium carbonate with moisture activation, as an alternative to chemical-based oxygen scavengers.

Methods: Ball milling was performed to increase the surface area for oxygen-scavenging reaction sites, verified through BET surface area analysis. Scavenging mixtures were prepared and tested for oxygen absorption under 95% RH at 25°C and 45°C. Kinetic studies were conducted to determine the activation energy of the developed system.

Results: Among the developed mixtures, AC1/NC1 demonstrated the highest performance, with oxygen absorption rates of 2.13 mL O₂/g·day at 25°C and 0.58 mL O₂/g·day at 45°C. The OSS scavenged nearly all oxygen (20.8% v/v) in the test chambers under various temperature and humidity conditions. The activation energy of 57.1 kJ/mol was 0.6 times lower than that of a previous system (94.9 kJ/mol), likely due to increased surface area, stronger alkalinity, and higher mixture mass.

Significance: The findings indicate that the AC-NC-based OSS with moisture activation is a promising natural solution for oxygen-sensitive, long-term stored foods. It offers a safe, effective, and sustainable alternative to synthetic scavengers.

Award Application: Developing Scientist Award

P3-35: ESTIMATING THE BURDEN OF FOODBORNE DISEASES IN JAPAN USING MEDICAL FEE RECEIPT DATA (2018-2023).

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract: Introduction: Despite establishing the surveillance system on foodborne diseases based on Food Sanitation Acts, which do not exactly reflect the real burden of foodborne diseases, robust and objective standards for ranking priorities are lacking in Japan. Purpose: This study aimed to estimate incidences of foodborne diseases and to provide the basis for developing a strategy for the prevention and control of foodborne diseases. Methods: Data on cases of foodborne diseases caused by five pathogens reported on Japanese national medical fee receipts were collected from 2018 to 2023. We estimated the burden of foodborne diseases using the burden pyramid reconstruction method. We used a Bayesian method to estimate the probability distributions of the proportion of incident cases who visited a physician, which was derived from population-based telephone surveys, and the proportions of infection attributable to foodborne disease, which were estimated using an expert elicitation. Uncertainty intervals were derived by Monte-Carlo simulation within the R statistical package. Results: Estimates of annual incidence in 2023 were approximately 2.18 (UI 2.1 to 2.3), 45.1 (UI 40.5 to 50.2), 11.4 (UI 10.2 to 12.8), and 72.4 (63.6 to 82.2) cases per 100,000 population for foodborne diseases caused by enterohaemorrhagic *Escherichia coli* (EHEC), *Campylobacter jejuni/coli*, *Salmonella* SPP, and Norovirus, respectively. These estimates were many-fold higher than the value indicated by the results of routine surveillance, which ranged from 0.2 to 4.4 cases per 100,000 population. Regarding *Listeria* spp., estimates of mean incidence from 2018 to 2023 ranged from 0.14 to 0.17 cases per 100,000 population despite no outbreak report caused by *Listeria monocytogenes*. Significance: This study estimates incidences of foodborne diseases caused by five pathogens based on the data from Japanese national medical fee receipt information and suggests the need to establish a complementary system for estimating the burden of foodborne diseases.

Award Application: Developing Scientist Award

P3-36: MICROBIOLOGICAL EXAMINATION OF YELLOW MEALWORM (*TENEBRIO MOLITOR*) LARVAE UNDER VARIOUS PROCESSING AND STORAGE CONDITIONS AS POTENTIAL FOR HUMAN CONSUMPTION

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Yellow mealworm (*Tenebrio molitor*) larvae have emerged as a novel and sustainable food source in the Western world, offering a well-balanced nutritional profile. However, their naturally high bacterial load poses substantial food safety concerns.

Purpose: This study aimed to investigate how various pre-processing, processing, and storage conditions influence the microbial load and bacterial diversity in mealworms.

Methods: Treatment methods simulating commercial insect processing—specifically a range of thermal interventions and freeze-drying—were applied and compared to fresh, untreated controls to assess their individual and combined impacts on microbial reduction. Microorganisms were quantified using serial 10-fold dilution and standard spread-plating (detection limit: 10 CFU/g) both immediately after treatment and following short-term (10-day) and long-term (28-day) vacuum-sealed refrigerated (4°C) storage. To better understand sample variability and its relation to microbial activity, water activity (A_w), pH, and moisture content (%) were also evaluated. Repeated measures ANOVA was conducted to compare the effects of thermal treatment types (hot vs. cold) and storage duration on microbial loads. Total mesophilic aerobic, anaerobic, psychrotrophic, and yeast and mold populations were assessed.

Results: Across all experimental groups, mesophilic aerobic counts ranged from 4.59 ± 0.35 log CFU/g (hot treatments) to 8.15 ± 0.43 log CFU/g (cold treatments). No statistically significant increase in microbial load was observed between day 0 and day 10 ($p > 0.05$) for any treatment. However, a significant increase was observed between day 0 and day 28 ($p = 0.006$) across all treatments. Microbial loads increased significantly ($p < 0.05$) during storage in heated treatments, while no significant growth was observed over time in cold-treated samples ($p > 0.05$). Anaerobic and psychrotrophic bacteria followed similar trends, maintaining higher levels throughout, whereas yeast and mold counts remained consistently low and exhibited no notable trends.

Significance: These findings underscore the critical role of effective heat processing in substantially reducing microbial contamination in yellow mealworm larvae, even under vacuum-sealed refrigeration, thus supporting safer integration into the food chain.

Award Application: Developing Scientist Award

P3-37: DEVELOPMENT OF HACCP PLAN FOR MOLO MILK COOLING PLANT

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: The development of a Hazard Analysis and Critical Control Points (HACCP) plan for the NKCC Molo cooling plant is essential to ensure the safety and quality of dairy products. This study employs a comprehensive research design integrating qualitative and quantitative methods to identify and address potential hazards in milk processing.

Purpose: The aim of this study was to develop, validate, and recommend a HACCP plan tailored for the NKCC Molo cooling plant to improve hazard control, ensure regulatory compliance, and enhance milk safety and quality.

Methods: Preliminary assessments, on-site observations, interviews, and hazard analysis were conducted to identify potential hazards at each processing stage. Data collection included reviewing quality assurance records, conducting inspections and testing, performing literature reviews, and interviewing experts. The HACCP plan was validated through peer review to ensure completeness and feasibility.

Results: The research highlighted the prevalence of hazards such as aflatoxins and antibiotic residues, emphasizing the need for tailored control measures. The proposed plan prioritizes enhanced monitoring and stakeholder collaboration. The study underscores the importance of regulatory compliance, commitment to consumer safety, and continuous improvement. Communication with stakeholders and research investment will support future milk safety initiatives. Adherence to safety standards and a culture of improvement remain essential to maintaining trust and health safeguards.

Significance: The HACCP plan plays a key role in mitigating risks and enhancing the credibility of the NKCC Molo cooling plant. Recommendations include regular review and revision of the plan, stakeholder collaboration, and technological investment to further reduce risks. These efforts will help ensure the plant continues to deliver safe, high-quality processed milk that meets consumer expectations and regulatory requirements.

Award Application: Undergraduate Student Award

P3-38: INACTIVATION KINETICS OF ESCHERICHIA COLI AND LISTERIA MONOCYTOGENES IN EDIBLE INSECTS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Edible insects provide alternative and highly nutritive proteins and lipids, but concerns about food safety, particularly pathogens like *Escherichia coli* and *Listeria monocytogenes*, hinder their acceptance.

Purpose: This study evaluated the effectiveness of thermal processing in inactivating these pathogens and their non-pathogenic surrogates in fresh and dehydrated edible insects.

Methods: Fresh mealworm larvae and crickets were inoculated with target bacteria and incubated to >9 log CFU/g as part of a challenge study. Reduced water activity (aw) samples were prepared using oven and freeze-drying dehydration methods. The inoculated samples in thin pouches (<1 mm thickness) were heated in a circulated water bath at 60–90°C for 1–3 minutes. Bacterial survivors were enumerated using serial 10-fold dilutions (detection limit of 10 CFU/g) and standard spread-plating on selective and non-selective media. Thermal inactivation kinetics, including D and z-values, were determined. The effect of aw on bacterial inactivation was also quantified. Experiments were statistically designed and evaluated.

Results: Thermal inactivation studies using a common pasteurization temperature of 70°C revealed similar ($p \geq 0.05$) heat resistance for *E. coli* O157:H7 (D-value 34.8 ± 5.6 s, z-value $25.2 \pm 3.7^\circ\text{C}$) and *L. monocytogenes* (D-value 32.6 ± 4.3 s, z-value $24.9 \pm 5.2^\circ\text{C}$) in fresh insects. Higher temperatures showed shorter ($p \leq 0.05$) times to inactivate target bacteria. However, at 60°C inactivation was relatively slow and may not be practical for commercial applications. Non-pathogenic surrogates *E. coli* JM109 (D-value 40.5 ± 5.5 s) and *Listeria innocua* (D-value 31.5 ± 0.9 s, z-value $26.1 \pm 6.6^\circ\text{C}$) exhibited similar ($p \geq 0.05$) heat resistance compared to their pathogenic counterparts. Therefore, *L. innocua* and *E. coli* JM109 may be used as surrogates for *L. monocytogenes* and *E. coli* O157:H7,

respectively, in thermal inactivation studies of edible insects; however, the higher resistance of *E. coli* JM109 may not reflect real-life conditions.

Significance: This research provides essential data to develop safe and effective thermal processing for the edible insect industry, supporting the creation of sustainable and safe food systems.

Award Application: Developing Scientist Award

P3-39: SALMONELLA BIOMAPPING AND QUANTIFICATION OF SOW AND BOAR LYMPH NODES AND TONSILS TO ASSESS RISK IN PORK PRODUCT ACROSS THE UNITED STATES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Pork biomapping studies, conducted at Texas Tech University, have previously indicated that lymph nodes in market hogs can serve as a reservoir for *Salmonella* in meat processing operations. However, *Salmonella* housed in the lymph nodes and tonsils of sows and boars have not been explored and can lead to potential contamination of the carcass and further processed pork products.

Purpose: The objective of this study was to determine the prevalence and quantification of *Salmonella* in the tonsils and various lymph nodes from sows and boars harvested throughout the United States in order to determine the risk of contamination that these structures hold and their potential impact on public health.

Methods: Sows and boars were harvested at 3 commercial pork processing facilities throughout the United States during a year long period. From each carcass, the subiliac, superficial inguinal, axillary, pre-scapular, mesenteric, and tracheobronchial lymph nodes (LN) were collected, as well as the corresponding tonsil (n=1,819). Each structure was trimmed of attached fat, tissue, and fascia before being weighed, boiled, and macerated. The samples were then processed for *Salmonella* detection and quantification using the Hygiena's BAX® System SalQuant™.

Results: The overall *Salmonella* prevalence for all collected samples was 10.55% (192/1819 samples). Sample type specific, the *Salmonella* prevalence was 25.49% in tonsils, 29.38% in mesenteric, 3.43% in axillaries, 0.76% in subiliac, 3.84% in both pre-scapular and superficial inguinal LN, and 7.63% in tracheobronchial LNs.

Significance: The results of this study indicate that the highest *Salmonella* prevalence was observed in mesenteric LN and tonsils. While neither of these

structures are directly incorporated into further processed products, they still pose a public health risk of potential cross-contamination during processing. The remaining variety of LN that have displayed a lower prevalence of *Salmonella* may be incorporated in ground product, therefore, further research is needed to determine how to mitigate the risk of contamination associated with lymph nodes and tonsils in sows and boars further processed pork products.

Award Application: Developing Scientist Award

P3-40: EFFECTIVENESS OF DIVERGICIN-PRODUCING CARNOBACTERIUM DIVERGENS M35 IN CONTROLLING LISTERIA MONOCYTOGENES USING A LIQUID ATOMIZATION DEVICE

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Category: Food Safety Systems

Abstract:

Introduction: *Listeria monocytogenes* is a serious food safety concern that can be specifically controlled with the bacteriocin divergicin produced by the probiotic *Carnobacterium divergens* M35 (CdM35).

Purpose: To demonstrate the listericidal action of CdM35 on smoked salmon slices using an industrial atomization device.

Methods: A lyophilized CdM35 powder was dispersed in water at 1×10^6 CFU/mL and kept at $<10^\circ\text{C}$. The dispersion was sprayed using an atomizer (40 psi; 0.3 gallon per minute) onto 25-gram slices of smoked salmon to obtain 1×10^6 CFU per gram or cm^2 . Then, 1×10^3 CFU of *Listeria monocytogenes* was evenly inoculated, or not, on corresponding slices. Controls consisted of slices non-inoculated, inoculated with only *Listeria monocytogenes* (unchallenged), or inoculated with only CdM35. Challenged conditions consisted of slices inoculated with both *Listeria monocytogenes* and CdM35. All slices were incubated at 4°C and bacterial counts were performed at baseline and at Day 4, 7, 11, 14, and 21.

Results: On unchallenged slices, *Listeria monocytogenes* grew by 4.9 log from 1×10^3 CFU/g (baseline count) to 7.6×10^7 CFU/g (Day 21). However, when challenged with CdM35, the growth of *Listeria monocytogenes* was reduced from the baseline count to 3.5×10^1 CFU/g at Day 21. This corresponded to significant ($p < 0.05$) 2.5 log and 6.3 log reductions at Day 7 and Day 21, respectively, when compared to unchallenged slices. CdM35 did not affect the growth of the endogenous food microbiota. Furthermore, CdM35 did not impact the organoleptic properties of the smoked salmon.

Significance: These results highlight the potential of using CdM35 as a listericidal bio-ingredient using an industrial liquid atomizing system. The use of a probiotic-

derived selective bacteriocin maintains the integrity of the food matrix. This is in line with consumer demand for natural food preservation and could enhance food safety standards while maintaining product quality.

P3-41: DEVELOPMENT OF A NOVEL PHAGE AMPLIFICATION-QPCR ASSAY FOR DETECTION OF *E. COLI* O157:H7

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Since Shiga toxin-producing *E. coli* is a dangerous foodborne pathogen that can cause severe illness and death, rapid and sensitive detection tools for this pathogen are crucial. Based on the high specificity and natural affinity of phages, phage-based approaches have been listed as potential rapid detection tools for foodborne pathogens without the need for additional equipment or reagents.

Purpose: To develop a sensitive phage amplification assay (PAA) for rapid detection of viable *E. coli* O157:H7 in food by using a novel Escherichia phage, CAM-21.

Methods: In amplification assays, CAM-21 was mixed with *E. coli* O157:H7, followed by the addition of ferrous sulfate (FeSO₄) as a virucide to inactivate any remaining phage in the sample. A neutralizer (trisodium citrate) was then added to neutralize the effect of the virucide and protect the progeny phages. After neutralization, progeny phages produced by PAA were detected by qPCR and the double agar overlay assay. A simple thermal lysis DNA extraction protocol was selected for progeny phage DNA extraction. CAM-21 primers were designed using Primer3. qPCR experiments were performed in 20 µL reactions under the following three-step cycling procedure: 95 °C for 5 min, (95 °C for 10 s, 60 °C for 30 s) × 40 cycles, and 60 °C for 15 s.

Results: Plate counting results indicated that 200 mM FeSO₄ effectively eliminated 5 log PFU/mL of free phage within 5 minutes. Additionally, 200 mM trisodium citrate neutralized the virucidal effect within 5 seconds. The real-time PCR method successfully detected phages using specific primers.

Significance: CAM-21 PAA demonstrates a rapid and reliable method for detecting *E. coli* O157:H7 using phage-based techniques, which can significantly enhance food safety monitoring and contamination control.

Award Application: Developing Scientist Award

P3-42: APPLICATION OF AN ENHANCED FIBER OPTICS SERS SENSOR FOR RAPID DETECTION OF SALMONELLA ENTERITIDIS IN TURKEY RINSE

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Detecting *Salmonella* in poultry is crucial, as it causes over 1 million infections annually in the U.S., with poultry being a major source. Rapid, sensitive detection and differentiation of live and dead *Salmonella* are vital for food safety.

Purpose: This study aims to validate an advanced fiber optics-based Surface Enhanced Raman Spectroscopy (SERS) sensor for sensitive detection and differentiation between live and dead *Salmonella* Enteritidis (SE) in turkey rinse.

Methods: Sensor sensitivity was determined with SE concentrations in turkey rinse of 1000, 100, 10, and 1 CFU/mL. Colony counts were determined using conventional plating. SE, at 1000 CFU/mL, was treated with heat at 95°C for 30 minutes, or 10 ppm, 50 ppm, and 100 ppm peracetic acid. Samples were analyzed with the SERS sensor, and spectra were recorded using OceanView 2.0.14-EZ software with a 900 ms integration time and 20 scans per sample. Untreated control samples were included.

Results: The SERS sensor successfully detected as low as 1 CFU/mL of SE in spiked turkey rinsate, producing a SERS spectrum with characteristic peaks. The reduction in peak intensity with lower SE concentrations correlated with colony counts from conventional plating methods. The sensor was also able to differentiate between live and dead SE cells treated with heat and peracetic acid. The initial inoculum produced distinct SERS peaks, which showed a complete peak disappearance after heat treatment and a gradual reduction in peak intensities with increasing peracetic acid concentration.

Significance: The results demonstrate that the enhanced fiber optics-based SERS sensor offers a rapid and sensitive method for detecting as low as 1 CFU/mL of SE in turkey rinse and distinguishing live and dead SE treated with heat and peracetic acid. It also offers a simpler alternative to traditional techniques, with potential for broader food safety applications, including serovar and species differentiation.

Award Application: Developing Scientist Award

P3-43: RAPID METHOD EVALUATION FOR RTE AND RTC QUICK-FROZEN FOODS IN 5-YEAR STUDY

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Traditional Chinese foods, such as steamed (stuffed) buns, fried bread stick and rice dumplings, have been involved in the cold chain and food industry. Although the products are stored and distributed under -18 °C, the GB 19295 (Quick-frozen wheat and Rice Products and Prepared foods) have specific acceptance criteria of aerobic plate count and coliforms. For product quality control high volume of testing is often necessary and thus, the application of rapid

Methods based on ready-to-use dehydrated media reduces the time and improves the efficiency of laboratory.

Purpose: To summarize the performance of alternative ready-to-use dehydrated media with the GB standards for the raw materials and final quick-frozen rice and wheat foods from 2018 to 2023. Method: All the samples were randomly collected from quick-frozen food product line from 2018 to 2023. Neogen® Petrifilm® Rapid Aerobic Count Plates and GB 4789.2 were performed to enumerate 17 raw materials (112 samples) and 6 products (423 samples). Both Petrifilm Coliform Count Plates and GB 4789.3 were used to test 19 raw materials (111 samples) and 4 products (51 samples).

Results: For aerobic plate count, 74.95% of the samples presented an acceptable log₁₀ difference (± 0.5) between alternative method and GB method. 15.32% of the samples had larger counts using alternative method while 9.72% of the samples had larger counts following GB method. For positive samples the comparison between methods was not significantly different ($P=0.417$). For coliform test, the log₁₀ differences for 92.59% samples were between ± 0.5 . only 11 raw materials and one product got larger than ± 0.5 log₁₀ difference. The P-value of positive samples was 0.826.

Significance: There is no significant difference between Petrifilm plates and the corresponding GB method. These alternative

Methods are rapid, convenient options for microbial testing within the quick-frozen cereal products industry.

P3-44: CULTURALLY TAILORED FOOD SAFETY CULTURE ASSESSMENT TOOL: A HALAL-FOCUSED APPROACH FOR MALAYSIA

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Food safety culture (FSC) has gained global attention and is being widely adopted into regulatory frameworks. However, the implementation of FSC remains ambiguous in Southeast Asia, particularly in Malaysia, where reliance on Codex Standards is prevalent. As a global leader in the halal food manufacturing industry, Malaysia's unique cultural and regulatory environment necessitate localized adaptations of existing FSC assessment tools to ensure their suitability for use in Malaysia.

Purpose: This paper presents the development of an FSC assessment tool tailored for the Malaysian food manufacturing industries.

Methods: A qualitative approach was adopted, involving a comprehensive literature review to identify the factors that shape FSC. Findings from the literature review were validated through group interviews with key food safety stakeholders, including regulatory authorities, quality managers, Halal auditors, and experts.

Results: Malaysia's robust Halal certification system and commitment to the Halal economy underscore the importance of integrating Halal practices into FSC. Top management plays a critical role in fostering a positive FSC by influencing internal factors such as leadership, communication, education programs, resource allocation, punish-reward systems, organizational norms, and compliance. External factors like national values, religion, regulatory requirements, and customer expectations are also pivotal. Halal compliance serves as a key driver in shaping Malaysia's food production practices. Malaysia's high-power distance and collectivist culture emphasize the need for an FSC tool adapted for this unique socio-cultural context. Additionally, Malaysia's diverse workforce in the food manufacturing industry, predominantly comprising foreign workers, necessitates the consideration of their cultural and national values in the implementation of FSC initiatives.

Significance: This study aims to develop an FSC assessment tool integrating Halal concepts that align with local values to promote a positive FSC in Malaysia's food

manufacturing industry. It highlights how national values and religious practices influence workplace dynamics, providing a foundation to develop socioculturally-fit strategies for Malaysia in strengthening FSC.

Award Application: Developing Scientist Award

P3-45: PROVIDING TECHNICAL ASSISTANCE TO THE FOOD INDUSTRY IN LOW-INCOME COUNTRIES HAS A POSITIVE IMPACT ON FOOD SAFETY REGULATION COMPLIANCE

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Foreign food facilities are required to meet FDA regulatory guidelines to export their goods to the United States. Smaller companies in low-income countries face challenges related to a lack of training and knowledge about U.S. regulations. USDA FAS sponsored a capacity-building proGram to promote international trade while contributing to guaranteeing food safety for American consumers.

Purpose: Evaluate technical assistance's impact on improving food safety performance and facilitating international food trade.

Methods: Three foreign processing facilities from Latin America were included in this proGram. in-person and virtual food safety technical assistance was provided. Two indicators were used: 1)Microbial environmental samples and 2)Food safety compliance (%) using a standardized performance checklist created according to the requirements of the FSMA-Preventive Controls for Human Food (PCHF) rule. Microbial indicators included Total Viable Count (TVC) and Enterobacteriaceae (EB); surfaces were swabbed using Hygiena's MicroSnap® Total and MicroSnap® EB swabs on 30 samples. Wilcoxon signed-rank test, paired t-test, and negative binomial regression models were performed with R-studio software.

Results: The checklist was applied during two in-person visits to evaluate regulatory compliance after technical assistance. Facilities 1 and 2 improved overall, from 43% to 66% and 72% to 79%, respectively. Facility 3 remained at 50% during both visits. No significant differences between companies were observed ($P>0.05$). Scores were dependent on visit and regulatory requirements ($p<0.05$) based on PCHF rule. Microbial indicators showed an overall significant reduction ($p<0.05$) of 78.15% for TVC after the second intervention, and it was dependent on the hygienic zone and company ($p<0.05$). only facility 2 significantly reduced EB counts ($p<0.05$).

Significance: Providing training and technical assistance to small industries

positively impacts food safety outcomes and performance. increasing the level of regulatory compliance by foreign food facilities will contribute to public health, representing a positive economic impact and maintaining a healthy import/export relationship.

P3-46: TARGETING “CONSISTENCY” IN A UK-BASED LOW-RISK FOOD AND DRINK MANUFACTURER TO STRENGTHEN FOOD SAFETY AND QUALITY CULTURE

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction:Food safety culture(FSC) encompasses business attitudes/behaviours relating to food safety/quality(FSQ)and research has shown it is comprised of multiple elements/dimensions.Weak FSC has been associated with being a root cause of foodborne disease incidents in large-scale food/drink manufacturing/processing(FDMP)businesses.Targeted interventions may be more effective in improving cognitive influences/behaviours incrementally,thus strengthening FSC.

Purpose:Targeted improvement of specific FSC dimension“consistency”,in a low-risk FDMP business,through the implementation/evaluation of a targeted FSQ improvement intervention.

Methods:Analysis FDMP business FSQ metrics(stock-on-hand/non-conformances)and qualitative(n=31)/quantitative(n=103)operative/management FSC insights informed identification of the FSC dimension“consistency”to be a focus for targeted improvement during 2024.Behavioural theories(COM-B/Nudge)underpinned intervention development;evaluation of intervention effectiveness included business FSQ metrics and operative/management quantitative 5-point Likert-scale responses(n=117)and qualitative(n=16)data relating to targeted behaviours/related cognitive influences.

Results:Historical FSQ metrics(2022-2023)indicated increased(+37.5%)non-conformances concerning labelling inconsistency.Identified key behaviours included operational consultation with digital specifications and precision when changing labelling programmes during manufacturing.The key target audience included operatives, reinforced by management support mechanisms.Bespoke,data-driven interventions(date-coding equipment nudges/coaching conversations/communication devices/training briefs)were

launched in February 2024. Evaluation of effectiveness (>3 months) evidenced an 18.1% reduction of labelling precision non-conformances, thus indicating behavioural improvement. Further improvements of cognitive influences were determined, for example, operative attitudes toward consistency with FSQ standards (3.83 ± 0.41), management practices (3.83 ± 0.41) and departmental consistency (4.00 ± 0.63).

Significance: Company bespoke, data-driven development of interventions that included management/operative components facilitated a focus on the FSC dimension 'consistency' associated with digital labelling. Overall, thus contributing to cognitive and behavioural improvement within the business and strengthened FSC.

P3-47: COMPARISON OF AEROBIC COUNT (AC) AND ENTEROBACTERIACEAE (EB) AS INDICATORS FOR MICROBIAL INTERVENTION VALIDATIONS IN BEEF TRIMMINGS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Microbial indicators such as Aerobic Plate Count (AC) and Enterobacteriaceae (EB) are vital for validating food safety interventions in meat processing. Recent regulatory changes provide flexibility in indicator selection, yet limited data exist comparing these measures.

Purpose: This study evaluated AC and EB counts on Chuck and Heel beef trimmings to assess intervention effectiveness and guide indicator selection.

Methodology: A total of 160 samples were analyzed, with 20 samples collected before and 20 after intervention for each trim type (Chuck and Heel) annually over two years. Trimmings were sampled from 100 cm² areas using 25 mL BPW pre-hydrated sponge swabs. Microbial counts were determined using the TEMPO® system. Interventions included Citrilow immersion and BioSafe Ozone treatment. Statistical analyses (t-tests, $\alpha = 0.05$) evaluated reductions, and box plots visualized microbial load distributions. A t-test was conducted to assess reductions in microbial loads, with significance set at $\alpha = 0.05$. Box plots were used to visualize microbial load distributions. Statistical analyses were performed using R (Version 4.0.4).

Results: Significant reductions ($p < 0.05$) were observed for both AC and EB. In 2023, Chuck trimmings had initial counts of 2.62 log CFU/cm² (AC) and 1.05 log CFU/cm² (EB), with reductions of 0.95 and 0.76 log CFU/cm², respectively. Heel trimmings showed reductions of 0.88 (AC) and 0.65 log CFU/cm² (EB). In 2024, initial counts increased, with Chuck trimmings reduced by 1.12 (AC) and 0.83 log CFU/cm² (EB) and Heel by 0.93 (AC) and 0.70 log CFU/cm² (EB).

Significance: Both AC and EB are effective validation tools. AC reflects overall hygiene and microbial load, aiding quality control, while EB indicates potential fecal contamination and pathogen presence. The study demonstrated consistent

and significant reductions for both indicators, allowing facilities to select the most suitable option for cost-effective, robust validation.

P3-48: NONDESTRUCTIVE FOODBORNE PATHOGEN DETECTION USING A COLORIMETRIC SENSOR ENABLED BY MACHINE LEARNING AND NON-TOXIC DYES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Current foodborne pathogen detection methods are unsuitable for use in the food supply chain post-packaging, but a non-destructive sensor can address this gap in food safety infrastructure by performing in-packaging continuous testing for foodborne pathogens.

Purpose: This project aimed to develop an AI-enabled sensor for non-destructive sensing of foodborne pathogens using non-toxic dyes' chromogenic reactivity to volatile organic compounds (VOCs).

Methods: Nineteen dyes were tested for their color changes to standard VOCs and three pathogens (*Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella enterica*) to select the dyes, pathogens, and food model for database construction. Food samples were weighed in Petri plates, inoculated (1 log CFU/g), and then a paper with the dyes was added to the Petri plate lid. The plates were incubated at 4°C, 12°C, 22°C, and 37°C for 0h, 4h, 24h, 48h. There were 60 replicates for each treatment and control group. The dye strips were scanned, and the red-green-blue (RGB) data was extracted from the scans. The RGB data and ground truth (bacteria identity, incubation temperature, and incubation time) were used to build a neural network in Python, with the color data being used to predict the ground truth with 5-fold validation.

Results: Seven dyes changed color in response to the VOCs and bacteria. The color changes varied with pathogen treatment and incubation conditions. The color changes were most apparent with longer incubation time and greater incubation temperature, but the color change patterns were best interpreted through neural networks.

Significance: This sensor's novelty lies in its non-destructive and non-toxic traits, which make it especially fit for smart packaging that can fill the current gaps in foodborne pathogen sensing post-processing. There is great potential for this sensor to be expanded to detect more pathogens in a wider range of food products, reducing foodborne illnesses and recalls.

P3-49: MAGNETIC CELLULOSE NANOCOMPOSITES COATED BY BENZALKONIUM CHLORIDE: ANTIBACTERIAL ACTIVITY AGAINST LISTERIA MONOCYTOGENES

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Magnetic nanocomposites have gained much interest for food safety and antibacterial treatments against hazardous pathogens. *Listeria monocytogenes*, a significant foodborne pathogen, poses severe health risks and requires innovative control strategies.

Purpose: This study aimed to synthesize and evaluate a magnetic cellulose nanocrystal composite functionalized with benzalkonium chloride (BC@CNC-Fe₃O₄) for its antimicrobial activity against *L. monocytogenes*.

Methods: Superparamagnetic iron oxide nanoparticles were synthesized on the surface of cellulose nanocrystals (CNC-Fe₃O₄) using a co-precipitation technique. The composite was further functionalized with benzalkonium chloride (BC), a broad-spectrum antimicrobial. Structural analysis was performed using XRD, TEM, SEM, and VSM to confirm magnetite formation, morphology, and magnetic behavior. Antibacterial activity against *L. monocytogenes* was evaluated using the standard disk diffusion method.

Results: XRD confirmed the crystalline magnetite (Fe₃O₄) phase. TEM and SEM revealed spherical-shaped nanoparticles (~20 nm) and rod-shaped CNCs. VSM analysis showed superparamagnetic behavior with high saturation magnetization. Disk diffusion assays demonstrated that BC@CNC-Fe₃O₄ exhibited notable inhibition against *L. monocytogenes*, with significantly improved antimicrobial activity compared to controls.

Significance: The synergistic interaction between magnetic nanoparticles and benzalkonium chloride enhances antimicrobial efficacy while enabling magnetic recovery and reuse. These findings support the potential of BC@CNC-Fe₃O₄ nanocomposites in food safety and biomedical applications addressing antibiotic resistance and pathogen control.

Award Application: Developing Scientist Award

P3-50: DEVELOPMENT AND EVALUATION OF AN OPEN-TUNNEL 222 NM FAR-UVC LIGHT DECONTAMINATION SYSTEM FOR FOOD-CONTACT SURFACES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Far-UVC light (200–230 nm) has been demonstrated to effectively inactivate microorganisms. More importantly, recent studies have shown that unlike conventional UVC light (254 nm), which can cause significant skin and eye damage, 222 nm far-UVC light does no such harm under comparable treatment conditions. This highlights far-UVC's potential for use in continuous decontamination processes, even in the presence of people.

Purpose: This study reports on the development of a far-UVC decontamination system with a UV-transmitting tunnel structure and evaluates its decontamination efficacy against bacteria on stainless-steel coupons, one of the most common food-contact surfaces.

Methods: The prototype was constructed mainly of UV-permeable fused-silica glass, with microplasma-based flat lamps emitting 222 nm far-UVC light on both their sides. Its decontamination efficacy was tested against two lethal foodborne pathogens, *Listeria monocytogenes* (Gram-positive) and *Escherichia coli* O157:H7 (Gram-negative), inoculated on stainless-steel surfaces both inside and outside the tunnel.

Results: After 2 seconds of treatment, significant reductions ($p < 0.05$) in *E. coli* and *L. monocytogenes* populations were observed, and longer treatment times led to greater log reductions. An approximately 5-log (99.999%) reduction was achieved after 10 seconds of treatment inside the tunnel. Outside the tunnel, a reduction of about 4 log was observed after 80 seconds. This difference is likely due to differences in lamp proximity and/or reduction of irradiation through the

UV-permeable glass.

Significance: The proposed far-UVC-based tunnel system, which can provide continuous decontamination functions both within the tunnel and outside it, has considerable potential for improving food safety. For example, it could be integrated with conveyor systems to inactivate pathogens on belts or product surfaces while simultaneously minimizing contamination risks in surrounding spaces, including potentially by decontaminating the surrounding air.

P3-51: FOOD SAFETY DATA IN AFRICA: AN OVERVIEW

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Food safety data serves as a significant resource for identifying food contamination and informing policies aimed at mitigating associated risks. While the collection and impact of food safety data have been significantly explored in Europe and other Western countries, similar data remains scarce across Africa.

Purpose: This study presents a comprehensive review of developments in food safety data collection within the African context.

Methods: Using the PRISMA approach for systematic literature reviews, we extensively screened 12,743 records from three academic libraries and identified 16 relevant articles for in-depth analysis.

Results: Our findings reveal that although many African countries have enacted food safety laws and policies, the collection and accessibility of food safety data remain limited. Additionally, the review highlights that existing food safety data in Africa is largely confined to academic publications, making it less accessible to policymakers and the public. Notwithstanding these challenges, the widespread use of mobile phones, the high penetration of mass media, the internet, and social media across the continent present unexploited opportunities for real-time food safety data collection and early warning systems.

Significance: Leveraging these technologies could enable African governments to improve food safety data collection and management, thus reducing foodborne risks and improving public health outcomes, as observed in other regions of the world.

P3-52: ANALYSIS OF THE INFLUENCE OF SOIL MOISTURE ON COLIFORM COUNTS IN WILD BLUEBERRY FARM SOILS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Soil microbiological analysis is essential for understanding how environmental factors influence the presence and proliferation of microorganisms, which directly impact food safety and public health.

Purpose: This study aimed to investigate the relationship between soil moisture, pH, mineral content, organic matter, and coliform counts in soils from wild blueberry farms. The hypothesis driving this research was that increased soil moisture would positively influence coliform counts, including *E. coli*, with potential effects on overall microbiological load.

Methods: Thirty-four soil samples were collected from six wild blueberry farms across Maine during the 2024 harvest season over a five-week period. Coliform enumeration was performed using specific microbial culture assays and moisture by oven-dry method. Soil testing (mineral analysis, pH, organic matter) was performed at the Maine Soil Testing Services. Linear regression modeling (log-transformed response variable), ANOVA and Pearson correlation were used to evaluate the relationships between soil attributes and coliform counts ($p < 0.05$).

Results: Soil pH (3.2- 5.1) and moisture (18.6- 57.8%) were key factors influencing coliform counts (mean 2.95 logCFU/g). Higher moisture ($r = 0.421$) and lower pH ($r = -0.399$) increased coliform levels, and organic matter was also strongly correlated ($r = 0.72$). ANOVA confirmed pH and moisture as significant predictors of coliform levels. Minerals and other factors analyzed did not show significant correlations. The regression model explained 85.86% of the variance (adjusted $R^2 = 0.3873$). Soil type, which was not assessed in this study, likely plays a significant role. Irrigation practices and crop management also require further investigation.

Significance: These results emphasize the importance of managing soil moisture and pH in blueberry farming to reduce microbial contamination. Controlling these levels could be a key strategy to improve food safety and crop quality.

P3-53: THERMAL INACTIVATION KINETICS (D- AND Z-VALUES) OF SALMONELLA IN HIGH MILK PROTEIN COOKIES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Salmonella* can survive in low water-activity foods and can be shielded from heat treatments by added fats and proteins. Therefore, a study was conducted to evaluate the impact of increased protein content on the thermal inactivation parameters of 3 serovar *Salmonella* cocktail in two cookie dough recipes.

Purpose: To determine the thermal inactivation parameters (D- and z-values) of *Salmonella* in high milk protein cookies.

Methods: Two laboratory-simulated high milk protein cookie recipes were prepared to evaluate the thermal inactivation parameters of *Salmonella* in high milk protein cookie doughs. Three batches of unbleached bread flour were mist inoculated with 3-serovar *Salmonella* cocktail to prepare MPC80, WPC80 and control cookie doughs. Bread flour was substituted with milk protein powders in the control samples. The inoculated flour was dried at 37°C for ~5 hours to restore pre-inoculation water activity levels. The dried flour was then used alongside unsalted butter, brown sugar, skim milk powder, pasteurized eggs, vanilla extract, salt, sodium bicarbonate, water, and MPC80/WPC80 to prepare the respective cookie doughs. Three temperatures (55, 58, and 61 °C) were used to determine the D- and z-values for *Salmonella*. Five TDT disks were employed for each temperature point, with one TDT disk connected to a T-type thermocouple to monitor the dough temperature.

Results: The D-values of *Salmonella* in MPC80, WPC80, and control recipes at 55, 58, 61 °C were 54.76 ± 10.65 , 19.67 ± 2.63 , 9.95 ± 1.02 min; 49.25 ± 3.03 , 26.06 ± 1.22 , 10.12 ± 1.13 min; and 41.17 ± 3.12 , 14.2 ± 0.25 , 5.6 ± 1.15 min, respectively. The z-values of *Salmonella* in MPC80, WPC80, and control recipes at 55, 58, and 61 °C were 8.8 ± 1.49 , 7.9 ± 1.14 , and 6.9 ± 0.44 °C, respectively.

Significance: The D- and z-values determined in this study will help in understanding *Salmonella*'s increased heat resistance characteristics with an increase in protein content in cookies and aid in optimizing the baking process

ensuring safety.

Award Application: Developing Scientist Award

P3-54: UTILIZING MODERATE ELECTRIC FIELD TREATMENT TO EXTEND THE ANTIMICROBIAL SPECTRUM OF LYSOZYME AGAINST GRAM NEGATIVE BACTERIA

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Category: Food Safety Systems

Abstract:

Introduction: Can the antimicrobial spectrum of innate food antimicrobials like lysozyme be extended to target Gram-negative bacteria using a non-chemical, non-additive method at mild temperatures?

Purpose: We recently demonstrated that a combination of mechanical shear stress (SS) and moderate electric field (MEF) applied to lysozyme-rich liquid whole eggs (LWE) inoculated with *Salmonella enterica* serovar Enteritidis (SE) effectively reduced bacterial counts by 3–5.1 log₁₀ cycles within 15 minutes at a mild temperature range of 46–52°C. We hypothesize that the MEF treatment induces electro-permeabilization of the Gram-negative bacterial outer membrane, allowing lysozyme to penetrate the periplasmic space and hydrolyze the peptidoglycan layer, leading to cell death.

Methods: To test this hypothesis, we performed SS+MEF treatment on phosphate-buffered saline (PBS) inoculated with SE, with and without the addition of hen egg white lysozyme.

Results: Our findings showed that lysozyme alone exhibited no antimicrobial activity against SE in PBS. However, the addition of lysozyme prior to MEF treatment at 37°C significantly enhanced the antimicrobial effect, achieving an additional reduction of over 5 log cycles, resulting in a total reduction of 7.5 log cycles at 2 minutes of treatment time ($P < 0.05$) compared to MEF treatment alone.

Significance: This novel approach presents a promising, non-chemical solution to enhance the safety of heat-sensitive food products such as eggs and milk, offering significant benefits to the food industry.

P3-55: CONVERGENCE SCIENCE APPROACH TO SOLVE COMPLEX FOOD SAFETY CHALLENGES IN THE COMING DECADES: A SALMONELLA-POULTRY FOOD SAFETY PROJECT CASE STUDY

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: The Convergence Science approach fuses knowledge, methods, and expertise from multiple disciplines and practices to form a unified framework for solving complex problems. It fosters deep collaboration while creating new paradigms that transcend traditional boundaries.

Purpose: We present a framework developed by our Sensor Enabled Decision Support System (SENS-D) team, demonstrating the significance of convergence science in mitigating *Salmonella* food safety risks.

Methods: Our convergent team comprises eighteen researchers from five U.S. universities and multiple disciplines. The team received National Science Foundation Convergence Accelerator funding to develop rapid *Salmonella* detection technologies and a decision support system (DSS) for safe and equitable food distribution. Using Team Science and Use-Inspired Research, SENS-D interviewed stakeholders from the poultry industry, supply chain, Extension, food banks, and policy makers to understand challenges in pathogen detection and tracking. Interviews informed the development of bioelectrical impedance and Raman spectroscopy-based sensors for pathogen detection and DSS. The DSS includes: (1) Descriptive: data visualization and maps (180 GIS data layers); (2) Predictive: pathogen growth and shelf-life modeling during the supply chain; (3) Prescriptive: optimizing sensor placement, intelligent distribution, and workforce planning to ensure food safety, cost efficiency, and reduced disparities and waste. Broader impacts are addressed through extension, outreach, and engagement with end-users and stakeholders.

Results: *Salmonella* could be detected within 10–70 minutes without rinsate enrichment. The DSS predicted shelf-life impacts caused by supply chain temperature fluctuations.

Significance: Convergence science is an innovative, transformational approach to solve complex food safety problems such as *Salmonella* in poultry.

P3-56: MICROBIAL DYNAMICS IN EARLY-STAGE FOOD WASTE COMPOSTING SUPPORT FOOD SAFETY

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Food waste composting is a sustainable method for recycling organic materials; however, its effects on mitigating risks for food safety and antimicrobial resistance (AMR) are not fully understood.

Purpose: This study aimed to explore changes in food safety indicators and AMR populations during food waste composting and to assess how environmental condition flux correlates with potential risk reduction.

Methods: Three compost piles at a local urban farm were prepared with a 1:4 ratio of food waste from local groceries (e.g., lettuce, bread, citrus) to other organic inputs and monitored over 42 days. Triplicate samples were collected on days 0 and 3, then at weekly intervals at pile depths of 0 cm (surface), 30 cm, and 60 cm. Temperature and pH were measured at each depth, and concentrations of total bacteria, tetracycline-resistant bacteria, *Escherichia coli*, and coliforms were enumerated. *E. coli* isolate diversity was further assessed using whole genome sequencing to track strain-level changes across piles and over time.

Results: Temperature and pH increased during the first week and stabilized, reflecting biological processes within the piles. Total bacterial concentrations fluctuated around 6-8 log CFU per Gram over the composting process. In contrast, tetracycline-resistant bacteria and *E. coli* began at about 3-5 log CFU per Gram and declined throughout the piles over time by approximately 2-3 log ($p < 0.05$). However, *E. coli* showed potential regrowth as temperatures decreased. Whole genome sequencing indicated that strain-level diversity of *E. coli* varied by pile, depth, and time.

Significance: This study demonstrates that food waste composting can lower food safety risks by reducing AMR bacteria and *E. coli* populations, though

recontamination risks may emerge after piles cool down. Future research will explore microbiome dynamics and multi-drug resistance patterns with the goal to improve compost quality and promote safe use of sustainable soil amendments.

P3-57: VALIDATION OF OATMEAL CHOCOLATE CHIP GRANOLA BAR MANUFACTURING PROCESS TO CONTROL SALMONELLA CONTAMINATION

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Outbreaks linked to oat-based granola bars highlight vulnerabilities in the safety of oat-based food products. Therefore, a study was conducted to validate the effectiveness of toasting oats to control *Salmonella* during the oatmeal chocolate chip granola bar manufacturing process.

Purpose: Evaluate the effectiveness of toasting old-fashioned oats at 325°F for 12 minutes as an effective kill-step to control *Salmonella* during the oatmeal chocolate chip granola bar manufacturing process.

Methods: Whole grain old-fashioned oats were mist inoculated with a 5-serovar *Salmonella* cocktail and dried back to its pre-inoculation water activity levels. The inoculated oats were toasted at 325°F for 12 minutes before mixing with melted unsalted butter, brown sugar, honey, rice crisp, salt, and vanilla extract. Once cooled, chocolate chips were added, and the final mixture was poured into eight cavity granola bar mold before refrigerating for an hour. Samples collected at regular intervals during the granola bar preparation were analyzed for *Salmonella* population using injury recovery media.

Results: Following the mist inoculation and at the time of preparing granola bars, the old-fashioned oats retained 8.89 ± 1.98 log CFU/g of *Salmonella* population. However, toasting oats at 325°F for 12 minutes reduced the initial *Salmonella* concentration to 2.43 ± 0.20 log CFU/g. After refrigeration for 1 hour, the microbial count further decreased to 1.62 ± 0.01 log CFU/g. By the end of the process, it resulted in > 7 log CFU/g in the *Salmonella* population.

Significance: The

Results demonstrate that toasting oats at 325°F for 12 minutes can act as an effective kill step in controlling *Salmonella* contamination originating from heavily contaminated oats during oatmeal chocolate chip granola bar manufacturing. The data provides valuable baseline information for food manufacturers to ensure the safety of oat-based granola bars.

Award Application: Developing Scientist Award

P3-58: SYNERGISTIC INACTIVATION OF BACTERIAL PATHOGENS USING FOOD-GRADE PHENOLIC DERIVATIVES WITH MILD HEAT IN WAX COATINGS ON CITRUS

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Category: Food Safety Systems

Abstract:

Introduction: Microbial contamination of fruit wax with bacterial pathogens poses a significant food safety risk, as the wax-coating of fruits is the final post-harvest step before the distribution of fruits in the supply chain.

Purpose: This study aims to develop a synergistic intervention strategy that can lower the microbial risks of wax-coated oranges using food-grade phenolics during the routine wax drying process. **Method:** Two carnauba oil-based waxes with (SF200; pH 8.6) and without morpholine (EF100; pH 9.5) were selected, and the potential inhibitory activities of the wax products were evaluated against *Escherichia coli* O157:H7 and *Listeria innocua*, respectively. An in vitro test was performed to evaluate the antimicrobial activities of the gallic acid (GA) and their derivatives with varying hydrocarbon chain lengths (methyl gallate [MG], ethyl gallate [EG], propyl gallate [PG], and octyl gallate [OG]) in the wax formulations at 40°C. An in vivo test was performed on orange skin to evaluate the synergistic antimicrobial activities of PG during the routine wax drying process with heated air (55°C).

Results: Both EF100 and SF200 showed stronger inhibitory activities against *L. innocua* than *E. coli* O157:H7, and the wax product containing morpholine (SF200) exhibited stronger activities against both bacterial strains. Based on the in vitro test, significant synergistic activities were exerted by the combined treatment of GA derivatives with mild heat (40°C) against *E. coli* O157:H7 in the wax formulations, and GA derivatives with shorter chain lengths (i.e. MG and PG) exhibited stronger antimicrobial activities. The combined treatment of EF100 supplemented with PG and dry heating (55°C) resulted in synergistic inactivation of *E. coli* O157:H7 cells on orange skin, achieving more than 3 log reductions within 2 min.

Significance: These findings highlight a novel and practical approach to enhance the microbial safety of wax-coated citrus by the synergistic combination of food-grade phenolics during the routine wax drying process.

Award Application: Developing Scientist Award

P3-59: TEMPERING OF WHEAT BY CHLORINE DIOXIDE SOLUTIONS TO REDUCE POPULATIONS OF PATHOGENIC BACTERIA

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Wheat is a globally important food crop but is frequently contaminated by pathogens such as *Salmonella enterica* and *Escherichia coli*. These contaminations pose public health risks and compromise product safety. Recent outbreaks from wheat-based products emphasize the need for effective microbial control strategies in food processing. Chlorine dioxide tempering presents a promising solution with potential for microbial safety enhancement and grain quality preservation.

Purpose: The purpose of this study was to evaluate the efficacy of chlorine dioxide as a tempering agent in reducing microbial contamination in wheat, while assessing its impact on enzymatic activity, antioxidant content, and quality metrics such as germination and seed vigor.

Methods: The study utilized bacterial strains, including *Salmonella enterica* (ATCC 6962), *S. typhimurium* (ATCC 14028), *S. enterica* serovar Kentucky (ATCC 9263), *Escherichia coli* O121 (ATCC 2219), and O26 (ATCC 2196). *Enterococcus faecium* was employed as a surrogate. inoculated wheat kernels were treated with chlorine dioxide concentrations of 3000–6000 ppm for varying durations (0 to 24 hours). Microbial populations were enumerated, and enzymatic assays (lipase, polyphenol oxidase, peroxidase, and lipoxygenase) and antioxidant tests were performed. Germination and vigor were also evaluated. Kinetic modeling and ANOVA with Tukey HSD were used to optimize treatment conditions and assess data.

Results: The chlorine dioxide treatments resulted in reduced microbial loads, validating the effectiveness of this approach in pathogen control. Surrogate bacteria exhibited similar or greater resistance than target pathogens, supporting robust treatment validation. Microscopic studies indicated that microbial inactivation was linked to oxidative damage at the cellular level. Additionally, enzymatic degradation was controlled, and antioxidant levels were improved without compromising seed germination or vigor. These results demonstrate that microbial safety can be achieved alongside preservation of grain quality.

Significance: to develop solutions for low moisture food safety in wheat systems.

Award Application: Developing Scientist Award

P3-60: EVALUATION OF ENVIRONMENTAL STRESSES CONTRIBUTING TO THE SURVIVAL OF SALMONELLA INFANTIS IN PROCESSING ENVIRONMENTS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Salmonella* Infantis (SI) has become an emerging serotype in poultry, and research indicates that environmental conditions can influence survival and persistence, posing food safety risks.

Purpose: This study evaluated phenotypic changes to SI in response to various environmental stresses relative to poultry processing.

Methods: A farm-isolated SI that had never been exposed to disinfectants was subjected to gradually increasing concentrations of peracetic acid (PAA) and quaternary ammonium compounds (QAC), starting at 40 ppm and 1 ppm, respectively, until growth ceased. Minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined. The stressed cells underwent two shock treatments at 4°C (60 min) and 55°C (6 min), simulating chiller and scalding conditions, resulting in three variants per antimicrobial. These included PAA/QAC-stressed (SI-P, SI-Q), cold-stressed (SI-P4, SI-Q4), heat-stressed (SI-P55, SI-Q55), and unexposed control (SI). Phenotypes assessed included tolerance to PAA/QAC, biofilm formation, recovery post-PAA or QAC treatments at MIC and MBC levels for 5 min, and antibiotic susceptibility. Biofilm data were analyzed using ANOVA in JMP Pro 18.

Results: The MBC of SI after daily exposure was 140 ppm (PAA) and 8 ppm (QAC). After stress, the MIC of PAA variants remained similar to the control (120 ppm), while QAC variants increased slightly (5 ppm to 6–7 ppm). Biofilm formation was consistent across all variants; however, bacterial recovery post-PAA and QAC treatments differed significantly ($p < 0.0001$). Dual-stressed (PAA and temperature) variants (SI-P4, SI-P55) exhibited greater bacterial recovery at MIC levels than SI-P and SI. At MBC levels, all stressed variants showed recovery, indicating strong PAA tolerance. In contrast, all variants responded similarly to QAC treatments. Notably, all stressed variants displayed altered antibiotic susceptibility, with increased tolerance to azithromycin and chloramphenicol, and reduced susceptibility to 1 (QAC) and 7 (PAA) antibiotics.

Significance: This study shows that environmental stress can influence the survival, persistence, and virulence of *Salmonella* Infantis, underscoring the need for further genetic analysis.

Award Application: Developing Scientist Award

P3-61: PERFORMANCE EVALUATION OF SPONGE STICK WITH NEUTRALIZING BUFFER

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Environmental monitoring of food industries has become an essential method to prevent contamination and foodborne disease. Good Manufacturing Practices (GMPs) have been developed as guidelines, and GB 14881 also clarifies the requirements of cleaning procedure, equipment, and hygiene in factories. All the instructions have mentioned the importance of an environmental sampling plan. Therefore, a suitable sampling tool would help factories receive more accurate data.

Purpose: To evaluate the performance of Neogen® Sponge-Stick with neutralizing buffer for environmental sampling.

Method: Three tests were performed using the sponge stick artificially contaminated with *Salmonella* paratyphi B CMCC 50094, *Escherichia coli* CMCC 44102, *Cronobacter sakazakii* NC 0010, *Listeria monocytogenes* NC 0005, and *Bacillus subtilis* CICC 6633. In the pick-up and release efficiency test, five strain suspensions were spiked on the stainless-steel surface and collected with sponge sticks. In the release efficiency test, all five strain suspensions were directly added into the sponge. In the holding time recovery test, *S. paratyphi*, *C. sakazakii*, and *L. monocytogenes* were contaminated into the sponge and stored at 4–8 °C for 48 and 72 hours. After pretreatments, 90 mL phosphate buffer was added into sample bags, mixed well, and counted with Petrifilm® Rapid Aerobic Count Plates. The strain suspensions were also counted as the reference.

Results: In the pick-up and release efficiency test, a recovery ratio of 97% was achieved for the *Salmonella*, *E. coli*, *Cronobacter*, and *L. monocytogenes* strains, while 79.73% was achieved for *B. subtilis*. In the release test, all five strains had at least a 99% release ratio. In the holding time test, all recovery ratios were higher than 89% and 79% in 48-hour and 72-hour storage, respectively.

Significance: The sponge stick achieved high recovery ratios for both indicator and pathogen microorganisms. This reliable sampling tool plays an important role in environmental monitoring in food factories.

P3-62: SYNERGISTIC EFFECTS OF ULTRASOUND, UV, AND CHEMICAL TREATMENTS ON FOODBORNE PATHOGENS AND BIOFILM FORMATION IN HYDROPONIC NUTRIENT SOLUTIONS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: The rapid growth of hydroponic production systems necessitates effective interventions to mitigate the risks of foodborne pathogen proliferation and biofilm formation in nutrient solutions.

Purpose: This study aimed to evaluate the survival characteristics of foodborne pathogens in hydroponic nutrient solutions (HNS) and synergistic effects of ultrasound (US), UV, and chemical treatments on inactivating foodborne pathogens and reducing biofilm formation.

Methods: Hoagland's nutrient solution (1L) was inoculated with *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. innocua* to reach $\sim 10^5$ – 10^6 CFU/mL. The samples were treated using US (20 kHz; 25, 50, and 75% amplitudes); antimicrobial blue light (aBL), UVA and UVC (ranging from 3 to 50 mJ/cm²), and chemical disinfectants (low doses of sodium hypochlorite and peracetic acid; 2–5 ppm) individually and in combination. For biofilm experiments, polycarbonate coupons were placed in a CDC biofilm reactor and exposed to inoculated HNS under continuous recirculation (0.1 mL/s) for up to 7 days. Surviving planktonic cells and biofilm-forming cells were quantified using selective and non-selective media. Physico-chemical properties, including pH, electrical conductivity, and dissolved solids, were also monitored. All experiments were conducted in triplicates, and the data were analyzed by ANOVA using SPSS™.

Results: Among the tested organisms, *Salmonella* better survived (5.5 log) in the HNS, followed by *E. coli* O157:H7 (5 log) and *L. innocua* (2.72 log) after 7 days of recirculation. Similar trends were observed in their ability to form biofilms. US treatments achieved significant reductions, with 75% amplitude reducing *Salmonella* and *L. innocua* by up to 1.05 and 1.62 log CFU/mL, respectively, after 60 min. Synergistic treatments combining US, UV, and PAA resulted in up to a 3.5 log reduction for planktonic cells and effectively reduced biofilm levels by ~ 2 – 3 log CFU/cm². Minimal changes in pH (~ 5.0), EC (47–72.3 mV), and dissolved solids (~ 952 ppm) indicated that treatments did not significantly alter HNS properties.

Significance: These findings demonstrate that combining US, UV, and chemical treatments effectively reduces foodborne pathogens in hydroponic systems, both as planktonic cells and biofilms. Future studies should focus on optimizing treatment parameters.

P3-63: TRANSFORMING HACCP THROUGH DIGITALIZATION: TOWARDS A CONSISTENT, INTUITIVE AND AGILE FOOD SAFETY MANAGEMENT SYSTEM

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Hazard Analysis and Critical Control Points (HACCP), is the systematic preventive approach to food safety. Many food & beverage (F&B) companies manage HACCP studies using documents or spreadsheets. These approaches lack the ability and flexibility to guide HACCP teams during HACCP plan development, with consistent use of available hazard analysis and control measure definitions – the master data. Manual HACCP plans across multiple product types are also prone to (unintentional) errors. Such errors & inaccuracies in master data can seriously impair the quality of HACCP studies. A digital-enabled HACCP system offers a leaner, agile, systems integrated approach, to develop and maintain HACCP plans, for compliance and safer food. Purpose: In this presentation, we provide our understandings, gained by working with leading F&B companies, on how digitalized HACCP systems help to improve the quality of HACCP plans. Methods: We'll demonstrate how a digital HACCP platform has been developed and matured to assist HACCP teams with automation and oversight during the creation of a HACCP plan. The platform uses a bespoke material hazard library to create ingredient hazard analysis records which are assessed through an in-built risk matrix, resulting in defined control measure(s) assignment and monitoring/verification outputs. Oversight is illustrated by an approach that checks the completeness of the HACCP plan during its creation. Results: Outcomes will showcase how a digital HACCP system helps with the correct processing of information on materials, hazards and their associated risks. This digital transformation of HACCP plans is complimentary to other initiatives in the digital era of food safety and improves the quality, consistency, and completeness of food safety risk assessment and management. Significance: HACCP is central to any Food Safety management system, and therefore systems that help F&B companies to significantly increase the quality of their HACCP plans is important to the whole F&B industry.

P3-64: PROBIOTICS AS BIOCONTROL TOOLS OF OCHRATOXIN A IN GRAPE JUICE

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Presentation type/Format: Technical

Category: Food Toxicology

Abstract:

Introduction: The occurrence of ochratoxin A (OTA) in grape juice is of public concern because of its stability against industrial detoxification techniques. Probiotics have been studied as a new OTA detoxification approach due to their ability to degrade through multienzyme and adsorption properties. Few studies have investigated the effect of probiotics on removing OTA from grape juice.

Purpose: This study evaluated the ability of *Lactobacillus casei* (LC-01) and *Lactobacillus acidophilus* (LA-05) to remove OTA in grape juice.

Methods: LC-01 and LA-05 strains suspended in saline solution (7 log CFU/mL) were added to grape juice spiked with OTA (10 ng/mL). The mixtures were incubated at 37°C for 24 and 48 h in different systems. A control system without probiotics was included. OTA extraction was performed using liquid-liquid partitioning (LLP) with chloroform, vortexed for 30 s, and sonicated for 3 min in a 25-kHz ultrasonic bath. The organic phase was removed, and LLP was repeated three times. The final volume was dried, resuspended in mobile phase, and analyzed via HPLC with a fluorescence detector. All experiments were done in triplicate and analyzed using $p < 0.05$ significance.

Results: OTA levels in grape juice were reduced by 9.2% (0.92 ng/mL) and 11% (1.1 ng/mL) after 24 and 48 h of incubation with LC-01, respectively. Incubation with LA-05 for 24 h decreased OTA levels by 12% (1.2 ng/mL), and a longer incubation (48 h) did not lead to further reductions.

Significance: Results show differential effects of two probiotic strains on OTA reduction in grape juice. Findings suggest the intrinsic ability of each probiotic strain to reduce OTA or convert it into a different form in grape juice.

P3-65: MEMBRANE INTEGRITY OF *SALMONELLA* ENTERICA IN STORED THINLY SLICED CARROTS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Carrots can be thinly sliced into spaghetti-like strands as a ready-to-eat topping for salads and sandwiches. Fresh-cut produce items like carrots have been linked to foodborne disease outbreaks. Temperature and relative humidity (RH) affect the survival of *Salmonella enterica* in sliced carrots, but the effects of temperature and RH on membrane integrity of *S. enterica* cells remains relatively unstudied.

Purpose: This study evaluates the membrane integrity of *Salmonella* cells in thinly sliced carrots during storage at different temperature and RH conditions.

Methods: Carrots were sliced into spaghetti-like strands of 2 mm diameter and inoculated with a *Salmonella enterica* cocktail (*S. Typhimurium*, *S. Enteritidis*, *S. Newport*, and *S. Saintpaul*) at approximately 7 log CFU/g by spot inoculation. Carrots were placed in desiccators over saturated salt solutions to achieve 75%, 85%, and 95% RH and stored at 6°C, 9°C, and 12°C for 7 days. *Salmonella* cells were harvested by mixing carrots with phosphate-buffered saline and centrifuging (4500 × g, 15 min, 4°C) at time zero and after 7 days. Cells were stained with propidium iodide (red) to assess membrane damage and bis-1,3-dibutylbutyric acid (green) to assess membrane polarization. Flow cytometry was used to collect 10,000 forward light scatter events, and density plots were generated to assess red and green fluorescence populations.

Results: When thinly sliced carrots were stored at 12°C and 95% RH, approximately 9.1±0.2% of *Salmonella* cells exhibited permeabilized membranes and 26.1±0.3% were non-polarized. When stored at 6°C and 75% RH, 42.3±0.4% of cells were permeabilized and 84.2±0.0% were non-polarized, indicating more

severe membrane damage at lower temperature and RH.

Significance: Storage at lower temperatures and RH conditions induces more membrane damage in *Salmonella* cells compared to higher temperatures and RH. These findings have implications for managing the risk of salmonellosis in thinly sli

P3-66: MAIZE VALUE CHAIN AND CONSUMER EXPOSURE TO AFLATOXIN IN RWANDA

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Presentation type/Format: Technical

Category: Food Toxicology

Abstract:

Introduction: Aflatoxin contamination remains a problem in tropical countries, particularly in sub-Saharan Africa, where production, harvesting, and storage practices contribute to the rapid contamination of food products.

Purpose: This study aimed to analyse aflatoxin incidence post-harvest along the maize value chain in Rwanda and understand its effect on consumer exposure to aflatoxin.

Methods: A total of 180 samples comprised of maize grains and flours (1:1) were collected from different nodes of the maize value chain, including farmers, wholesalers, and millers in two agro-ecological zones: the Northern and Eastern provinces of Rwanda. A structured questionnaire was used to collect data on maize handling practices. Maize samples were analysed for total aflatoxin contamination using a validated AflaTest fluorometric method. SAS® software version 9.4 was used for statistical analysis. Differences in aflatoxin contamination levels were determined using LSD at a 5% significance level.

Results: Aflatoxin contamination in maize grains was significantly higher in the Eastern province ($p < 0.05$), with levels of 72.45 ± 39.9 , 51.35 ± 22.6 , and 13.08 ± 6.1 $\mu\text{g/kg}$ for farmers, wholesalers, and millers, respectively. In the Northern province, aflatoxin levels were 8.23 ± 1.6 , 10.33 ± 9.8 , and 10.40 ± 6.1 $\mu\text{g/kg}$ for farmers, wholesalers, and millers, respectively. In maize flour samples, aflatoxin content was 12.6 ± 43 $\mu\text{g/kg}$ in the Eastern province and 24.75 ± 23.1 $\mu\text{g/kg}$ in the Northern province. Sun drying was the most used drying method at farm and miller levels (66.6%), and polypropylene bags were the most common for transport and storage at seller (85%) and miller (100%) levels. The storage period was one month at seller level and two months at farm level.

Significance: The results infer that post-harvest practices currently used are inadequate and unsuitable for protecting consumer health against aflatoxin

contamination and the associated risks in maize.

P3-67: GROWTH AND PLASMID STABILITY OF GFP-LABELED CRONOBACTER SAKAZAKII ISOLATES IN NUTRIENT RICH MEDIUM

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Cronobacter sakazakii* is a pathogen associated with infant formula. GFP-labeled pathogens can be used for improved traceability in the presence of background microbiota.

Purpose: The purpose of this study was to compare the growth rates and plasmid stabilities of three *C. sakazakii* isolates transformed with pGFPuv plasmid encoding green fluorescent protein and ampicillin resistance.

Methods: *C. sakazakii* cultures isolated from the environment (CSE), infant formula (CSIF), and a clinical infection (CSC) were used. TSB (25 mL) was inoculated to ~ 2.5 log CFU/mL and was incubated at 37°C. To assess growth rate and plasmid stability, samples (1 mL) were collected in 2 hour intervals until 12 hours followed by 4 hour intervals until 24 hours and enumerated on TSA. Fluorescent and non-fluorescent colonies were counted. Each isolate was performed in triplicate. The maximum growth rates of wild type and transformants were compared using student's T-test. The log CFU/mL were compared between wild type and transformants of all three isolates at all time points.

Results: Approximately 6.3 log CFU/mL growth was achieved by all isolates within 24 hours. The percentage of fluorescent colonies at the starting and end points were $85.3 \pm 14.3\%$ and $78.8 \pm 36.7\%$ (CSE), $29.7 \pm 11.7\%$ and $1.6 \pm 1.6\%$ (CSIF), and $98.1 \pm 2.9\%$ and $95.2 \pm 2.5\%$ (CSC). The maximum growth rate of wild type and transformant CSE (2.22 ± 0.03 , 2.37 ± 0.25 gen/h) and CSC (2.54 ± 0.05 , 2.21 ± 0.30 gen/h) were not significantly different ($p > 0.05$). The growth rate of CSIF transformant could not be assessed due to rapid plasmid loss. The log CFU/mL were significantly different between wild type and transformant CSE until 10 hours of growth, between CSIF until 12 hours, and between CSC until 10 hours; wild type counts were higher at all significantly different time points.

Significance: Transformant CSE and CSC isolates had stable plasmids and similar growth rates compared to the wild types, indicating feasibility in infant formula studies.

Award Application: Developing Scientist Award

P3-68: GROWTH AND PLASMID STABILITY OF GFP-LABELED SALMONELLA ENTERICA SEROVAR NEWPORT ISOLATES IN NUTRIENT RICH MEDIUM

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Salmonella enterica* serovar Newport is a pathogen associated with outbreaks linked to onions. GFP-labeled pathogens can be used for improved traceability in the presence of background microbiota.

Purpose: This study was to compare the growth rates and plasmid stabilities of a *S. Newport* isolate from an onion outbreak transformed with pGFPuv plasmid encoding green fluorescent protein and ampicillin resistance.

Methods: TSB (25 mL) was inoculated to 4.1 ± 0.3 log CFU/mL (wild type) and 3.0 ± 0.2 log CFU/mL (transformant) and incubated at 37°C. To assess growth rate and plasmid stability, samples (1 mL) were collected in 2 hour intervals until 12 hours followed by 4 hour intervals until 24 hours and enumerated on TSA. Fluorescent and non-fluorescent colonies were counted. Each isolate was performed in triplicate. The maximum growth rates of wild type and transformants were compared using student's T-test. The log CFU/mL were compared at all time points.

Results: Growth of 4.7 log CFU/mL (wild type) and 5.2 log CFU/mL (transformant) was achieved in 24 hours. The percentage of fluorescent colonies at the starting and end points were $98.3 \pm 2.8\%$ and $94.7 \pm 9.2\%$. The maximum growth rates between wild type and transformant (1.84 ± 0.03 , 1.74 ± 0.06 gen/h) were not significantly different ($p > 0.05$). The log CFU/mL were significantly different until 16 hours of growth; wild type counts were higher at all significantly different time points.

Significance: The onion outbreak *S. Newport* isolate had similar growth rates between wild type and transformant, indicating feasibility for use in further experiments.

Award Application: Developing Scientist Award

P3-69: IMPACT OF FETAL BOVINE SERUM IN MEDIA ON THERMAL RESISTANCE OF PATHOGENS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: To date, no research delves into the intricate realm of thermal tolerance of foodborne pathogens within the context of cellular agriculture.

Purpose: This study evaluated the thermal tolerance of various foodborne pathogens cultured in a universal cell culture media at varying fetal bovine serum (FBS) concentrations.

Methods: Bacterial cultures consisting of three or four strains of *E. coli* O157:H7, *L. monocytogenes*, *S. enterica*, and *S. aureus* were grown in L-15 media containing 0%, 2%, and 10% FBS for 24 hours at 36°C. These cultures were exposed to thermal treatments at 55, 60, 65, 70, and 75°C for various durations to evaluate survival rates from three independent trials. Destruction rate curves were created by plotting bacterial survivors on a logarithmic scale versus exposure time on a linear scale with a detection limit of 1 log CFU/ml, and decimal reduction times (D-values) and thermal destruction temperatures (z-values) were calculated.

Results: This study revealed that the addition of FBS to L-15 media promotes the proliferation of all tested foodborne pathogens. Cultures with 10% FBS showed the greatest thermal inactivation gradients, indicating increased susceptibility to thermal treatment. *E. coli* O157:H7 cultured in 10% FBS and subjected to 55°C exhibited the significantly highest D-value compared to those cultured in 0% and 2% FBS. However, *Salmonella enterica* cultured in 10% FBS showed opposite trends with the lowest D-value. Among the bacteria evaluated, *E. coli* O157:H7 exhibited the highest z-value across all tested media, signifying its exceptional thermal tolerance compared to the other bacteria. Gram-negative bacteria (*E. coli* O157:H7 and *Salmonella enterica*) had decreasing z-values with higher FBS, while Gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) showed opposite trends.

Significance: While acknowledging the need for further research, this study underscores the substantial impact of serum concentrations on bacterial responses, particularly within cell culture-based food processing and storage.

P3-70: SANITIZER SENSITIVITY AND VIRULENCE: GENOMIC INSIGHTS INTO ESCHERICHIA COLI

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Escherichia coli* is one of the most studied bacteria worldwide due to its genetic plasticity. This bacterium includes both non-pathogenic strains and those that cause diseases in humans, whether through food or clinical transmission. Recently, in addition to characterizing its pathogenic potential, research has focused on understanding the resistance profile to antimicrobial agents, such as antibiotics and sanitizers.

Purpose: The present study investigated six of the main serogroups of *E. coli* linked to foodborne infection (O26, O45, O103, O111, O121, and O157) to understand the dynamics of heterogeneity in resistance to sanitizers derived from quaternary ammonium compounds (QAC) and peracetic acid (PAA) using whole genome sequencing.

Methods: Whole-genome sequencing of 24 *Escherichia coli* strains, selected based on serogroups and MIC variability for QAC and PAA, was performed using Illumina NovaSeq6000 and Shovill for assembly. Serogroup identification, virulence and resistance genes, and phylogenetic analyses were conducted using in silico tools. pHage insertions and the presence of the stx gene within the genome were investigated. Multivariable statistical analyses, including Multiple Correspondence Analysis (MCA), explored associations between genomic traits, phenotypic data, and environmental factors.

Results: Four *E. coli* strains were stx-positive and one was qacE-gene-positive. MCA

Results indicated that strains harboring genes encoding Shiga toxin (stx) presented profiles more likely to be sensitive to QAC. In contrast, the presence of the cbtA gene (toxin-antitoxin system) was more closely associated with increased tolerance to PAA. Furthermore, analyses of the integration sites of stx genes suggested a potential genomic trade-off between the presence of stx and QAC

resistance genes.

Significance: Findings highlight the complexity of bacterial resistance mechanisms and hypothesize that non-stx strains of *E. coli* may act as a shield to protect more pathogenic *E. coli* strains within biofilms, particularly under exposure to sanitizers.

P3-71: PHYLOGENETIC ANALYSIS SUPPORTS INVA FOR SALMONELLA SPECIES CLASSIFICATION

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Presentation type/Format: Poster**Category:** General Microbiology**Abstract:**

Introduction: Nontyphoidal *Salmonella* causes significant foodborne illnesses globally, with 93.8 million cases and 155,000 deaths annually. Molecular detection methods and diagnostic assays commonly target *invA*, encoding a critical type III secretion system component, yet its suitability across diverse *Salmonella* species and subspecies remains unassessed.

Purpose: The overall goal of this study was to characterize the genetic variability of *invA* among *Salmonella* species and subspecies.

Methodology: We analyzed *invA* sequences from 224,483 high-quality genomic assemblies (<300 contigs, N50 > 50 Kbp, SISTR2-predicted serotypes) of *Salmonella* isolates from clinical and environmental samples across Europe, Asia, and the Americas. Among 637 serovars (spanning subspecies enterica, salamae, arizonae, diarizonae, houtenae, indica, and species bongori), we identified 1,495 allelic types (AT). Sequences contained 1–189 single nucleotide polymorphisms compared to *invA* from *S. enterica* serovar Typhimurium LT2, with an average nucleotide identity of 90.8–100%.

Results: Phylogenetic analysis of unique *invA* ATs revealed clear clade separation by species and subspecies. These findings demonstrate *invA*'s sequence conservation within *Salmonella* phylogenetic clades, supporting its potential as a single-gene tool for species and subspecies classification.

Significance: This study highlights *invA*'s sequence diversity and its continued suitability as a molecular target for *Salmonella* detection and diagnostics. Additionally, *invA* typing may enable simplified, robust subspecies differentiation in next-generation detection methods.

Award Application: Developing Scientist Award

P3-72: WGS REVEALS DYNAMIC CAMPYLOBACTER POPULATION STRUCTURE IN CHICKEN ABATTOIR

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Campylobacter* is a leading cause of human gastroenteritis worldwide and is commonly identified in poultry products. Current knowledge of its dissemination patterns in poultry production largely relies on the less sensitive traditional genotyping

Methods.

Purpose: The overall objective of this study is to characterize the *Campylobacter* population present in chicken abattoirs using whole genome sequencing (WGS) and to investigate its diversity and dynamics.

Methods: WGS was applied to 324 *Campylobacter* isolates sampled from a chicken abattoir in the Greater Vancouver area throughout 2020.

Results: Core genome MLST analysis revealed a highly diverse and dynamic *Campylobacter* population containing 27 distinct lineages. A wide range of plasmids was characterized, and a high prevalence of antibiotic resistance was observed among these isolates. Distinct subpopulations were identified in 10 lineages, suggesting that some *Campylobacter* populations may have diversified within the local agricultural environment. Some lineages were frequently reintroduced to the abattoir, suggesting the potential presence of hidden *Campylobacter* reservoirs upstream of slaughter. Comparisons between biological and environmental samples suggest a high probability of between-batch cross-contamination. Locally sourced public *Campylobacter* isolates showed strong genomic correlations with the lineages identified in this study. Notably, lineages 1629a and 1629b were identified to have persisted within the local poultry production ecosystem for several years, explaining their recurrent detection.

Significance: This study enhances our understanding of *Campylobacter* population dynamics in the chicken abattoir environment, providing insights for controlling this foodborne pathogen in poultry production systems.

Award Application: Developing Scientist Award

P3-73: PERSISTENCE OF CRONOBACTER SAKAZAKII AND SALMONELLA ENTERICA ON FOOD CONTACT SURFACES BASED ON CELL STATE AND RELATIVE HUMIDITY LEVELS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Cronobacter sakazakii* and *Salmonella enterica* are foodborne pathogens often associated with low-moisture foods and associated processing environments. Both pathogens are desiccation tolerant and have been shown to persist for long periods on certain surfaces. However, limited data exist on their survival on surfaces based on cell state or relative humidity (RH) levels during storage.

Purpose: To examine the long-term survival of sessile and planktonic *C. sakazakii* and *S. enterica* on food contact surfaces at different RH.

Methods: Sessile and planktonic cells of *C. sakazakii* and *S. enterica* were inoculated onto 1 in. coupons of stainless steel (SS), plastic (HDPE), and rubber (Buna-N) at 8 log CFU. Coupons were dried for 2 h and then stored at 25°C and 33% or 53% RH. At intervals up to 90 days, triplicate coupons for each condition were removed and placed into bags with Butterfield's phosphate buffer, massaged by hand, and plated onto brain heart infusion agar. Three independent trials were conducted. Differences in population reductions were determined using ANOVA; $p < 0.05$ was considered significant.

Results: After 1 day of storage, minimal reductions were observed for both pathogens (<1.30 log CFU). After 7 days, *S. enterica* reductions were significantly higher for planktonic cells (2.10 ± 0.54 log CFU) than sessile cells (0.14 ± 0.17 log CFU) on all surfaces at 53%; no differences were observed for *S. enterica* at 33% RH or *C. sakazakii* at either RH. After 90 days, sessile *S. enterica* reductions were 3.06 ± 0.32 log CFU on all surfaces, whereas planktonic reductions were >5 log CFU, except for SS at 33% (3.05 log CFU). For *C. sakazakii*, the highest reductions were observed for planktonic cells on HDPE and Buna-N at 53%: 4.97 and 4.56 log CFU, respectively.

Significance: Results of this study aid in understanding the persistence of *C. sakazakii* and *S. enterica* on food contact surfaces at RH levels associated with dry

environments.

Award Application: Developing Scientist Award

P3-74: BIOFILM FORMATION OF CRONOBACTER SAKAZAKII IN THE PRESENCE OF POWDERED INFANT FORMULA ON FOOD CONTACT SURFACES

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Cronobacter sakazakii* is a ubiquitous environmental pathogen and has been identified in powdered infant formula (PIF) manufacturing facilities. *C. sakazakii* can form biofilms on food contact surfaces used in the manufacture of PIF. However, no information is available on its biofilm forming potential in the presence of PIF residue.

Purpose: To examine the biofilm formation of *C. sakazakii* on surfaces in the presence of different PIF formulations at different aw levels.

Methods: PIF with probiotics (PIF-A) and without (PIF-B), were inoculated with *C. sakazakii* at 8 log CFU/g, resulting in aw 0.3. PIF was diluted in water to aw 0.5, 0.7, or 0.8 and placed into 50-mL tubes with 1-in. stainless steel (SS), plastic (HDPE), glass, or rubber (Buna-N) coupons. Samples were incubated at 25°C for 48 h. Coupons were washed with water and examined for a visible biofilm. Coupons were then placed into Butterfield's phosphate buffer, hand-massaged for 1 min, and plated onto brain heart infusion agar. Differences in biofilm formation or populations were determined using Fisher's exact test or ANOVA, respectively; $p < 0.05$ was considered significant.

Results: No visible biofilms were observed on any surface at aw 0.3. At aw 0.5, biofilms were observed on all surfaces except for HDPE (78% for both PIF types). At aw 0.7, 100% of surfaces contained biofilms. For aw 0.8, HDPE contained no biofilms, while 100% of SS and Buna-N contained biofilms with both PIF types. on glass, biofilms were observed on 100 and 56% of surfaces for PIF-A and PIF-B, respectively. *C. sakazakii* populations on surfaces were significantly ($p < 0.05$) higher with aw 0.8 PIF (6.20-7.58 log CFU/coupon) compared to aw 0.5 (2.82-4.14 log CFU/coupon); however, no significant difference ($p > 0.05$) between PIF types was observed.

Significance: The results of this study aid in understanding the biofilm potential of *C. sakazakii* on food contact surfaces containing PIF matrix at different aw levels.

P3-75: INACTIVATION OF CRONOBACTER SAKAZAKII IN RECONSTITUTED POWDERED INFANT FORMULA

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Cronobacter sakazakii* is capable of persisting in low moisture foods, including powdered infant formula (PIF). If present in PIF, *C. sakazakii* may not be inactivated during PIF reconstitution. However, little information is available on how *C. sakazakii* responds when different water temperatures for reconstitution are used.

Purpose: To determine the inactivation of *C. sakazakii* in reconstituted PIF depending on the water temperature, preparation volume, and PIF formulation.

Methods: Milk-based (MB) and soy-based (SB) PIF were inoculated with *C. sakazakii* at 8 log CFU/g. A high (HV) or low (LV) volume of reconstituted PIF was prepared, in duplicate, by adding 4 or 1 scoop of PIF to 8 or 2 oz. water, respectively. After addition of water at 25, 45, 70, 85, or 100°C, samples were mixed by hand and left for 60 min. *C. sakazakii* was enumerated at various timepoints by plating serial dilutions onto brain heart infusion agar. Three independent trials were conducted. Differences in *C. sakazakii* reductions were determined using ANOVA ($\alpha=0.05$).

Results: No *C. sakazakii* population reductions were observed when either PIF type was reconstituted with 25 or 45°C water, regardless of the volume. Reconstitution with 70°C water resulted in *C. sakazakii* reductions of 2.77 ± 0.10 and 1.39 ± 0.42 log CFU/mL after 5 min in MB-PIF HV and LV, respectively, whereas reductions

were >4 and 1.23 ± 0.91 log CFU/mL in HV and LV SB-PIF. Reconstitution with 85°C water significantly increased the reduction of *C. sakazakii* in MB-PIF HV and LV after 5 min: 3.17 ± 0.20 and 3.29 ± 0.02 log CFU/mL, respectively. Reconstitution of SB-PIF at 85°C and both PIF types at 100°C resulted in >4 log CFU/mL *C. sakazakii* reduction for both volumes after 5 min.

Significance: Results of this study provide information on the survival of *C. sakazakii* in reconstituted PIF depending on water temperature.

Award Application: Developing Scientist Award

P3-76: SELECTION FOR RIFAMPICIN RESISTANCE DOES NOT ALTER STRESS RESPONSE IN SALMONELLA AND STEC: IMPLICATIONS FOR EXPERIMENTAL DESIGN

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: In low-moisture food experimental designs, inoculum is introduced into food matrices containing complex background microflora. While sterilizing the matrices can alter their physical characteristics, using rifampicin-resistant mutants for inoculation provides a more controlled approach. This strategy enhances the reliability and interpretability of microbiological studies by enabling precise differentiation and analysis of bacterial populations.

Purpose: This study aimed to evaluate whether rifampicin-resistant mutants of *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) exhibit altered responses to acid and osmotic stress.

Methods: Five strains each of *Salmonella* (serovars Enteritidis PT30, Typhimurium, Mbandaka, Tennessee, and Montevideo) and STEC (serotypes O157:NM, O157:H7, O26:H11, and O121:H19) were sequenced and assembled. SNPs were identified between wild-type and rifampicin-resistant mutants using Snippy to assess affected genes. pHenotypically, *Salmonella* and STEC strains were prepared to ~ 9 log CFU/mL in 0.5 mL PBS and treated with 0.5% lactic acid (pH= 3.8) or 20ppm sodium hypochlorite (pH= 6.8) to evaluate pathogen inactivation.

Results: Mutations in *rpoB*, which indicates rifampicin resistance, were detected in all 10 strains. Non-synonymous mutations were observed in hypothetical proteins ECs_2941 and ECs_2746 in two STEC strains. pHenotypically, after treatment with 0.5% lactic acid, *Salmonella* and STEC populations were reduced by 2.29 ± 0.42 and 0.55 ± 0.16 log CFU/mL; Reductions by 20ppm sodium hypochlorite were 0.74 ± 0.09 and 0.61 ± 0.10 log CFU/mL, respectively. No significant differences ($p > 0.05$ in pathogen inactivation) were observed between wild-type and rifampicin-resistant mutants for any strain under either treatment. No significant differences ($p > 0.05$) were observed within each species under either treatment.

Significance: These findings demonstrate that selection for rifampicin resistance did not significantly alter the phenotypic response of *Salmonella* and STEC to acid and osmotic stress. Genotypic changes were limited, although further study is warranted to fully understand their implications under other stress related

conditions.

Award Application: Developing Scientist Award

P3-77: BACTERIOLOGICAL ANALYSIS AND ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATED FROM FARMERS' MARKET FRUITS AND VEGETABLES IN ALLEGANY COUNTY, MARYLAND

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Eating fruits and vegetables protects us from free radicals produced by metabolic reactions and safeguards us from cardiovascular diseases and cancer. However, there has been an increased concern about foodborne diseases tied to contaminated farmers' market produce.

Purpose: The purpose of this research is to determine the prevalence of bacteria on fruits and vegetables sold in farmers' markets and analyze antibiotic susceptibility and resistance of isolated bacteria.

Method: Tomatoes, lettuce, strawberries, and cucumbers were collected from three farmers markets in Allegany County, Maryland and transported to the microbiology lab at Frostburg State University, Maryland for bacteriological analysis. Pure cultures were made for the identification of bacteria began with using the Gram-staining technique following biochemical tests. The Kirby- Bauer technique was used to test antibiotics resistance of isolated bacteria using Ticarcillin, Triclocarban, Polymyxin B, Ampicillin, Chloramphenicol, Streptomycin, Penicillin, and Tetracycline.

Results: The most prevalent bacteria in the isolates in tomatoes, cucumbers, tomatoes, lettuce, and strawberries were as follows: Cucumbers; *Salmonella enterica*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Bacillus subtilis*. Tomatoes; *Salmonella enterica*, *Escherichia coli*, *Enterobacter aerogenes*, *Serratia marcescens*, *Staphylococcus epidermidis*, *Serratia marcescens*, and *Enterobacter aerogenes*. Lettuce; *Enterococcus faecalis*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. Strawberries; *Enterobacter aerogenes* and *Enterobacter cloacae*. Kirby Bauer test results for antibiotic testing indicated that Penicillin 10mg and Ampicillin 10 mg disks had less than 6 mm of inhibition zones. Further identification of culturable and nonculturable bacteria will also be identified using Bacterial 16S gene extractions.

Significance: The data obtained from this research will be useful to educate and train farmers and individuals involved in pre-and post-harvest processes.

P3-78: CHARACTERIZATION OF THE SALMONELLA SURFACE PROTEOME USING A BIOTINYLATION-BASED SURFACE PROTEIN EXTRACTION METHOD: A PROOF-OF-CONCEPT STUDY

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Despite sustained public health initiatives, the ongoing prevalence of salmonellosis highlights the critical need for novel control strategies. Key to *Salmonella* pathogenesis is its use of surface structures including fimbriae, outer membrane proteins, flagella, and the type III secretion system needle complex to mediate host interactions. However, extracting these proteins is challenging due to their diverse properties and the absence of a universal method for isolating all surface components.

Purpose: in this proof-of-concept study, we investigated the surface biotinylation method for extracting cell surface-exposed proteins of *Salmonella enterica* serovar Typhimurium strain SL1344 (S. Typhimurium SL1344).

Methods: Surface-localized proteins from the SL1344 proteome (n = 4,712 protein sequences) were characterized in silico using subcellular localization prediction tools: (i) PSORTdb 4.0, (ii) SOSUIGramN, and (iii) BUSCA. Surface proteins were extracted from SL1344 cells in the early stationary phase at 37°C using the Pierce Cell Surface Protein Biotinylation Kit. Total proteome extraction using mechanical lysis with sonication was conducted as a control. Proteins extracted using each method were assessed using SDS-PAGE. Immunofluorescence staining of biotinylated SL1344 cells was performed to confirm surface biotinylation of *Salmonella*.

Results: The in silico analysis identified 156 potential surface proteins on SL1344, comprising 92 extracellular proteins (e.g., fimbriae, flagella) and 64 outer membrane proteins. SDS-PAGE analysis of the surface biotinylated protein extract supported that multiple proteins were successfully extracted with this method, suggesting that most. Immunofluorescence imaging further demonstrated successful biotinylation of surface proteins.

Significance: Our results indicate that cell surface biotinylation-based extraction may enable rapid isolation of surface-exposed *Salmonella* proteins, potentially

informing novel control strategies and enhancing our understanding of *Salmonella* pathogenesis.

Award Application: Developing Scientist Award

P3-79: SURVIVAL OF LISTERIA MONOCYTOGENES DURING SEED GERMINATION

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* (Lm) is a Gram-positive bacterium that causes listeriosis following consumption of food contaminated with Lm. Outbreaks of listeriosis have been linked to fresh produce, which can be contaminated by several routes. one route is exposure during germination, which can be caused by Lm presence in irrigation water or soil.

Purpose: Evaluation of the potential for lettuce to be contaminated by Lm during germination will enhance understanding of what stages of growth are vulnerable to contamination.

Methods: Iceberg lettuce (Crispino) was exposed at planting to LS1061, a rifampicin resistant Lm isolate derived from the 2014 caramel-covered apple outbreak. Seeds were planted in two different soils: a sandy soil from a Maryland farm and a commercial soil for raised beds (Miracle-Gro). At planting, each pot was watered with either sterile dH₂O, as a control, or the same dH₂O standardized to add 7.35-7.5 log₁₀ cfu. All subsequent watering used dH₂O with no Lm. Seedlings were harvested after 3-8 weeks and tested for Lm using a modified version of ISO11290. Additionally, an aliquot was plated on a custom Oxford-based agar containing nalidixic acid, acriflavine, cycloheximide, and rifampicin. Final detection was determined using Rapid'L.mono agar (Bio-Rad).

Results: No samples yielded Lm by direct plating. Also, no control seedlings (n=33) tested positive for Lm. Five of 36 test seedlings yielded Lm. While sample numbers are too low to draw final conclusions, 4 of the 5 positive samples were grown in the raised bed soil, suggesting soil type might influence Lm transfer to the seedling.

Significance: This preliminary study verifies the possibility of Lm transfer to seedlings during germination and that this transfer may be impacted by soil type. Further studies are needed to assess the potential risk to harvested lettuce after Lm transfer during germination, as well as the impact of soil type.

P3-80: EVALUATION OF LISTERIA MONOCYTOGENES SOIL SURVIVAL AND TRANSFER TO ROMAINE LETTUCE

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* (Lm) is the causative agent of listeriosis, a severe, foodborne illness. Outbreaks have been linked to fresh produce, including lettuce. While Lm is known to reside in soil, there are few evaluations on how residence could lead to transfer to produce.

Purpose: This study will improve understanding of contamination during harvest by evaluating strain survival in different agricultural soils and quantifying transfer of Lm from soil to Romaine via a harvest knife.

Methods: Three different soils were inoculated with one of five Lm strains at ~6 log₁₀ CFU and sampled at defined time points for ≥8 weeks. Lm was enumerated via direct plating and/or MPN using a variant of ISO11290. Separately, soil was inoculated with LS1061 at 4.7 or 5.7 log₁₀ CFU/g and used to contaminate a harvest knife. The knife was used to sequentially harvest four heads of Romaine lettuce, and the cut site was enumerated via direct plating.

Results: All Lm strains decreased in all soil types, with a faster decline in sandy soil. While survival appeared highest in clay soil, survival differences by soil type or strain were not significant. All cuts with the contaminated harvest knife resulted in Lm transfer to lettuce. Knives contaminated with soil containing the higher Lm inoculum resulted in transfer of, on average, 1300 CFU/g to the first cut and a stable, 10-fold lower transfer of Lm subsequently. Reduction of Lm in contaminating soil by 1-log resulted in an equivalent reduction in the initial Lm transfer. Critically, due to variability, the observed differences were not significant.

Significance: This study showed limited differences in Lm survival by soil type or strain. Additionally, use of a contaminated harvest knife demonstrated a consistent pattern for Lm transfer, suggesting that harvest knife contamination may lead to stable transfer events affecting many lettuce heads.

P3-81: STABILITY STUDY AFTER X-RAY IRRADIATION OF A CONCENTRATED HALF-FRASER BROTH FOR LISTERIA DETECTION IN FOOD SAMPLES

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: A first-of-its-kind 5x concentrated Half-Fraser medium has been developed to enhance workflow efficiency, convenience, and sustainability in labs testing for *Listeria* in food samples. The product allows preparing 50 L of ready-to-use broth from the 5x concentrate.

Purpose: The study evaluated the stability of an X-ray irradiated 5x concentrated Half-Fraser broth held at room temperature for 10 days prior to sterility, productivity, and selectivity evaluation.

Methods: Dehydrated Half-Fraser bags were X-ray irradiated before production of the 5x concentrated broth using the ReadyStream® instrument. The ready-to-use broth was dispensed into 1 L bottles for sterility testing and 10 mL tubes for productivity and selectivity tests. For sterility evaluation, bottles were incubated at 30 °C for 2 days. For productivity testing, 11 strains of *Listeria monocytogenes* and non-monocytogenes were inoculated into Half-Fraser broth tubes and incubated at 30 °C for 25 hours. After incubation, 100 µL were spread onto ALOA agar plates and incubated at 37 °C for 24 hours. For selectivity, 14 non-*Listeria* strains were inoculated into Half-Fraser broth tubes and incubated at 30 °C for 25 hours. After incubation, 10 µL were spread onto non-selective media and incubated according to the requirements of the tested strain. Concentrated Half-Fraser media bags were then stored for 10 days at room temperature, and sterility, growth promotion, and selectivity tests were repeated.

Results: After X-ray irradiation, the Half-Fraser broth showed no growth after 2 days of incubation for the sterility evaluation, supported strong growth of the 11 strains of *Listeria*, and demonstrated good selectivity against the 14 non-*Listeria* strains. The same results were observed after 10 days of storage of the 5x concentrated bag at room temperature.

Significance: X-ray irradiation and 10 days of storage at room temperature have no effect on the performance of the 5x concentrated Half-Fraser media bags.

P3-82: ENHANCEMENT OF LISTERIA MONOCYTOGENES BIOFILM FORMATION BY HIGH CONCENTRATIONS OF BENZALKONIUM CHLORIDE

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* is a foodborne pathogen regularly isolated from food facilities, causing frequent recalls and outbreaks, despite stringent disinfection procedures. Benzalkonium chloride (BAC) is a commonly used disinfectant with demonstrated activity in preventing the growth of *L. monocytogenes*. However, *L. monocytogenes* has shown increasing rates of adaptation to this disinfectant, which may lead to the use of higher concentrations of BAC.

Purpose: There is a lack of research on higher concentrations of BAC. This study focused on determining the effects of higher BAC concentrations on *L. monocytogenes* biofilm formation.

Methods: This research utilized a standard microplate biofilm assay to further understand the role of biofilms in *L. monocytogenes* BAC susceptibility and persistence.

Results: Using crystal violet staining, we observed that higher concentrations of BAC (0.1% [wt/vol] or higher), applied either during or post biofilm formation, were associated with significantly higher ($p = 0.0001$) levels of biofilm regardless of temperature, surface materials, or oxygen availability. The enhancement from higher BAC concentrations is not due to abiotic interactions between crystal violet and BAC but requires the presence of *L. monocytogenes* cells. Despite the enhanced biofilm levels, the biofilm-associated metabolic activity ($p = 2.9E-14$) and colony-forming units ($p = 0.01$) were inhibited by BAC. Based on microscopic and survival assays, this enhancement was likely due to bacterial lysis followed by

clumping of bacterial cells—both of which contributed to increased adhesion to surfaces. All experiments were performed with at least three independent trials, each with triplicate samples.

Significance: These findings suggest that higher BAC levels result in the buildup of biofilm materials that contain residual BAC. Such a buildup of biological macromolecules could potentially contribute to persistence by promoting subsequent biofilm formation by environmental pathogens and fostering resistance to BAC. Therefore, appropriate BAC concentrations, as well as the removal of residual BAC from the environment, are both critical factors to consider in developing cleaning and disinfection procedures.

P3-83: ANTI-BIOFILM MECHANISM OF BACILLUS AMYLOLIQUEFACIENS D747 AGAINST FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Bacillus amyloliquefaciens* is widely used in agriculture, aquaculture, and hydroponics to combat plant root pathogens. However, its interactions with foodborne pathogens, particularly in biofilm formation, remain poorly understood.

Purpose: This study aimed to evaluate the effects of *B. amyloliquefaciens* D747 on the biofilm formation of *Salmonella enterica* (Se) and *Listeria monocytogenes* (Lm) and to elucidate the potential underlying mechanisms.

Methods: The *B. amyloliquefaciens* D747 strain, isolated from a commercial fungicide, was cocultured with either Se or Lm at a 1:1 ratio in 10% tryptic soy broth (TSB). Biofilm formation was assessed on stainless-steel coupons after 48 h of incubation at 25 °C. The inhibitory effect of cell-free supernatants from *B. amyloliquefaciens* was examined using an inhibition zone assay. Additionally, the role of physical separation between the *Bacillus* strain and pathogens was tested using specialized cell culture plates.

Results: Coculturing with *B. amyloliquefaciens* significantly ($p < 0.05$) reduced the biofilm populations of Se and Lm by 3.9 and 5.5 logs, respectively. An inhibition zone was observed for Lm but not for Se. Physical separation weakened the inhibitory effects, particularly for Se, where the removal of separation led to an additional 3.0-log reduction. For Lm, removing physical separation resulted in a further 1.4-log reduction in biofilm cell counts.

Significance: These findings demonstrate that *B. amyloliquefaciens* D747 exhibits significant antibiofilm activity against Se and Lm, providing promising insights for developing novel biocontrol strategies to combat foodborne pathogens.

P3-84: EFFECT OF GRAIN VARIETIES AND MICROBIAL CULTURES ON SOLID-STATE FERMENTATION OF GRAIN SORGHUM

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Sorghum, a gluten-free cereal, is the fifth most-produced grain worldwide, valued for its resilience to harsh environments and strong agronomic performance. However, its potential as a food ingredient is limited by anti-nutritional factors. Solid-state fermentation (SSF) offers a promising solution to these challenges by modifying macronutrient composition and improving digestibility.

Purpose: The objective of this study was to evaluate performance of SSF on different sorghum grain varieties using homofermentative and heterofermentative lactic acid bacteria (LAB) cultures at varying moisture levels to enhance sorghum nutritional quality.

Methods: Three varieties of whole sorghum grains (White, Sumac, Black) were subjected to SSF using commercially available *Pediococcus acidilactici*, *Leuconostoc mesenteroides*, and their combination as starter cultures. Fermentation was carried out at 30°C for 72 hours with two moisture levels (30% and 50%). Microbial growth, protein digestibility, and crude protein content were monitored throughout the fermentation period. Two-way ANOVA was used to assess statistical significance and Tukey's HSD test was applied for multiple comparisons.

Results: When using a single starter culture, microbial count reached 9 ± 0.36 log CFU/g by the end of the fermentation across all samples. A decrease ($p < 0.05$) in the final population was observed when both bacteria were used (8 ± 0.17 log CFU/g). Moisture significantly affected fermentation ($p < 0.05$). Crude protein content was influenced by moisture level ($p < 0.05$): at 50%, protein content decreased across all samples, while no differences ($p > 0.05$) were observed at 30%. Protein digestibility improved ($p < 0.05$) across samples from 12% to 20% over time. A significant increase of digestibility ($p > 0.05$) was observed in fermented samples after 72 hours (70–75%).

Significance: SSF with LAB cultures improved protein digestibility of sorghum, enhancing its nutritional value. These findings suggest that SSF can be considered a promising method to improve the quality of sorghum as a functional food ingredient.

Award Application: Developing Scientist Award

P3-85: EFFICACY OF BLOSSOM HONEYS FROM MEXICO ON THE PRODUCTION OF BACTERIAL BIOFILMS RELEVANT TO THE FOOD INDUSTRY

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Honey from bees (*Apis mellifera*) is used as a nutritional and therapeutic product. Its physical properties, such as osmotic pressure, acidity, hydrogen peroxide formation, and active components also strengthen its antimicrobial activity.

Purpose: To determine the effect of ten blossom honeys from Mexico on biofilm production of *Escherichia coli* O157:H7, *Bacillus cereus*, *Staphylococcus aureus*, and *Acinetobacter baumannii*.

Methods: Concentrations of 6.25, 12.5, 25, and 50 mg/mL from the blossom honeys of avocado, runner-bean, canyon live-oak, common-iceplant, orange-blossom, husk-tomato, beebrush, dzidzilche, goldeneye, and mesquite were used to test bacterial growth by turbidity measurement and biofilm production by crystal violet assay. Results were normalized to 1.

Results: All honeys tested at 25 and 50 mg/mL reduced bacterial growth from 0.62–0.97-fold ($p < 0.05$) in the four strains studied. However, concentrations of 6.25 and 12.5 mg/mL induced bacterial growth from 1.06 to 1.64-fold ($p < 0.05$). All concentrations of honeys from goldeneye, common-iceplant, beebrush, orange blossom, and dzidzilche blossom reduced biofilm production ($57 \pm 35\%$; range: 0.09 to 0.78-fold; $p < 0.05$) in *E. coli* O157:H7. Avocado, canyon live-oak, and mesquite honey at 25 and 50 mg/mL reduced biofilm formation by $68 \pm 18\%$ (range: 0.50 to 0.14-fold; $p < 0.05$). For *S. aureus*, all concentrations of runner-bean, canyon live-oak, and mesquite honeys decreased biofilm production by $35 \pm 25\%$ (range: 0.4 to 0.8-fold; $p < 0.05$). In contrast, all honeys tested promoted biofilm production of *A. baumannii* and *B. cereus* by 1.2 to 8.2-fold ($p < 0.05$).

Significance: These honeys reduce biofilm production in *E. coli* and *S. aureus*, but promote it in *A. baumannii* and *B. cereus*. This work demonstrates the potential of

honey to be used as a natural antibiofilm agent in specific cases, while also cautioning against unintended bacterial promotion in food processing environments.

Award Application: Undergraduate Student Award

P3-86: ZIF-8 NANOSTRUCTURE CARRYING ANTISENSE OLIGONUCLEOTIDES TARGETING RECA MRNA REDUCE VIRULENCE FACTORS ON DIARRHEAGENIC E. COLI

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Diarrheagenic *E. coli* is cause of foodborne diseases producing gastrointestinal infections in humans and animals. Its genomic plasticity has developed six pathotypes with diversified pathogenesis mechanisms, responsible of the controversy that surround the antibiotic-therapy.

Purpose: To develop a gene therapy using antisense oligonucleotides (ASO) as a silencer of *recA* gen carried in Zif-8 nanostructure, for the control of virulence factors on diarrheagenic *E. coli*.

Methods: *E. coli* pathotypes: enterotoxigenic (ETEC), enterohemorrhagic (EHEC), and enteroaggregative (EAEC) were exposed to 32, 64, and 128 nM/ml of Zif-8/ASO-*recA* by 24h to determine growth, swarming motility, and biofilm production. In addition, the expression of genes *recA* (encoding RecA protein), *stx2a* (encoding Shiga toxin 2a- EHEC), *It* (encoding Heat-labile toxin- ETEC), and *pic* (encoding Pic toxin- EAEC) were analyzed by qPCR (control was normalized to 1).

Results: The *recA* gene expression of ETEC, EHEC and EAEC was downregulated by the exposure to 64 and 128 nM/ml (from 0.029 to 0.92-fold; $p < 0.05$) of Zif-8/ASO-*recA*. Similar, these concentrations also reduced the growth in all studied pathotypes in range from 2.13 to 3.62 UFC log₁₀/ml ($p < 0.05$); decreased the swarming motility between 15% and 67% (ranged from 0.6 to 2.7 cm; control average 4.0 ± 0.3 ; $p < 0.05$); and inhibited biofilm production (from 0.57 to 0.88-fold; control normalized to 1; $p < 0.05$), except in EAEC which promote it up to 1.89-fold caused by the aggregative adherence. Toxins-related genes such as *stx2* (0.01-fold); *it* (0.02-fold), and *pic* (0.04-fold), ($p < 0.05$) were downregulated by the exposure to 128 nM/mL of Zif-8/ASO-*recA*.

Significance: The silence of *recA* gene by Zif-8/ASO-*recA* inhibit the expression of

virulence factors on *E. coli* pathotypes. This work showed a promising preliminary data for a potential gene's therapy that could be use in food industry and/or public health.

Award Application: Developing Scientist Award

P3-87: PRESENCE OF INVA GENE AND KANAMYCIN RESISTANCE IN SALMONELLA ISOLATED FROM CATTLE FECES FROM CENTRAL AND NORTHEASTERN MEXICO

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Salmonella* is a pathogen that causes gastroenteritis in animals and humans. The *invA* gene is a virulence gene in *Salmonella* that allows the bacteria to invade and survive in cells. In the last decade, *Salmonella* isolates from stool samples have shown a high prevalence of kanamycin-resistance. Little information is available on these characteristics of the microorganism in cattle from Mexico.

Purpose: The aim of this study was the isolation and characterization of kanamycin-resistant *Salmonella* strains from fecal samples of beef (BC) and dairy (DC) cattle, from the states of Aguascalientes (AG) and Nuevo Leon (NL) in Mexico.

Methods: A total of 110 bovine stool samples were collected (BC-AG n=30; DC-AG n=20; BC-NL n=30; DC-NL n=30) in spring of 2021. Kanamycin-resistant *Salmonella* strains were isolated by suspending 1g of feces in PBS and streaked onto selective/differential media with 50 µg of kanamycin. Colonies were confirmed by end point PCR, by presence of the genes *gusA* (encoding a transcriptional regulator) and *invA* (encoding an invasive protein).

Results: Eighty-seven percent of stool samples (98/110) were positive for the presence of *Salmonella* spp. strains. A total of 186 presumptive strains were isolated and the genus STM309 gene, that confirms the *Salmonella* genus, was present in 60% of isolates (111/186; AG=51 and NL=60; BC=65 and DC=46), from which 98% (109/111) were kanamycin-resistant. Gene *invA* was identified in 16.2% (18/111) of strains, being more prevalent in DC-NL (14/18) stool samples. No correlation was found between the confirmed kanamycin-resistant *Salmonella* isolates and the productive purpose ($r=0.028$) or geographic region ($r=-0.007$); however, a significant positive correlation was observed between the *invA* gene

($r=0.203$; $p<0.05$) and isolates from DC-NL.

Significance: This study revealed that cattle harbor *invA* gene *Salmonella* spp. strains, especially in DC from NL. These results highlight the risks to the animal food industry and emphasize the importance of implementing strategies to control antibiotic-resistant pathogens responsible for outbreaks.

Award Application: Undergraduate Student Award

P3-88: RATE OF CLOSTRIDIUM BOTULINUM TOXIN PRODUCTION IN COLD BREW COFFEE WITH DAIRY COFFEE CREAMER

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Guy Skinner, USFDA

Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Canned cold brew coffee is considered a low-acid canned food (LACF), at ambient temperature, and must adhere to LACF regulations. Recent cold brew coffee recalls have been the result of improper processing and filing with FDA. Previous studies were focused on botulinum toxin formation in black cold brew coffee, the aim of this study is to determine the rate of toxin production when dairy coffee creamer is added to black cold brew coffee to identify risks of improper processing or storage.

Purpose: Determine the rate of botulinum toxin production in cold brew coffee with added coffee creamer.

Methods: A 10-strain cocktail of proteolytic *C. botulinum* spores (5 Type A and 5 Type B strains) was heat-shocked (80°C for 10 minutes) and inoculated into 10 mL of 2 shelf-stable cold brew coffee products (#2 and #24), containing 5%, 10%, and 20% coffee creamer, at a concentration of 10³ spores/ml. inoculated samples were stored at 27°C under anaerobic conditions. Sampling was performed monthly during a 6-month incubation and tested for production of botulinum toxin using the Endopep-MS method.

Results: Coffee #2 (pH 4.7, Brix 4.3) produced botulinum toxin in the 20% creamer samples (Type A at 6 months, Type B at 1 month) and 10% creamer (Type A at 6 months, Type B at 2 months), but no toxin was produced in coffee containing 5% creamer. Coffee #24 (pH 6.1, Brix 1.7) produced botulinum toxin in the 20% creamer samples (Type A and B at 1 month), 10% creamer (Type A and B at 1 month), and 5% creamer (Type A at 6 months, Type B at 1 month).

Significance: The results of this study show *C. botulinum* can produce toxin in cold brew coffee with added cream within 1-month outside refrigerated conditions. This shows the importance of controlling *C. botulinum* in cold brew coffee with added cream.

P3-89: PHAGE BIOCONTROL OF LISTERIA MONOCYTOGENES ON READY-TO-EAT (RTE) MEAT PRODUCTS

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Continued recalls of ready-to-eat (RTE) foods for *Listeria monocytogenes* (Lm) contamination highlight the need for a diverse arsenal of intervention strategies to control this pathogen in fully cooked meat products. Bacteriophages are a natural, targeted approach to controlling foodborne pathogens without affecting the physiochemical and organoleptic properties of foods.

Purpose: We tested the efficacy of ListShield™, a commercial bacteriophage preparation containing six naturally occurring, lytic bacteriophages, against Lm contamination of three RTE meat products: hot dogs, sliced deli-style ham, and chorizo links.

Methods: Each product was inoculated with approximately 4 log CFU/g of Lm ATCC 19115 and allowed to attach for 20min. pHage solution delivering 6.7, 7.0, 7.7, or 8.0 log PFU/cm² or equivalent volume of sterile water was spray-applied on each food product. Samples were stored at 4°C either bagged or under vacuum seal. Viable Lm were enumerated via direct plate count 1h and 24h post-phage application.

Results: For all foods, phage efficacy significantly ($p < 0.05$) increased with the concentration of phage applied. pHage significantly ($P < 0.01$) reduced Lm contamination of hot dogs by 0.8-2.3 log CFU/g after 1h and 1.0-2.4 log CFU/g after 24h. At 7.7 and 8.0 log PFU/cm², phage efficacy was significantly greater ($p < 0.05$) on bagged hot dogs than vacuum sealed. For deli sliced ham and RTE chorizo, phage-mediated reductions after 1h ($P < 0.01$) ranged from 0.9-2.2 and 1.0-2.6 log CFU/g, respectively, and 1.2-2.8 and 1.4-2.9 log CFU/g after 24h ($P < 0.01$), respectively. pHage efficacy was similar ($P > 0.05$) for bagged and vacuum packaged deli sliced ham and chorizo. For all foods, phage-mediated Lm reductions at 24h were greater than or equal to reductions at 1h, indicating no bacterial regrowth or resistance development.

Significance: Bacteriophages are an effective intervention for Lm contamination of RTE meat products under both ambient atmosphere and vacuum-sealed conditions.

P3-90: GROWTH OF *LISTERIA MONOCYTOGENES* IN BIOFILMS WITH *PSEUDOMONAS* IS DEPENDENT ON SPECIFIC *PSEUDOMONAS* SPECIES

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Controlling *Listeria monocytogenes* (Lm) colonization in ready-to-eat food production environments is key to preventing product contamination. *Pseudomonas* is a major biofilm former and has been shown, at genus-level, to protect Lm against cleaning and sanitation stressors. Understanding the differences in biofilm produced by various *Pseudomonas* species and their impact on the Lm growth in biofilm requires further investigation.

Purpose: The objective of this study was to assess the impact biofilm produced by different *Pseudomonas* species on Lm growth in biofilm grown at 12°C.

Methods: Fourteen *Pseudomonas* and seven Lm strains were individually screened for biofilm production by crystal violet using Minimum Biofilm Eradication Concentration (MBEC) devices. Based on differences in biofilm production, six *Pseudomonas* strains were selected for further experiments alongside two Lm strains. Different combinations of *Pseudomonas*-Lm dual culture biofilms were grown in TSB at 12°C for 72 hours alongside monoculture controls (n=8). *Pseudomonas* and Lm were quantified using CFC agar and ALOA agar, respectively. Biofilm biomass was quantified using crystal violet after 24, 48, and 72 hours. Statistical differences were determined using ANOVA with Tukey post hoc tests.

Results: Concentration of *Pseudomonas* did not significantly differ in mono- and co-culture biofilms ($P>0.05$). However, concentration of Lm was significantly greater in co-culture compared to monoculture biofilms ($P<0.05$) in 6/12 of the dual-culture combinations after 72-hour incubation. Remaining combinations were not significantly different. Compared to monocultures, the amount of biofilm biomass increased in dual-cultures with *Pseudomonas weihenstephanensis* (PS03054), but not the other five *Pseudomonas* strains.

Significance: These findings suggest that the ability of Lm to colonize *Pseudomonas* biofilms is not fully explained by total biofilm production and that additional biofilm mechanisms may play a role. Further evaluation of the

interactions of Lm and *Pseudomonas* in biofilms is required to evaluate the food safety risks of this genus in the context of *Listeria* control.

P3-91: BIOCONTROL OF SHIGA-TOXIGENIC ESCHERICHIA COLI ON RAW SLIVERED ONIONS USING A LYTIC PHAGE PREPARATION

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: A recent outbreak of Shiga-toxigenic *Escherichia coli* (STEC) serotype O157:H7 in raw yellow onions has resulted in a massive recall which resulted in over one hundred illness, 34 hospitalizations, and one death. Controlling STEC and other foodborne pathogens while preserving organoleptic properties of fresh produce and meeting the demand for chemical-free food underscores the need for natural antimicrobial solutions. Bacteriophages offer one such approach.

Purpose: Efficacy of EcoShield™ PX, a commercial bacteriophage preparation containing three naturally occurring, lytic bacteriophages, was tested against *E. coli* O157:H7 contamination of raw slivered onions.

Methods: Raw slivered onions were inoculated with a nalidixic acid resistant mutant of *E. coli* O157:H7 strain G5101 at approximately 4 log CFU/g and allowed to attach for 20 min at room temperature. pHage at 7 log PFU/g or 8 log PFU/g, or equivalent amount of sterile water was sprayed at the rate of 4 mL/lb. pHage- and water-treated onion samples were placed in bags or vacuum packed and stored at 4°C. *E. coli* were enumerated via direct plate count using MacConkey agar supplemented with 25 ug/mL nalidixic acid at 1hr and 24hr post-treatment. The data was analyzed using two-way ANOVA tests.

Results: pHage applications of 7 log PFU/g and 8 log PFU/g significantly ($P < 0.01$) reduced *E. coli* populations after 1hr and reductions were sustained for 24hr post-treatment for both packaging methods. in bagged onions, reductions in *E. coli* populations were significantly ($P < 0.05$) greater when treated with 8 log PFU/g (1.07 and 1.17 log CFU/g at 1h and 24h, respectively) compared to 7 log PFU/g (0.56 and 0.70 log CFU/g at the same time points). Similar reductions ($P > 0.05$) were observed for vacuum packed onions suggesting packaging method did not affect phage efficacy.

Significance: Together, these data show that phage biocontrol is an effective intervention for managing *E. coli* O157:H7 contamination of raw slivered onions.

P3-92: EXPLORING THE LINK BETWEEN MICROBIAL CONTAMINATION, PATHOGENS, AND CONSUMER HYGIENE PRACTICES IN DOMESTIC KITCHENS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Most foodborne diseases at home result from poor hygiene and improper food handling. This study aimed to assess the presence of microbiological indicators and pathogens in domestic kitchens in Querétaro, Mexico, and explore their relationship with hygiene practices.

Purpose: To determine the prevalence of microbiological indicators and pathogens on various kitchen surfaces in domestic environments, and evaluate the correlation between hygiene practices and microbial contamination.

Methods: Hygiene practices were surveyed and visual inspections were conducted in 50 kitchens. A total of 250 samples were collected from sponges, kitchen cloths, cutting boards, refrigerators, and food preparation surfaces. Samples were tested for microbial indicators, including total plate count (TPC), total coliforms (TC), and *E. coli*, as well as pathogens such as *S. enterica* and *L. monocytogenes*. Statistical analyses, including contingency tables, Pearson tests, and Kendall's rank correlation, were used to assess associations between hygiene practices, surface types, and pathogen presence.

Results: The study found significant gender differences in the organization of refrigerators, the presence of spills, and the overall condition of sinks and dishcloths ($p < 0.05$). The prevalence of *L. monocytogenes* and *S. enterica* was 1.6% and 0.4%, respectively. The average TPC concentration was 7.2 ± 1.5 log CFU/surface, with significant variation by surface type ($p < 0.05$). Sponges had the highest concentration (8.6 ± 1.3 log CFU). Positive samples for TC and *E. coli* were found in 76.8% and 20.8% of cases, with concentrations of 4.9 ± 1.4 and 3.9 ± 1.4 log CFU/surface, respectively. *E. coli* was most frequently found on sponges (54% of samples, $p < 0.05$). Significant correlations revealed that as TPC increased, the visible cleanliness grade of the refrigerator decreased ($p < 0.05$, $\tau = -0.3$). A significant correlation was found between disinfection frequency of the

refrigerator and lower *E. coli* concentrations ($p < 0.05$, $\tau = 0.3$). A weak but significant negative correlation was observed between surface condition and *E. coli* concentrations on cutting boards and food prep surfaces ($p = 0.02$, $\tau = -0.3$ and $p = 0.04$, $\tau = -0.3$ respectively).

Significance: The findings emphasize the critical role of proper hygiene practices in reducing microbial contamination and preventing foodborne diseases in domestic kitchens.

Award Application: Undergraduate Student Award

P3-93: LETHALITY OF SALMONELLA SPP. AND LISTERIA MONOCYTOGENES FOR COOKING PROCESS VALIDATION OF FRESH-MADE AND DRIED VARIETIES OF PASTA

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Pasta reformulations require validation of blanch times to ensure food safety. Differences in processing parameters and components necessitate product-specific hazard analysis to mitigate pathogen risks.

Purpose: Determine blanch times to achieve a minimum 5-log reduction of *Salmonella* spp. and *Listeria monocytogenes* in various pasta formulations.

Methods: Lo Mein, penne, rotini, and linguini pasta formulations were inoculated with *Salmonella* and *L. monocytogenes* and then blanched at 180°F for 90 seconds to 20 minutes (n=36 per formulation). Dry pasta was dried overnight, while fresh pasta was made on the day of blanching. Samples (11 g) were enumerated on tryptic soy agar overlaid with xylose lysine deoxycholate and modified Oxford listeria agar for *Salmonella* spp. and *L. monocytogenes*, respectively.

Results: Fresh Lo Mein inoculated with *Salmonella* spp. and *L. monocytogenes* resulted in average log reductions of 6.85 ± 0.40 and 5.90 ± 0.56 , respectively, when blanched for 3 minutes. Fresh penne inoculated with *Salmonella* spp. and *L. monocytogenes* resulted in log reductions of 7.78 ± 0.34 and 6.61 ± 0.34 when blanched for 2 minutes, respectively. Fresh Lo Mein with xanthan gum inoculated with *Salmonella* spp. and *L. monocytogenes* resulted in log reductions of 7.51 ± 0.66 and 6.87 ± 0.20 when blanched for 90 seconds, respectively. Fresh rotini inoculated with *Salmonella* spp. and *L. monocytogenes* resulted in log reductions of 7.48 ± 0.48 and 6.12 ± 0.46 when blanched for 90 seconds, respectively. Dry linguini inoculated with *Salmonella* spp. and *L. monocytogenes* resulted in log reductions of 7.92 ± 0.22 and 6.64 ± 0.36 when blanched for 10 minutes and 5 minutes, respectively.

Significance: This study validates individual blanching times required to achieve a minimum 5-log reduction of *Salmonella* spp. and *Listeria monocytogenes* in fresh and dry pasta formulations, highlighting the importance of customized hazard analysis to ensure effective pathogen control and mitigate food safety risks.

P3-94: ONE HEALTH PERSPECTIVE ON SALMONELLA MBANDAKA: UNVEILING TRANSMISSION DYNAMICS BETWEEN EQUINES, FOOD, AND HUMANS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Salmonella* Mbandaka is one of the major *Salmonella* serotypes, responsible for foodborne outbreaks identified, as by the Centers for Disease Control and Prevention. Horses can be one of the major hosts of this *Salmonella* serotype and can spread this through the fecal-oral route.

Purpose: This study aimed to uncover the genomic characteristics of *Salmonella* Mbandaka from foals in Kentucky and discover their source of transmission through whole genome sequencing (WGS).

Methods: *Salmonella* isolates were obtained from horses and confirmed using PCR and MALDI-TOF. Serotyping was performed based on the Kauffmann-White classification scheme and antimicrobial resistance profiling was performed by broth microdilution method against 11 antibiotics. *Salmonella* Mbandaka isolates (n=3) were selected for WGS on the Illumina MiSeq System. Raw reads were subjected to de novo assembly on SPAdes version 4.0. Assembled contigs were annotated using PGAP version 6.7. pHylogenetic analysis was performed using the core genome alignment in comparison with other publicly available *Salmonella* Mbandaka genomes obtained from GenBank.

Results: These isolates were found resistant to five groups of antibiotics including aminoglycosides, penicillin, cephalosporins, chloramphenicol, and sulfonamides. Whole genome sequencing revealed that sequenced isolates belong to pathogenic sequence type ST413. Virulence profiling revealed more than 127 genes linked to host cell invasion, immune evasion, biofilm formation, and systemic infection, indicating significant pathogenic potential. Alarmingly, our isolates harbor genes conferring resistance to five antibiotic classes, including colistin which is considered a last-resort antibiotic for human infections. pHylogenetic analysis revealed their relatedness with an outbreak-causing isolate obtained from chicken breast.

Significance: The circulation of *Salmonella* Mbandaka in the environment, with transmission occurring between food, animals, and humans, poses a significant public health concern. Studying the complete genomes of other isolates and

analyzing transmission patterns can provide valuable insights, ultimately aiding in the development of effective prevention and control strategies.

Award Application: Developing Scientist Award

P3-95: INTERACTIONS BETWEEN LISTERIA MONOCYTOGENES AND PSEUDOMONAS FLUORESCENS IN DUAL-SPECIES BIOFILMS UNDER SIMULATED FOOD PROCESSING CONDITIONS

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Biofilms in the food processing environment typically comprise multiple microbial species, including pathogens and spoilage bacteria such as *Listeria monocytogenes* and *Pseudomonas fluorescens*. Biofilm formation is an adaptative response to various environmental stresses including disinfectants, pH, oxidative and osmotic. To date, the dynamics of dual-species biofilm under simulated food processing conditions remain poorly understood.

Purpose: This study aims to investigate the interactions between *Pseudomonas fluorescens* and *Listeria monocytogenes* in dual-species biofilms under controlled food processing conditions.

Methods: *L. monocytogenes* strains (dairy isolates) were combined with *P. fluorescens* individually or as a mixed strains cocktail. Biofilms were formed at $30 \pm 2^\circ\text{C}$ for 96 h in a CDC Biofilm Reactor on polycarbonate and stainless-steel coupons. Samples were collected on day 0, 1, and 4, and attached cells dislodged by sonication and vortexing. Cells counts were determined via spread plating. Scanning Electron Microscopy was used to visualize biofilms. Two-way ANOVA and Tukey's HSD test were applied used to assess overall statistical significance and identify significant differences between treatment pairs.

Results: Both *L. monocytogenes* and *P. fluorescens* populations increased over time across all conditions tested. in mature biofilms on polycarbonate coupons, *L. monocytogenes* reached an average of 7.50 log CFU/coupon, while *P. fluorescens* was enumerated at 5.11 log CFU/coupon. A similar trend was observed on stainless-steel coupons. *L. monocytogenes* cells were significantly ($P < 0.05$) more abundant than *P. fluorescens* cells. Images revealed the distribution of *L. monocytogenes* and *P. fluorescens* within the biofilm, with *L. monocytogenes* mostly located towards the outer environment.

Significance: This study offers insights into dual-species biofilms of *L. monocytogenes* and *P. fluorescens* on common food contact surfaces in dynamic conditions, emphasizing the persistence of *L. monocytogenes* and guiding the development of improved cleaning and disinfection strategies to control biofilm produced by this foodborne pathogen in the food processing environment.

Award Application: Developing Scientist Award

P3-96: COMPARATIVE ANALYSIS OF OPTICAL DENSITY AND COLONY-FORMING UNITS FOR BACTERIAL GROWTH ASSESSMENT: A COMPREHENSIVE OVERVIEW

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Selecting a method for bacterial quantification is essential to provide reliable and accurate cell growth data. Optical density (OD) is used to report bacterial growth; however, how that measure correlates with the real cell count/colony-forming units (CFU) is underreported.

Purpose: Evaluate the correlation between OD and CFU for four foodborne pathogens by testing growth kinetics.

Methods: Growth curves were created for *Listeria monocytogenes*, *Escherichia coli* O157, *Salmonella*, and *Staphylococcus aureus*. OD and CFU values were measured simultaneously. Each pathogen was tested independently; three strains per organism were used and grown under equal conditions (TSB broth, 37°C). Growth was monitored every three hours for 48h. OD600 was measured using a spectrophotometer UV-1600PC, and CFU estimated by spread plating. initial OD600 was standardized to ~0.100 for a measurable lag-phase. Doubling time (DT), growth rate (GR), and area under the curve (AUC) were calculated. The OD-CFU correlation and reliability were estimated through linear regression using R Statistical Software and GraphPad Prism10.

Results: DT using OD was 26.28, 39, 41.04, and 56.28 min for *E. coli*, *Salmonella*, *S. aureus*, and *L. monocytogenes*, respectively. OD values for GR were 1.5837, 1.254, 1.0128, and 0.8883, *E. coli*, *Salmonella*, *S. aureus*, and *L. monocytogenes*, respectively. CFU data was comparable, with *E. coli* growing fastest (DT=29.16 min, GR=1.4264), followed by *Salmonella* (DT=31.22 min, GR 1.145), *S. aureus* (DT=43.08 min GR 0.9545), and *L. monocytogenes* (DT=52.13 min GR: 0.91264). Linear regression revealed a strong correlation between OD and CFU during the initial growth phase ($R^2=0.89$, $p<0.05$); however, this correlation weakened over time ($R^2=0.45$, $p>0.05$).

Significance: This report translates the OD values into CFU, becoming a valuable reference document for pathogen growth/death studies. CFU appears more

reliable for tracking bacterial growth as cell count seems more tangible. OD may be affected by conditions influencing turbidity of the media.

P3-97: UTILIZING PINEAPPLE BYPRODUCTS AS SUSTAINABLE SUBSTRATES FOR PROBIOTIC CULTIVATION AND ANTIMICROBIAL PRODUCTION

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Probiotics play a critical role in human health, food safety, and reducing antibiotic dependence. However, the high cost of commercial media for probiotic cultivation limits their broader applications.

Purpose: This study explored the potential of pineapple peel as a sustainable, cost-effective substrate for probiotic growth and antimicrobial compound production.

Methods: Pineapple peel was dehydrated, ground into powder, and tested at concentrations of 5%, 10%, and 20% to determine the optimal conditions for culturing *Lactococcus lactis*, starting from approximately 5 log CFU/ml. Growth performance, pH, and titratable acidity in pineapple peel-based media were compared with standard MRS broth. The antimicrobial properties of probiotic supernatants and bacteriocins were assessed against *Listeria monocytogenes*.

Results: Among all conditions, *L. lactis* grown in 10% pineapple peel broth reached the highest cell density of 8.98 log CFU/ml within 48 hours. Similarly, *Lactobacillus plantarum* and *Pediococcus acidilactici* achieved 9.52 and 9.41 log CFU/ml, respectively, under the same conditions. This enhanced growth was associated with increased titratable acidity ($p < 0.05$) and decreased pH ($p < 0.05$). Supernatants from *L. lactis* cultured in pineapple peel broth reduced *L. monocytogenes* by 7.55 log CFU/ml ($p < 0.01$) within 48 hours, demonstrating inhibition comparable to MRS broth. Supernatants from *L. plantarum* and *P. acidilactici* completely eliminated *L. monocytogenes* within 48 hours. Additionally, bacteriocins from *L. lactis*, *L. plantarum*, and *P. acidilactici* reduced *L. monocytogenes* by 2.65, 3.2, and 1.35 log CFU/ml ($p < 0.05$), respectively, within 24 hours.

Significance: This study highlights an eco-friendly approach to probiotic cultivation, offering potential applications in sustainable food production and natural food preservation.

Award Application: Developing Scientist Award

P3-98: MICROBIAL PROFILING OF COMMERCIALY PACKED TORTILLAS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: in Mexico, the consumption of packaged tortillas has increased in recent years. These products generally have a longer shelf life compared to freshly made tortillas, although packaging deterioration remains a challenge.

Purpose: To evaluate the microbiological profile of indicator groups in vacuum-packed and traditionally-packed corn tortillas.

Methods: Between January and February 2024, 45 samples from 15 brands of corn tortillas (27 vacuum-packed and 18 traditionally-packed) were collected from supermarkets in Querétaro, México. First, a label analysis was conducted to assess shelf life and additives used. indicator microorganisms (aerobic plate count [APC], molds and yeasts [MY], coliforms, and *E. coli*) were quantified using MC-Media Pad® plates. A multiple correspondence analysis was performed to classify the tortilla brands based on microbial data. Finally, regression tree analysis was applied to develop a shelf-life model for tortillas based on the content of indicator microorganisms.

Results: The median counts of APC were 2.40 and 2.11 log CFU/g for traditionally packed and vacuum-packed tortillas, respectively. MY counts were 1.60 and <1 log CFU/g for traditionally packed and vacuum-packed tortillas, respectively. Coliform concentrations were 1.15 and 1.70 log CFU/g for traditionally packed and vacuum-packed tortillas, respectively, with no significant differences observed using the Kruskal-Wallis test ($p > 0.05$). *E. coli* was detected in three vacuum-packed brands (~1.26 log CFU/g). The multiple correspondence analysis revealed clustering of brands based on MY and coliform content. in the regression tree model for shelf life, MY content was identified as the significant variable.

Significance: While vacuum packaging technology can extend the shelf life of food products by controlling aerobic microbiota, it may also create favorable conditions for anaerobic microorganisms, such as *E. coli*.

P3-99: EVALUATION OF SUB-LETHAL INJURY AT SINGLE CELL VERSUS POPULATION LEVEL IN LISTERIA MONOCYTOGENES DURING POST-PACKAGE THERMAL TREATMENT OF FRANKFURTERS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* is a foodborne pathogen that may contaminate the surface of frankfurters, thus many manufacturing companies apply a post-package treatment through immersion in hot water. However, such techniques may be sub-lethal for the pathogen, inducing injury and viable-but-non-culturable (VBNC) state that is stochastically expressed at the single-cell level.

Purpose: (i) To evaluate the sub-lethal thermal injury of *L. monocytogenes* on frankfurters' surface at the single-cell versus population level, and (ii) to comparatively estimate pathogens' physiological status (viable, injured, dead), using plate counting, fluorescent, and confocal microscopy.

Methods: Frankfurters were inoculated with *L. monocytogenes* (EGDE-e; 7.0 log CFU/cm²) and individually vacuum packaged. Thermal treatment was simulated in a water bath at 61°C (max. 60 min) and 64°C (max. 12 min), while frankfurters' surface temperature was monitored using K-type thermocouples. Determination of injured sub-populations was performed by subtracting the number of colonies on Tryptic Soy Agar with 0.6% Yeast Extract (TSAYE) supplemented with 5% NaCl (5 days; 37°C) from those on TSAYE with 0.5% NaCl (2 days; 37°C). Sub-lethally injured cells assessed by plating were compared to those determined by fluorescent and confocal microscopy, coupled with CFDA/PI staining, and quantified using Image J.

Results: *L. monocytogenes* population on TSAYE showed significant reduction after 12 and 2 min of treatment at 61°C and 64°C, respectively ($p < 0.05$). At population level, injury was recorded after 2 and 4 min at 64°C (1.0 and 0.7 log CFU/cm², respectively). Under the same conditions, the percentage of CFDA+/PI+ cells increased from 31% to 68%, indicating sub-lethal injury at the single-cell level.

After exposure at 61°C for 60 min, the whole population was below the enumeration limit (0.3 log CFU/cm²); however, 47% of the cells were CFDA+/PI- and 8% were CFDA+/PI+, suggesting the induction of VBNC state and injury, respectively.

Significance: Culture-based enumeration methods may overestimate the efficacy of post-packaging heat treatments.

Award Application: Developing Scientist Award

P3-100: OPTIMAL SPORULATION OF THREE CLOSTRIDIUM PERFRINGENS STRAINS AT DIFFERENT INCUBATION TIMES: 10 AND 14 DAYS FOR VALIDATION RESEARCH

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Clostridium perfringens* is a spore-forming foodborne pathogen that has the ability to duplicate itself every 6 minutes under ideal conditions.

Laboratory-based studies must be conducted but a critical portion of the studies is developing a spore count appropriate for validation evaluating both strains and incubation time. Little information has been published on spore development for validation research.

Purpose: This study was to compare different strains and incubation times for growing *Clostridium perfringens* spores to optimize spores for use in validation studies.

Methods: All spores were prepared similarly. Frozen cultures of *C. perfringens* ATCC 8239, ATCC 10244, and ATCC 10388 were sampled in duplicates at 100 µL and put into cooled de-aerated Fluid Thioglycolate Medium (FTM), placed in an anaerobic chamber, incubated for 24 hours at 37°C. Cultures were transferred into fresh FTM tubes and incubated for 4 hours, each tube was transferred into 1.0 Liter of Duncan Strong Medium, supplemented with caffeine (0.1 µg per liter), and incubated for 10 or 14 days. Spores were harvested and confirmed through centrifugation and microscopy. Spore titer was quantified through four technical replicates of each sample, spread plated then evaluated.

Results: The 10-day spore titer showed ATCC 8239 had the highest spore/ml concentration at 6.05 log₁₀ followed by ATCC 10244 which yielded 6.06 log₁₀, and finally ATCC 10388 at 4.8 log₁₀. The 14-day spore titer had a similar trend with

ATCC 8239 yielding 6.36 log₁₀, but ATCC 10388 was lower at 5.4 log₁₀, and ATCC 10244 at 4.4 log₁₀. According to the Kruskal-Wallis test, strain ATCC 8239 yielded the highest spore titers even though there was no significant difference between strains ($p = 0.5647$). Furthermore, there wasn't any significant difference between incubation time ($p = 0.5127$).

Significance: This data confirms a more suitable strain for future validation research studies to ensure safe chilling conditions for meat products.

P3-101: EVALUATING THE PERSISTENCE STATE OF *LISTERIA MONOCYTOGENES* IN RESPONSE TO GENTAMICIN, BENZALKONIUM CHLORIDE, NaCl SOLUTIONS, AND BACTERIOCIN SB83

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* persister cells are a major food safety concern because they can contribute to bacterial persistence and survival in food processing environments. Persisters are described as a subpopulation of growth-arrested cells that possess non-heritable, transient tolerance to lethal doses of antibiotics (e.g., gentamicin) and can revert to an active state under favourable conditions. However, the effect of food industry-specific stresses on their formation remains unclear.

Purpose: The aim of this study was to investigate whether food industry-related stresses induce persister cell formation in *L. monocytogenes* strains isolated from Portuguese cheese factories.

Methods: Four *L. monocytogenes* strains were exposed to high doses of gentamicin, increasing concentrations of benzalkonium chloride, high salt concentrations (20% and 34% NaCl solutions), and SB83 bacteriocin. Dose-dependent killing curves were generated to assess persister formation. Viability in saline was monitored for over one month and both culturable and non-culturable forms were examined. Controls and treatment levels were explicitly defined for each stress condition tested.

Results: Gentamicin exposure induced a persister state in all four strains as evidenced by biphasic killing curves. In contrast, benzalkonium chloride, NaCl solutions, and bacteriocin SB83 did not induce detectable persister formation

under the conditions tested. *Listeria monocytogenes* cultures remained viable in NaCl solutions for more than 30 days in both culturable and viable but non-culturable states. Statistical analysis of survival rates and persister frequencies was performed for each stress condition ($p < 0.05$).

Significance: Although these results do not indicate that common food industry stresses independently promote *L. monocytogenes* persister formation, further research is needed to clarify the factors influencing persister formation and their implications for food safety and public health.

P3-102: ASSESSMENT OF PATHOGEN BURDEN IN MARKET READY LAMB, GOAT AND PORK PRODUCED IN PROVINCIALLY LICENSED FACILITIES IN ONTARIO

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: The pathogen burden in market ready lamb, goat and pork produced in provincially licensed facilities in Ontario is relatively unknown, leaving an information gap which may impact food safety and policy.

Purpose: To obtain baseline data on the microbiological safety and quality of market ready lamb, goat and pork. **Methods:** Ground or cut lamb, goat and pork meat were purchased from 57 meat plants in Ontario. Sixty-five gram portions of the samples were screened for *Listeria monocytogenes*, *Salmonella*, *Yersinia enterocolitica*, *Campylobacter* spp., Shiga toxin-producing *Escherichia coli* (STEC) including *E. coli* O157:H7 by PCR methods, followed by confirmation using Health Canada or ISO culture methods. Bacterial and generic *E. coli* counts were assessed using Petrifilm-based methods and overall bacterial composition was determined by meta-barcoding targeting the 16S rRNA gene.

Results: from 471 samples (265 pork, 159 lamb, 47 goat), the number of culture-confirmed positives were 122 (25.9%) for *L. monocytogenes* (76 pork, 38 lamb, 8 goat), 19 (4.0%) for *Salmonella* (13 pork, 3 lamb, 3 goat), 18 (3.8%) for *Y. enterocolitica* (14 pork, 4 lamb), 10 (2.1%) for *Campylobacter* (4 pork, 5 lamb, 1 goat), 18 (3.8%) for STEC (9 pork, 9 lamb, 0 goat), and 0 for *E. coli* O157:H7. The positive rates for *Y. enterocolitica* and STEC may be underestimated due to failure in isolating these organisms from approximately 75% of the PCR-positives. The meta-barcoding data revealed the presence of other pathogens such as *Clostridium perfringens*, *C. botulinum* and *Vibrio parahaemolyticus*; the microbial signatures of the meat types; and the predominance of spoilers, such as *Pseudomonas*, *Photobacterium*, *Lactococcus*, *Carnobacterium* and *Brochothrix*

species.

Significance: This research fills in a knowledge gap that can help inform risk-based food safety and policy in ontario for lamb, goat and pork and shows the importance of pathogen monitoring in market ready meat.

P3-103: THE ROLE OF ENVIRONMENTAL BACTERIA IN PROMOTING LISTERIA MONOCYTOGENES BIOFILM STABILITY ON ABIOTIC FOOD SURFACES

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Biofilms significantly contribute to microbial persistence by firmly adhering to food contact surfaces. Environmental bacteria, such as *Ralstonia insidiosa* (Ri) and *Brevundimonas naejangsanensis* (Bn), can enhance the persistence of *Listeria monocytogenes* (Lm), posing a heightened risk to consumers.

Purpose: This study aimed to evaluate the persistence and stability of Lm in multi-species biofilms co-cultured with Ri and Bn under the influence of sterile cantaloupe, lettuce, and peach juices, representative of fresh produce and fruit matrices.

Methods: Mono-, dual-, and multi-species biofilms were developed in 96-well plates which bacterial growth and biofilm optical density (OD) were quantified. A drip flow reactor, mimicking food industry conditions, was used to investigate Lm persistence in mono- and multi-species biofilms on stainless steel coupons. The spatial arrangement and morphology of bacteria in biofilms were analyzed using fluorescence imaging.

Results: In all matrices, multi-species biofilms showed significantly higher OD₅₉₅ values, ranging from 0.36 to 0.41, and *L. monocytogenes* counts between 4.47 and 6.01 log CFU/mL, compared to monocultures with OD of 0.24 and undetectable cells after 7 days. Imaging indicated enhanced persistence through spatial arrangement of Ri and Bn.

Significance: This study highlights the critical role of environmental bacteria in promoting Lm biofilm stability and growth. Their presence in food environments increases the risk of contamination and persistence of human pathogens. Additionally, Ri and Bn may serve as potential indicators of biofilm hotspots, enabling targeted interventions to mitigate consumer risks.

P3-104: IMPACT OF ENVIRONMENTAL STRESSES IN THE AGRI-FOOD SYSTEM ON ANTIBIOTIC TOLERANCE AND SURVIVAL OF CAMPYLOBACTER JEJUNI

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Campylobacter* is a major foodborne pathogen and one of the four leading causes of global diarrheal diseases. Among its species, *Campylobacter jejuni* is the primary cause of human infections, primarily transmitted through contaminated food, particularly poultry, and contact with infected animals. As a microaerophilic bacterium, *C. jejuni* is highly sensitive to environmental stresses, which can either eliminate the pathogen or induce a dormant state, enhancing its resistance to stresses, including antibiotics, and increasing the risk of treatment failure.

Purpose: This study investigated the survival of *C. jejuni* strains under various environmental stresses and assessed their potential to develop antibiotic tolerance.

Methods: *C. jejuni* strains were incubated under optimal, starvation, aerobic, cold, and high salt conditions. To identify cells with increased antibiotic tolerance, bacterial populations were exposed to lethal concentrations of ampicillin and ciprofloxacin, and survivors were quantified using plating assays. All experiments included at least three biological replicates.

Results: Starvation significantly enhanced antibiotic tolerance, increasing survivors by 2.5 log and 1.5 log after 5 hours of exposure to ampicillin and ciprofloxacin ($p < 0.05$), respectively. Cold stress did not consistently increase antibiotic tolerance across strains. Strain-specific responses were observed under high salt and aerobic conditions. For example, under high salt conditions, the F38011 strain showed increased survival, while ATCC 33560 did not. Conversely, under aerobic conditions, the ATCC 33560 strain exhibited a 2-log increase ($p < 0.05$) in survivors after ampicillin treatment compared to optimal growth conditions, while the 81116 showed no significant change.

Significance Understanding how environmental stresses affect *C. jejuni* survival and antibiotic tolerance is critical for controlling its prevalence in food commodities, such as poultry, dairy, and fresh produce. Targeted mitigation

strategies, such as optimizing storage and processing conditions for specific commodities, can reduce contamination risks and the likelihood of antibiotic treatment failures.

P3-105: ATTACHMENT AND BIOFILM FORMATION BY AUTOCHTHONOUS PACKING FACILITY ISOLATES OF *L. MONOCYTOGENES* AND *P. AERUGINOSA*

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* can persist in challenging environments by adhering to surfaces and forming biofilms. Adherence and biofilm formation by *L. monocytogenes* is influenced by nutrient availability, temperature, and synergistic microbiota.

Purpose: Biofilm formation by *L. monocytogenes* 573-035 (LMC), *L. monocytogenes* drain isolate (LMD), *Pseudomonas aeruginosa* packing house isolate (PA), and combinations- LMC+PA, and LMD+PA at low, ambient and high temperature and nutrient availabilities were analyzed.

Methods: Trypticase Soy Broth (TSB), nutrient-limited medium (1: 10 TSB), and sterile distilled water (SDW) were added (180 μ L) to the wells of a 96-well plate and inoculated with approximately 5 log CFU/ml of the respective isolates. The plates were incubated at 4, 25, and 37 $^{\circ}$ C for 48 h. The population of attached *L. monocytogenes* and *P. aeruginosa* was determined through enumeration on MOX and PIA plates respectively. Biofilm biomass was quantified using Crystal Violet staining.

Results: At 25 $^{\circ}$ C in TSB, PA (OD_{570nm} 4.21 ± 0.74), LMD+PA (OD_{570nm} 5.04 ± 0.86), and LMC+PA (OD_{570nm} 4.77 ± 0.67) were able to form significantly higher biomass than individual *L. monocytogenes* isolates. LMD, LMC, LMD+PA and LMC+PA produced higher biomass than PA (0.31 ± 0.05 OD_{570nm}) in 1: 10 TSB at 25 $^{\circ}$ C whereas at 37 $^{\circ}$ C in 1: 10 TSB, only LMD had higher biomass than PA. While cell attachment occurred in SDW at 4 $^{\circ}$ C, biofilm biomass was not detected. No significant differences occurred between the attached cell population (6.7- 8.3 log CFU/ ml) in TSB and 1: 10 TSB at 25 $^{\circ}$ C and 37 $^{\circ}$ C. However, at 4 $^{\circ}$ C, LMD (4.44 ± 0.22 log CFU/ ml) and LMC (4.51 ± 0.39 log CFU/ ml) attached significantly better than PA (2.93 ± 0.96 log CFU/ ml) in SDW.

Significance: *L. monocytogenes* can form synergistic biofilms with *P. aeruginosa* under certain conditions.

Award Application: Developing Scientist Award

P3-106: PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE OF SALMONELLA INFECTIONS IN CATTLE

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Non-typhoidal *Salmonella* is estimated to cause the highest economic impact of any foodborne illness in the United States, with associated costs of approximately \$17.1 billion. Antibiotic therapy is frequently used as the initial strategy to manage infections caused by *Salmonella* spp. Nevertheless, the extensive misuse of antibiotics has raised significant concerns regarding the emergence of antimicrobial resistance.

Purpose: Our study aims to comprehensively characterize *Salmonella* isolates from necropsied cattle by analyzing their antimicrobial resistance profiles, biofilm-forming abilities, and distribution of virulence genes and antimicrobial resistance genes (ARGs).

Methods: A total of 1008 Samples were collected from necropsied cattle. Genotyping and serotyping characterization confirmed 23 isolates as *Salmonella enterica* subspecies. Molecular characterization was conducted to detect the profiles of virulence and ARGs. The antimicrobial resistance patterns were identified against different antibiotics. The biofilm-forming ability of the isolated bacteria was assessed using a crystal violet assay and the motility phenotypes of the isolates were assessed on soft-agar plates.

Results: *Salmonella* Dublin was the most common stereotype (34.8%). All isolates with a MAR index higher than 0.2 indicate multidrug-resistant (MDR). Most of the isolates showed high resistance to chloramphenicol, tetracyclines, beta-lactams, and macrolides. For the virulence genes, *sopB*, *spvC* and *hliA* were detected in 94.7%, 86.96% and 82.6% of isolates, respectively. Additionally, these isolates produced biofilm at different levels of intensity, and 47.8% of isolates were strong biofilm producers. Eighteen isolates (78.3%) exhibited swarming motility, and sixteen isolates (72.7%) displayed high swimming activity.

Significance: The increasing prevalence of MDR *Salmonella* poses significant public health risks. These resistant strains can reduce the effectiveness of

standard treatments and elevate outbreak risks. Strengthening surveillance, regulating antibiotic use in livestock, and improving infection control are essential to mitigating these threats.

Award Application: Developing Scientist Award

P3-107: HIGH-THROUGHPUT PHENOTYPIC CHARACTERIZATION OF MICROBES WITH TIME-LAPSE IMAGING

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Time-lapse imaging is one of the most powerful tools for phenotypic characterization and visualization in microbiological studies. However, the large footprint of Petri dishes presents a significant challenge for time-lapse imaging, particularly in high-throughput settings.

Purpose: This study aimed to demonstrate phenotypic characterization of 96 microbial samples in parallel with time-lapse imaging. As a proof of concept, we evaluated 16 microbial strains on XLT4 (Xylose-Lysine-Tergitol 4) agar, a widely used selective agar for differentiation and isolation of *Salmonella*.

Methods: The study was conducted on MagiCyte MB, the world's first microplate-based microbial culture and time-lapse imaging platform. The platform achieves significant reductions in instrument complexity, cost and footprint by replacing Petri dishes with a standard ANSI/SLAS 96-well plate. Sixteen microbial strains, including 8 *Salmonella* strains and 8 non-*Salmonella* strains including *E. coli*, *Citrobacter*, and *Klebsiella*, were used in this study. A 96-well "sample" plate containing the samples was loaded into the MagiCyte MB, together with an empty 96-well "culture" plate and a trough containing molten XLT4 agar. Inside MagiCyte MB, each sample is serially diluted into 6 wells on the sample plate. All 96 diluted samples were then plated into the "culture" plate with XLT4 agar and incubated for 12 hours. During incubation, MagiCyte MB took an image of each well every hour. Time-lapse images of each well were automatically analyzed to assess microbial growth, morphological change, and metabolic activities.

Results: The time-lapse images clearly showed the growth of microcolonies. All 8 *Salmonella* strains showed *Salmonella*'s "signature" dynamic metabolic activity: xylose fermentation followed by lysine decarboxylation. Non-*Salmonella* strains could be easily differentiated from *Salmonella* strains by their morphological and metabolic dynamics.

Significance: The high-throughput time-lapse imaging tool can be easily adapted

to other microbiological studies, providing the research community with a cost-effective, space-saving, yet powerful phenotypic characterization solution.

P3-108: INVESTIGATING PATHOGEN GROWTH IN A BOVINE CELL CULTURED MEAT MODEL

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Cultured meats (e.g., cellular agriculture, lab-grown meats) are an emerging food sector with the potential to support growing global protein demands. Many unknowns remain about the biological hazard risk of this developing food system.

Purpose: The purpose of this study was to investigate the degree to which *Staphylococcus aureus* contamination impacts bovine skeletal muscle cell growth in a cell culture model representing cultured meat tissue components.

Methods: Immortalized bovine skeletal muscle cells (iBSCs) were grown in Dulbecco's Modified Eagle Medium with 20% Fetal Bovine Serum and seeded at a concentration of 0.05×10^6 cells/mL. *S. aureus* was grown to approximately 108 CFU/mL in TSB overnight then diluted to an initial inoculation concentration of approximately 10 CFU/mL. *S. aureus* was inoculated into iBSCs at 0, 24, 48, 72, and 96 h post iBSCs seeding. *S. aureus* concentration was determined at 0, 3, 6, 9, 12, 24, 48, and 72 h by serial dilution and plating on TSB. Trypan blue cell staining was used to determine iBSCs viability and cell counts at each 24 h period.

Results: *S. aureus* grew to a concentration of up to 107 CFU/mL after 24 h in a cell culture environment and remains at that concentration through the 72 h period. No iBSCs were viable 24h after each *S. aureus* inoculation in iBSCs cultures (24, 48, 72, 96 h).

Significance: Contamination of a cultured meat system with *S. aureus*, a common human and foodborne pathogen, will likely be evident at scale and inhibit production of cellular components (e.g., muscle cells) integrated into product.

P3-109: A COMPARISON OF THE MICROBIAL QUALITY OF ORGANIC AND CONVENTIONAL STRAWBERRIES

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Strawberries are nutritionally and economically important but highly perishable agricultural commodities. Methods to extend their shelf-life are needed, and understanding the microbiological variability across individual strawberries is crucial.

Purpose: This study aimed to assess the microbiological quality of fresh strawberries from different layers within boxes, across two brands, and between organic and conventional samples.

Methods: A total of 168 samples were obtained from 28 boxes, including 7 boxes each of conventional and organic strawberries from brands A and B. Six strawberries of similar size and ripeness were selected from each box, with two samples taken from the top, middle, and bottom layers. Each strawberry was weighed and diluted from 10^{-1} to 10^{-4} in peptone water. Serially diluted samples were spread plated on Tryptic Soy Agar (TSA), MacConkey (MAC) agar, and Dichloran Rose-Bengal Chloramphenicol (DRBC) agar to enumerate aerobic plate count, coliforms, and yeasts and molds, respectively. TSA and MAC plates were incubated at 37°C for 48 and 24 hours, respectively, while DRBC plates were incubated for up to 5 days at 25°C in darkness.

Results: Coliform counts on organic samples ranged from <1 -5.7 log CFU/g (average 3.2 ± 1.6 log CFU/g) compared to 2.1-5.5 log CFU/g (average 4.1 ± 0.77 log CFU/g) on conventional samples. Total aerobic plate counts ranged from 2.3-6.5 log CFU/g (average 4.8 ± 0.92 log CFU/g) for organic and 3.0-5.7 log CFU/g (average 4.4 ± 0.63 log CFU/g) for conventional samples. Yeast and mold counts ranged from 4.3-6.1 log CFU/g (average 5 ± 0.30 log CFU/g) on organic samples and 3.8-6.4 log CFU/g (average 4.9 ± 0.52 log CFU/g) on conventional samples.

Significance: These findings highlight the natural variability in microbial populations of individual strawberries, even within a box. Accounting for this variability in experimental designs can help improve the outcomes of shelf-life studies for strawberries.

Award Application: Developing Scientist Award

P3-110: PREVALENCE AND CHARACTERIZATION OF VAGOCOCCUS LUTRAE IN SEAFOOD

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: *Vagococcus lutrae* is an emerging human pathogen attributed to increasing hospitalization cases in humans; however, its investigation-relevant biology and epidemiology are still limited.

Purpose: The present study explored *V. lutrae*'s isolation and cultivation conditions, prevalence, and biology.

Methods: Raw (n=28) and cooked, ready-to-eat (n=17) seafood products were acquired from estuarine environments, wet markets, retail stores, and a local restaurant. Using a two-step enrichment method, presumptive *V. lutrae* contaminant in enriched alone (1-step) and enriched followed by heated (80 °C) (two-step) seafood was selectively cultured on thiosulfate–citrate–bile salts–sucrose (TCBS) agar. Bacterial colonies were streaked for purification before 16S rRNA bacterial identification. Confirmed *V. lutrae* isolates were further analyzed for their glucose, oxygen (parafilm-wrapped vs. non-parafilm-wrapped agar plates), and salinity requirements; thermal tolerance (63 °C and 80 °C); antibiotic sensitivity profiles; urease activity; and hemolytic virulence.

Results: Of 135 bacterial isolates from raw (94) and cooked (41) seafood organisms, 19 (13.5%) confirmed *V. lutrae* were exclusively recovered from crab (2), shrimp (10), lobster (6), and cooked shrimp (1). Of these, 3 (15.8%) and 16 (84.2%) were regular (1-step) and heat-resistant (2-step) isolates with exclusive black or yellow colonies on TCBS agar. They were more prevalent in raw (17.9% of 28) than cooked seafood (5.9% of 17). Growth assays revealed tolerance to glucose (0–2%), oxygen, salinity (0.5–20.5%), and extended high-temperature exposure (63 °C for 8 h). These conditions may dictate its culturable viability in suspension or on agar. Antibiotic-sensitivity, urease, and hemolysis assays showed the presence of gentamycin-resistant, urease-negative, and hemolysis-negative *V. lutrae*, respectively.

Significance: Emerging foodborne pathogen contaminants could readily invade the established food safety regime. The present study improves knowledge of *V. lutrae*

isolation, cultivation, prevalence, and biology to enhance its detection and inform food safety and public health efforts.

P3-111: ISOLATION OF BACTERIOPHAGES FROM WASTEWATER AGAINST LISTERIA MONOCYTOGENES AND SALMONELLA AND APPLICATION ON RTE MEAT PRODUCTS.

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* is a foodborne pathogen of concern in ready-to-eat (RTE) deli and luncheon meats and regulatory limits for *Salmonella* contamination target <1 cfu/gm on raw poultry. Bacteriophages specific to *L. monocytogenes* or *Salmonella* holds potential for control strategies for both RTE and raw meats.

Purpose: Our objectives were to isolate bacteriophages from wastewater sources that could be used to control *L. monocytogenes* and *Salmonella* on raw and RTE meats.

Methods: Samples from city sewage and dairy wastewater were collected and added to Tryptic Soy Broth (TSB) containing 0.1% CaCl₂. Each sample was enriched with specific *L. monocytogenes* strains and incubated at 30° C, 150 RPM for 12 hrs. Enriched samples were centrifuged and double filtered using 0.22µm syringe filters. Phage lytic activity was tested using a spot-on-lawn method and efficiency-of-plating (EOP) on Tryptic Soy Agar + 0.1% CaCl₂. Several lytic phages were also detected that demonstrated strong lytic activity against a bacterial sewage isolate and was tested on RTE ham during 2 trials, each comprising 5 treatments (2 controls and 3 experimentals); each treatment included triplicate sample replications/day, spanning day 0, 1, 2, and 3. Significant differences were determined by 1-way analysis of variance (ANOVA). Bacteriophage extracts were examined by Transmission Electron Microscopy (TEM).

Results: The sewage isolate was identified as *Salmonella* Typhimurium by 16S rRNA PCR and isolated phages provided up to a 4-log reduction of *Salmonella* when tested on deli ham slices. The phages showed inhibition of *S. Typhimurium*, *S. Infantis*, and *S. Thompson* with equivalent high EOP regardless of host. Anti-listerial bacteriophage were also detected against *L. monocytogenes* using sewage enrichment with specific strains. High titer enrichment provided levels as

high as 10^9 - 10^{10} pfu/ml. Visualization by TEM demonstrated that both *L. monocytogenes* and *Salmonella* phages had typical icosahedral capsids and striated sheaths.

Significance: Recent outbreaks of *L. monocytogenes* on sliced deli meats and upcoming regulations limiting *Salmonella* levels in raw poultry will require additional approaches to prevent contamination on these food items. We feel these bacteriophages have potential as antimicrobial interventions on raw and processed RTE meats.

Award Application: Developing Scientist Award

P3-112: ASSESSING LISTERIA MONOCYTOGENES BLOOD-BRAIN BARRIER CROSSING AND ANTIBIOTIC RESISTANCE ACROSS FOOD AND CLINICAL ISOLATES

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* (Lm) from contaminated food products can cause neurolisteriosis in humans and ruminants; however, the impact of Lm's biodiversity on tropism to penetrate the blood-brain barrier (BBB) remains poorly understood.

Purpose: This study evaluates fifty (50) Lm isolates from food or animals and clinical cases for their ability to cross the BBB and antibiotic resistance profiles.

Methods: Fifty food or animal (22) and clinical (28) Lm strains were analyzed for adhesion, invasion, translocation, cytotoxicity, and antibiotic susceptibility using HBEC-5i cells. Assays included adhered bacterial CFU quantification, gentamicin protection, Transwell systems, lactate dehydrogenase measurement, and Kirby-Bauer tests. An MOI of ~100 was used, and *Listeria innocua* and *Listeria ivanovii* served as controls. Experiments were performed in triplicate and analyzed using one-way ANOVA with Tukey's multiple-comparison test.

Results: The clinical isolates 19115, 171, and CAP-D05 and the food/animal isolates ATCC 19117, and EGD exhibited the highest adhesion rates (4%; $P < 0.05$). The clinical F4243 (0.25%) and food-isolated F45 (0.27%) were the most invasive isolates ($P < 0.01$). The food-isolated ATCC 19116 and the clinical 171 isolates translocated most efficiently (2.7%; $p < 0.01$). Clinical 19115 and CHLR1 strains induced the most cytotoxic effects (47%; $P < 0.01$) on HBEC-5i barriers. Serotype-based analysis suggested that clinical serotype 4b isolates were the most invasive. Food and clinical Lm isolates were most sensitive to amoxicillin and gentamicin, while several isolates showed resistance to ampicillin, oxacillin, and cefotaxime. Several clinical isolates showed resistance to penicillin.

Significance: A comparison of phenotypic abilities to invade the BBB indicates that clinical isolates of serotype 4b exhibited the most hypervirulent traits, many

showing antibiotic resistance to ampicillin, oxacillin, cefotaxime, and penicillin. Since Lm causes neurolisterosis in humans and animals, understanding the impact of Lm biodiversity in BBB invasion between foodborne and clinical isolates can help prevent zoonotic transmission through animal-derived food products.

Award Application: Undergraduate Student Award

P3-113: IMPACT OF LACTIC ACID ON PLANKTONIC AND SESSILE CELLS OF WILD-TYPE AND RIFAMPICIN-RESISTANT O157 AND NON-O157 SEROGROUPS OF SHIGA TOXIN PRODUCING ESCHERICHIA COLI ON TWO ABIOTIC SURFACES

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Microbial biofilms are the leading cause of bacterial infections. The O157 and non-O157 serogroups of Shiga toxin-producing *Escherichia coli* are important pathogens of public health concern associated with various food and food contact surfaces.

Purpose: The current study compared biofilm formation of O157 and six non-O157 serogroups (O26, O45, O103, O111, O121, and O145) of wild-type and rifampicin-resistant *E. coli* on surface of rubber and stainless steel (finish 2b, type 304).

Methods: The study was a complete randomized block design with two biologically independent repetition, each considered as blocking factor for studying biofilm formation of six-strain mixture of wild-type and rifampicin-resistant O157 and non-O157 *E. coli*. Strains were inoculated on the surface of the above-mentioned two abiotic surfaces and after removal of loosely attached cells, the biofilms were recovered from coupons via sonication before and after treatments (immersion for 60 seconds) with 3% and 30% lactic acid. The log-transformed data were then statistically analyzed using Analysis of Variance, followed by Tukey and Dunnett mean separation at type I error level of 5%.

Results: Lactic acid at 3% and 30% were effective ($P < 0.05$) for reduction of various serogroups and phenotypes of the pathogen in planktonic state by more than 99% and close to the detection limit. However, the same treatments lost considerable efficacy against one- and two-week-mature biofilms. As an example,

after treatments with 3% lactic acids, more than 4 log CFU/cm² of wild-type *E. coli* O157:H7 were recovered from surface of stainless-steel coupons for two-week-mature biofilms, while the same treatment reduced the planktonic cells nearly to the detection limit.

Significance: A treatment highly effective against planktonic cells of the pathogen illustrated diminished capability for eliminating the sessile cells of Shiga toxin-producing *E. coli*. These results emphasize the importance of preventive measures against the proliferation of bacterial biofilms on abiotic food contact surfaces.

Award Application: Developing Scientist Award

P3-114: ANTIMICROBIAL POTENTIAL OF PSYCHROTROPHIC LACTIC ACID BACTERIA ISOLATED FROM KIMCHI AGAINST FOODBORNE PATHOGENS

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: The use of lactic acid bacteria (LAB) as fermentation starter cultures has emerged as an effective strategy to regulate fermentation by minimizing the influence of variables; however, most research has focused on dairy-derived mesophilic strains, neglecting the distinct fermentation characteristics required for low-temperature products including kimchi and fruit-vegetable beverages.

Purpose: This study aimed to evaluate the antimicrobial efficacy of psychrotrophic LAB against foodborne pathogens in comparison to mesophilic LAB.

Methods: A total of 99 psychrotrophic LAB strains isolated from cabbage kimchi were tested for antibiotic resistance using E-test strips. Strains without antibiotic resistance were further evaluated for their antimicrobial activity against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes* and *Staphylococcus aureus* using a disc diffusion assay. Additionally, the organic acid content produced by psychrotrophic and mesophilic LAB under their optimal cultivation conditions was analyzed using high-performance liquid chromatography. All experiments were conducted in triplicate, and statistical analysis was performed using one-way analysis of variance.

Results: Based on assessments of antibiotic resistance and antimicrobial activity, two psychrotrophic LAB strains, *Dellaglistia algida* and *Leuconostoc gelidum* subsp. *aenigmaticum*, were selected for further study. The cell-free supernatants of these strains exhibited significantly higher antimicrobial activity against foodborne pathogens ($p < 0.05$), with inhibition zones ranging from 12.5 to 19.2 mm, compared to those of mesophilic *Leu. mesenteroides* (9.4 to 15.8 mm). Furthermore, under optimal cultivation conditions, the psychrotrophic LAB produced up to 26.6% more lactic acid and 89.3% more malic acid than mesophilic LAB.

Significance: These findings suggest that psychrotrophic LAB isolated from kimchi have potential as effective starter cultures for low-temperature fermented foods by improving microbiological safety and fermentation processes.

P3-115: MODELING THE INACTIVATION KINETICS OF LISTERIA MONOCYTOGENES IN MODEL ACIDIFIED SOLUTIONS USING HIGH-PRESSURE PROCESSING

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: High Pressure Processing (HPP) has emerged as a promising non-thermal technology for enhancing food safety by effectively reducing microbial populations. It is commonly used for processing refrigerated juices to achieve a 5-log reduction in pathogens and extend the shelf life. In this study, we investigated the inactivation kinetics *Listeria monocytogenes* in model acidified solutions using HPP.

Purpose: The aim was to develop predictive models to better understand and optimize the HPP process parameters for ensuring microbial safety in fruit juices. Inactivation kinetics of *Listeria monocytogenes* was determined at various combinations of acid type (Malic, Citric and Ascorbic Acid), pH (3.5, 4.0 and 4.5), pressure (500, 600 and 700 MPa) and time using a laboratory-scale HPP system.

Methods: A standard model solution was prepared using three different organic acids with sterile Tryptic Soy Broth. Trials were performed in triplicate followed by microbial count determination at sampling times.

Results: Greater than 5 log reduction was achieved under specific conditions, using high pressure processing. The results indicate that both higher pressures and lower pH levels significantly reduce the D-values for *Listeria* inactivation. The mANOVA

Results showed a $R^2 = 0.974$ for Citric acid indicating a good-fit of the model to the data.

Significance: The findings suggest that high-pressure processing, especially at 700 MPa, in combination with malic acid at lower pH levels, could be an optimal strategy for enhancing the microbial safety of acidic food products. This approach could be beneficial for industries looking to minimize microbial contamination while maintaining product quality.

P3-116: EVALUATING THE EFFECTIVENESS OF COMMERCIAL LOW SODIUM ORGANIC ACID SALTS FOR CONTROLLING LACTOBACILLUS SAKEI IN CURED AND UNCURED TURKEY DELI MEAT

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The consumer trends for low sodium food products present an opportunity to explore innovative solutions that maintain food quality while reducing sodium content.

Purpose: This study evaluates the effectiveness of sodium and potassium based organic acid salts in combating *Lactobacillus sakei*, a common spoilage bacterium, in reformed turkey deli meat at refrigerated storage (4°C).

Methods: Ten different treatments were applied to the turkey deli meat: Cured (100 ppm sodium nitrite) and uncured no antimicrobial control, 0.25–0.75% potassium acetate/diacetate dry powder blend (Provian K), 1.25%–3.50% liquid potassium lactate/sodium diacetate commercial blend, and 100 ppm sodium nitrite in the highest concentration of both antimicrobials. These deli meats were inoculated with *L. sakei* to make 2.5–3.5 log CFU/g, vacuum packed, and stored at 4°C for 42 days. At each sampling point, samples were plated onto MRS agar for enumeration of lactic acid bacteria. 6 log CFU/g was considered as the spoilage limit. Data was analyzed using ANOVA in SAS JMP 17 at $P < 0.05$.

Results: Moisture content and water activity of no antimicrobial control were 77.66% and 0.9730–0.9732, respectively. Potassium acetate/diacetate formulations had 74.69–77.23% moisture and water activity 0.9612–0.9690, while potassium lactate/sodium diacetate had 76.61–77.45% moisture and 0.9654–

0.9732 water activity. The level of *L. sakei* on Day 0 was 2.5–3.5 log CFU/g. The shelf life of the deli meat was highly increased with 0.75% potassium acetate/diacetate blend compared to the control, giving 14 days of extension to 29 days. The *L. sakei* load of highest concentration of potassium acetate/diacetate and potassium lactate/sodium diacetate commercial blend were significantly lower ($P < 0.05$) than all other treatments on Day 29, exhibiting 5.68 log₁₀ CFU/g and 5.75 log₁₀ CFU/g respectively, while all other treatments had exceeded 7 log₁₀ CFU/g.

Significance: The performance of potassium acetate/diacetate blends is comparable to the commercial potassium lactate/sodium acetate solution, providing significant shelf-life extension in turkey deli meat contaminated with *L. sakei*.

P3-117: SODIUM-FREE ALTERNATIVES IN FRESH PORK SAUSAGE PRESERVATION

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The use of lactate-based solution as an antimicrobial has been widely adopted in fresh sausage. However, sodium-free acetate has the potential to minimize cost and enhanced antimicrobial efficacy by substituting lactate from the formulation.

Purpose: This study was performed to determine antimicrobial solutions that can replace lactate-based solutions in fresh pork sausage microbial shelf life.

Methods: Fresh pork sausages were prepared with no-antimicrobial control, 2.20% potassium lactate control, 0.50% potassium acetate/diacetate treatment (Provian K), and 0.50% potassium acetate/diacetate with (0.30%) natural flavor and (0.10%) natural flavor, packed (100g per package) and placed into dark storage at 4°C for evaluation of uninoculated shelf-life. At each sampling points, samples were plated on TSA for aerobic plate count, MRS for lactic acid bacteria (LAB), VRBA for Enterobacteriaceae and CFC for *Pseudomonas* spp. Additionally, sausages were injected with *Lactobacillus sakei* to make 2-3 log CFU/100g. The results were evaluated in SAS JMP 17 using a generalized linear model, significance set at $p \leq 0.05$.

Results: The no antimicrobial control passed spoilage threshold ($\sim 6 \log_{10}$ CFU/g) at Day 16, while all antimicrobial treatments had significantly lower ($P < .0001$) counts. At Day 18, no spoilage defects were observed in antimicrobial containing treatments. Visible mold growth on Day 25 led to the termination of sampling. Moreover, lactate-based samples were dominated by Pseudomonads and Enterobacteriaceae, while acetate-based solutions favored growth of LAB. This shift is beneficial, as LAB are less fastidious and produce fewer unpleasant odors.

However, controlling these organisms by assessing the growth of inoculated *L. sakei* is important. At Day 16, natural flavor treatment had significantly less ($P=0.0002$) outgrowth of *L. sakei* than the other treatments, exhibiting 3.69 log₁₀ CFU/g, compared to 5.72-6.42 log₁₀ CFU/g.

Significance: Potassium acetate/diacetate is a viable replacement for lactate-based antimicrobial in fresh pork sausage. Natural flavors with secondary microbial inhibition can provide additional protection against *Lactobacillus sakei*.

P3-118: CLEAN-LABEL ANTIMICROBIALS FOR INHIBITING SPOILAGE MICROORGANISMS ISOLATED FROM FRESH MEAT SYSTEM

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Lactic acid bacteria (LAB) are recognized as the primary spoilage microorganisms in fresh meat. To address this issue and extend the shelf life of fresh meat, current market trends emphasize the use of clean-label solutions.

Purpose: This study evaluates the antimicrobial properties of natural, clean-label agents against spoilage bacteria isolated from fresh meat system.

Methods: Antimicrobial solutions were prepared in deMan Rogosa and Sharpe broth with no antimicrobial, 0.6% market control (dry vinegar), 0.6% and 0.8% dry vinegar, 1.5% sodium-potassium buffered liquid vinegar, 1.5% potassium buffered vinegar, 1.5-3.5% vinegar-fermentate system, and combination of natural flavor system 0.3% with 1.2% sodium-potassium buffered liquid vinegar, 1.5% potassium buffered liquid vinegar, and 0.6% and 0.8% dry vinegar. The pH of the solution was adjusted to 6 and aliquoted to 100 well plates inoculated with lactic acid bacteria (6 log CFU/mL) isolated from the fresh meat system. The plates were then incubated at 30°C using Bioscreen-C Pro for 7 days. Growth curves (600 nm) were fitted using modified Gompertz equation to determine the maximum growth rate ($\mu_{\max}$; d⁻¹) and lag time (days, d). Generated $\mu_{\max}$ values were used to determine the MIC. The MIC was defined as the lowest concentration at which no growth occurred. Data was analyzed using one-way ANOVA at $p < 0.05$.

Results: All the tested antimicrobial exhibited significant inhibition ($p < 0.05$) compared to the no antimicrobial control. All the vinegar-natural flavor (MIC 0.9-1.8%) and vinegar-ferment systems (MIC 1.5%) showed no growth for the lactic acid bacteria tested. For liquid and dry vinegar alone, there was a significant increase in lag time (~ 2 d) and reduction of growth rate of LAB ($\mu_{\max}$ 0.4-0.8 d⁻¹) compared to control ($\mu_{\max}$ 1.04 d⁻¹, 1 d).

Significance: This study demonstrated the potential of utilizing vinegar-natural

flavor and vinegar-ferment systems to achieve inhibition of spoilage lactic acid bacteria from the fresh meat.

P3-119: EFFECT OF VINEGAR AND VINEGAR-NATURAL FLAVOR SYSTEM ON OUTGROWTH OF LISTERIA MONOCYTOGENES AND LEUCONOSTOC MESENEROIDES IN FRANKFURTERS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Vinegar systems can be implemented as clean-label multifunctional systems for food preservation and *Listeria monocytogenes* control.

Purpose: The objective of this study was to reduce food safety risks and food spoilage due to outgrowth of *Listeria* and *Leuconostoc* in frankfurters using a clean-label multifunctional system consisting of vinegar and natural flavor.

Methods: Four treatments of frankfurters were prepared, including a control without preservatives and samples containing 0.90% IsoAge DV110 (dry vinegar) and 0.75% and 0.90% dry vinegar-natural flavor system. Each treatment was divided into two portions, of which one was inoculated with a five-strain *Listeria monocytogenes* inoculum (serotypes 1-2a, 1-2c, 4a, 4b, 6a/6b) and the other was inoculated with slime-producing *Leuconostoc mesenteroides*, targeting 2–3 log CFU/g. Samples were vacuum-packed in 100 g portions of two links (50 g each) and stored at 4°C for 24 days (*Leuconostoc*) and 120 days (*Listeria*). At each sampling, samples were homogenized and plated onto deMan, Rogosa, Sharpe agar for *Leuconostoc* and Modified Oxford medium for *Listeria*. The effect of treatment was evaluated using a generalized linear model with significance set at $P \leq 0.05$.

Results: For both *Listeria* and *Leuconostoc*, the 0.75% and 0.90% dry vinegar-natural flavor systems showed significantly ($P < 0.05$) improved antimicrobial performance compared to IsoAge DV110 and control. For *Listeria*, the 0.90% dry

vinegar-flavor system performed better than 0.90% dry vinegar, reaching 2 logs outgrowth after 100 and 80 days at 4°C, respectively. However, both 0.75% and 0.90% concentrations of the dry vinegar-flavor system were significantly effective in controlling *Leuconostoc* for 17 days (6 log CFU/g), compared to 0.75% dry vinegar alone (9 days).

Significance: This study demonstrates the possibility to increase food safety and shelf life using a clean-label multifunctional system of dry vinegar-flavor system.

P3-120: ASSESSING THE EFFICACY OF LIQUID SMOKE IN COMBINATION WITH ORGANIC ACID SALTS IN INHIBITING LACTIC ACID BACTERIA AND LISTERIA MONOCYTOGENES GROWTH IN FRANKFURTERS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Given the market trend of sodium reduction, potassium-based solutions are preferred antimicrobials.

Purpose: To evaluate synergy between Cloud 230 and potassium acetate/diacetate (Provian K) for shelf-life extension of frankfurters stored at 4 °C.

Methods: Five treatments including control (0.5-1.5% Cloud 230 + 0.55% Provian K) were prepared for microbiological challenge test and sensory panel respectively. The samples (two frankfurters in-package) were inoculated with *L. monocytogenes* or *L. sakei*, vacuum packaged, and stored at 4°C for up to 120 days. At each sampling, frankfurters were homogenized and plated onto Modified Oxford medium for *L. monocytogenes* and Man, Rogosa and Sharpe for *L. sakei* enumeration. The result was compared using ANOVA ($p < 0.05$). Sensory analysis was conducted to assess the impact of Cloud 230 on frankfurter attributes using a 9-point scale.

Results: The control treatment exhibited fastest outgrowth (>2 log CFU/g) of *L. monocytogenes* by 21 days at 4 °C. The 0.55% Provian K treatment significantly ($p < 0.05$) controlled *L. monocytogenes* outgrowth (2 log CFU/g) until 49 days. Addition of 1-1.5% Cloud 230 further extended this control to more than >63 days. For *L. sakei*, the control sample (no antimicrobial) spoiled by day 15 while 0.55% Provian K kept the *L. sakei* counts below 7 log CFU/g until day 22. In addition, combination of 1.0% Cloud 230 and 0.55% Provian K significantly extended the shelf life to >35 days at 4°C. Addition of Cloud 230 at 1% enhanced meaty, smoke and umami perception on day 7. Overall, smoky perception reduced

over storage period. Provian K and Cloud 230 do not deliver enhanced smokiness perception in hot dogs (day 7, 14, 30) Conclusion: At storage temperature of 4°C, 1-1.5% Cloud 230 was effective in enhancing the efficacy of 0.55% Provian K by providing significantly higher shelf life of frankfurters when combined as compared to Provian K by itself.

P3-121: EVALUATION OF SMOKE FOR SALMONELLA INTERVENTIONS IN FRESH POULTRY

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The USDA's stringent policies aimed at reducing *Salmonella* in chicken to mitigate foodborne illnesses, which affect millions annually.

Purpose: The objective of this study is to assess the efficacy of smoke for the control of *Salmonella* in fresh chicken application.

Methods: Skinless chicken breast, weighing on average 100 ± 3 g, were used for the study. The samples were spot inoculated with a two-strain cocktail of *Salmonella* Typhimurium and *Salmonella* Enteritidis at 1 mL/100 ± 3 g to achieve a target inoculation level of 2–3 log CFU/g. The inoculum was then spread using a sterile L spreader and rested for one hour in a biosafety cabinet for attachment. The samples were then treated with liquid Smoke A and B by spray application using an airbrush and ground after 30 minutes. Treated ground chicken samples (~50 g) were air flushed with 70% nitrogen and 30% carbon dioxide and stored at 4°C for up to 30 days. At each sampling point, duplicate samples were homogenized and plated onto XLT4 agar selecting for *Salmonella*, and TSA for total aerobic plate count. Means of each treatment group were compared by Tukey-Kramer adjustment (Tukey's Honestly Significant Difference test) at a 5% significance level. Treatment effect was evaluated with one-way ANOVA in SAS JMP 17. Significance in this study is set at $P \leq 0.05$.

Results: All inoculated samples reached the target inoculum levels of 2–3 log CFU/g. The smoke solutions, Smoke A and Smoke B, showed cidal effects on *Salmonella* in ground chicken. On Day 0, 1.5% Smoke A significantly ($P \leq 0.05$) lowered the *Salmonella* levels from 2.80 log CFU/g in untreated samples to 1.85 log CFU/g, whereas 2.0% Smoke A and 0.3–1.2% Smoke B provided levels below the detection limit for *Salmonella*. The antimicrobial efficacy of Smoke B was significantly greater ($P \leq 0.05$) than Smoke A.

Significance: The spray application of Smoke A and Smoke B provides an effective

intervention strategy and clean-label solution for *Salmonella* control in ground poultry applications.

P3-122: EFFICACY OF ANTIMICROBIAL INTERVENTIONS AGAINST SALMONELLA IN FRESH BEEF

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Category: Meat, Poultry and Eggs

Abstract:

Introduction: Combining functional components and natural flavor effectively reduces *Salmonella* in fresh beef, offering a promising intervention strategy for food safety.

Purpose: To evaluate the efficacy of combination functional components and natural flavor for control of *Salmonella* in fresh beef.

Methods: Beef chuck rolls (4×4 inches, 100±3 g) were spot inoculated with a rifampicin-resistant three-strain cocktail of *Salmonella* Typhimurium, *Salmonella* Infantis, and *Salmonella* Enteritidis at 1 mL/100±3 g to achieve an inoculation level of 2–3 log CFU/g with one hour of attachment. The samples were then treated with vinegar (1.5%), lauric arginate (200 ppm), and propionic acid (0.25%) in combination with natural flavor (0.3%) by spray application using an airbrush and ground after 30 minutes. Treated ground beef samples (~50 g) were air-flushed with 70% nitrogen and 30% carbon dioxide and stored at 4°C for up to 30 days. At each sampling point, duplicate samples were homogenized and plated onto XLT4 agar for *Salmonella* enumeration and TSA for total aerobic plate count. Means of each treatment group were compared using Tukey-Kramer adjustment (Tukey's Honestly Significant Difference test) at a 5% significance level. Treatment effects were evaluated with one-way ANOVA in SAS JMP 17. Significance in this study was set at $P \leq 0.05$.

Results: Overall, the combinations tested in the study showed cidal efficacy against *Salmonella* in fresh beef. The natural flavor at 0.3% was effective in reducing *Salmonella* by 0.5–0.6 log CFU/g. Vinegar at 1.5% provided a 0.3–0.4 log reduction, while combining it with natural flavor enhanced the reduction to 1 log CFU/g. The maximum log reduction of 1.4 log CFU/g was achieved using a combination of 0.3% natural flavor and 0.25% propionic acid. The combination of lauric arginate (200 ppm) and natural flavor (0.3%) resulted in a 1–1.2 log reduction of *Salmonella*.

Significance: The spray application of vinegar, lauric arginate, and propionic acid

in combination with natural flavor provides an effective intervention strategy for *Salmonella* control in ground beef.

P3-123: SPOTTING ALLIES AND ADVERSARIES: NATURAL BACTERIA AFFECT PATHOGEN SURVIVAL

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract: Introduction: Biofilm formation by foodborne pathogens may play an important role in meat contamination. Previous studies suggested that via mixed-species biofilm formation, the environmental microorganisms may enhance or inhibit pathogen tolerance and survival.

Purpose: Isolate individual bacterial strains from natural mixed biofilms collected at meat plants and investigate the impact of pathogen-environmental species interactions on pathogen sanitizer tolerance.

Methods: individual environmental bacterial strains in floor drain samples collected from 3 beef plants were isolated and their biofilm forming ability evaluated. Strong or weak biofilm formers were selected and individually paired with one pathogen strain (*S. enterica* or *E. coli* O157:H7) to form mixed biofilms, which was established either by co-incubating the two strains simultaneously or by inoculating the pathogen strain on the solid surface pre-colonized by the environmental strain. The mixed biofilms were treated with quaternary ammonium compound or chlorine, and pathogen survival was measured on selective agar plates and compared to pathogen survival in its sanitizer-treated single-strain biofilm.

Results: Multiple environmental bacterial strains (n=31) were isolated. The biofilm forming ability of these isolates varied significantly depending upon the strains. The pathogens were able to form mixed biofilms with the environmental companion strains. Temperature, sequence of colonization, and biofilm forming ability of the companion strains affected the pathogen cell densities in mixed biofilms. The diverse environmental bacterial strains exhibited distinct impact on pathogen survival within the mixed biofilms, either enhancing, decreasing, or exerting no significant influence on pathogen tolerance against sanitization.

Significance: This study indicates that the unique interactions between the pathogens and the individual environmental species could influence pathogen persistence, therefore, may provide a natural alternative approach for biofilm control.

P3-124: EVALUATING ANTIMICROBIAL RESISTANT BACTERIA IN GOAT, SHEEP, AND LAMB

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The demand for goat, sheep, and lamb meat is increasing in the U.S. and globally; however, the prevalence of bacterial antimicrobial resistance (AMR) in these animals at slaughter is poorly understood. AMR is a recognized public health threat in the U.S. and across the globe. It impacts the ability to treat common infections, reduces antimicrobial drug effectiveness and limits treatment options.

Purpose: To address knowledge gaps, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) conducted its first nationwide cecal (intestinal) content sampling study for goat, sheep, and lamb under the National Antimicrobial Resistance Monitoring System (NARMS).

Methods: From February 2020 to September 2022, FSIS collected 1,025 cecal content samples from goat, sheep, and lamb at 449 FSIS-regulated slaughter establishments across the U.S. Samples were cultured and tested for the presence of *Salmonella*, *Campylobacter*, *Enterococcus*, and generic *E. coli*. Isolates were evaluated for resistance to various antimicrobial agents using standard susceptibility testing methods. Data were analyzed to determine pan-susceptibility, resistance to individual classes, and the presence of multi-drug resistance profiles.

Results: Analyses showed that 91% of *Salmonella*, 23% of *Campylobacter*, 61% of *Enterococcus*, and 48% of generic *E. coli* were not resistant (pan-susceptible) to the antimicrobials tested. Resistance to 1–2 antimicrobial classes was highest in *Campylobacter* (74%) and lowest in *Salmonella* (8%). Resistance to quinolones (ciprofloxacin and nalidixic acid) was exhibited in *Campylobacter*. Resistance to tetracycline was highest among *Salmonella*, generic *E. coli*, and *Enterococcus*. Multi-drug resistance (resistant to three or more classes of antimicrobial drugs) was highest in generic *E. coli* (9%). A host-adapted *Salmonella* IIIb 61: K: 1,5, (7) (enterica subspecies diarizonae) that can cause serious illnesses in sheep and lamb was recovered in disproportionately higher numbers from cecal samples of sheep (63%) and lamb (52%) compared with goats (2%).

Significance: This study provides a representative national snapshot of AMR occurrence in pathogens (*Salmonella*, *Campylobacter*) and indicator bacteria (generic *E. coli*, *Enterococcus*) from goat, sheep, and lamb collected from cecal content at the time of slaughter. This study may serve as a model for future studies among other food-producing animals and commodities.

P3-125: CONSUMER KNOWLEDGE, ATTITUDES, AND PRACTICES REGARDING MEAT AND POULTRY SAFETY AT FARMERS' MARKETS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Although fruits and vegetables are the mainstays of farmers' market sales, there is a growing trend of consumers purchasing meat and poultry directly from farmers.

Purpose: This study was to investigate consumers' knowledge, attitudes, and practices regarding the safety of meat and poultry products sold at Kentucky farmers' markets.

Methods: A 24-question, paper-based survey was developed and administered to Kentucky farmers' markets shoppers. The data were statistically analyzed using Stata17 software through multinomial logit and ordered probit models to examine factors associated with level of knowledge, attitude, and practice towards meat and poultry safety among consumers. Significant differences were determined at 1%, 5%, and 10% probability levels.

Results: Results revealed that perceptions of better product quality are significantly influencing consumer attitudes. Consumers who believe the quality of products at farmers' markets is superior are more likely to trust the safety of meat and poultry sold at these markets (0.593, $p < 0.01$). Female consumers are significantly more likely to support government regulation of meat and poultry products sold at Kentucky farmers' markets (0.653, $p < 0.1$). With regard to consumer practices, females (0.756, $p < 0.05$) are 75.6% more likely to use disposable plastic bags for poultry at farmers markets to contain any leakage that could cross-contaminate cooked foods or fresh produce, indicating a gender difference in concern for cross-contamination during shopping. Higher education is positively associated (0.960, $p < 0.1$) with always cooking poultry to a safe internal temperature.

Significance: This research has revealed a low level of risk perceptions and meat and poultry safety practices amongst the consumers. Risk perceptions and meat and poultry safety practices were influenced by gender, age, and education.

P3-126: FOODBORNE CAMPYLOBACTERIOSIS IN NEW ZEALAND: A SUCCESSFUL RISK REDUCTION STORY

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Reduction in the rate of campylobacteriosis attributable to food has long been a food safety goal for New Zealand (NZ). While previous risk management activities have been very successful, because foodborne campylobacteriosis remains the predominant notifiable foodborne illness, NZ in 2020 set a new public health goal to reduce disease incidence by a further 20% (88 to 70 cases/100,000) by the end of 2024.

Purpose: To implement and evaluate a new *Campylobacter* Action Plan (CAP) focused on chicken meat, the predominant source of campylobacteriosis in NZ, with the goal of reducing incidence through targeted regulatory, industry, and consumer interventions.

Methods: The CAP incorporated regulatory and non-regulatory measures across the food chain, from production to consumption. Oversight was provided by a governance group with representatives from NZ Food Safety (NZFS), the Poultry Industry Association, Ministry of Health, Consumer NZ, and major supermarket retailers. Since 2006, broiler chicken processors participated in daily *Campylobacter* monitoring programs. Performance was assessed against a *Campylobacter* performance target (CPT), which was tightened under the CAP. Consumer education campaigns on safe meat handling were run during high-incidence summer months.

Results: The prevalence of *Campylobacter* on carcasses decreased from 14.5% in 2019 to 10% in 2023. High-count samples ($>\log_{10}$ 3.78 CFU/carcass rinsate) decreased from 1.7% to 1.1%. The 2023/24 consumer campaign achieved a 39% recall rate and a 31–38% cognitive response rate, with viewers reporting learning something new from the videos.

Significance: The downward trend in foodborne campylobacteriosis from 2019 through Q3 of 2024 indicates that collaboration among producers, retailers, consumers, and NZFS is likely to achieve the national goal of a 20% reduction in disease incidence by the end of 2024.

P3-127: COMPARATIVE ANALYSIS OF TRADITIONAL SALMONELLA SEROTYPING AND DEEP SEROTYPING FOR ENHANCED SEROTYPE DIVERSITY DETECTION IN SWINE BARN BOOT SOCK SAMPLES

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* serotyping involves isolation, PCR to detect genes encoding somatic and flagellar antigens, and subsequent confirmation through antisera agglutination (hereafter “PCR-Agg”). Alternatively, Deep Serotyping (DST) by CRISPR-SeroSeq is not dependent on isolation and characterizes the serotype diversity within a sample by leveraging high-throughput sequencing of the spacer sequences in the native *Salmonella* CRISPR arrays.

Purpose: Comparison of PCR-Agg and DST serotyping for characterizing 50 pen surface boot-sock swab samples obtained from swine finishing barns.

Methods: Fifty boot-sock samples were processed and suspended in Buffered Peptone Water and enriched in BAX-MP for PCR-Agg or in Rappaport-Vassiliadis R10 (RVR10) or tetrathionate (TT) broth for DST. For PCR-Agg, enrichments were plated on selective media and up to nine presumptive colonies per sample were serotyped. For DST, DNA was extracted from 1 ml of enriched cultures using the Wizard Genomic DNA Purification Kit, followed by amplification of the *Salmonella* CRISPR spacers that were pooled and sequenced on an Illumina NextSeq with 150 cycles. Reads were analyzed using BLAST against a CRISPR spacer database for 150 serotypes. A generalized linear mixed model with a binomial distribution and Bonferroni-adjusted pairwise comparisons was applied to evaluate difference in detection of serotype diversity among methods, with samples treated as random effects.

Results: Among samples with *Salmonella* detection, PCR-Agg identified more than one serotype in 10/50 samples, DST-RVR10 in 6/38 samples, and DST-TT in 17/43 samples. DST-TT detected a significantly higher ($p=0.024$) and numerically greater ($P = 0.266$) number of samples with multiple serotypes compared to DST-RVR10 and PCR-Agg, while no significant ($P = 0.737$) difference was observed between PCR-Agg and DST-RVR10. Senftenberg and Typhimurium (including

monophasic Typhimurium) were the most frequently detected serotypes across all methods.

Significance: DST-TT offers a more comprehensive alternative to PCR-Agg, capable of resolving serotype diversity within mixed populations.

P3-128: SALMONELLA LIMITS (SALLIMITS™) LOD10 FOR VARIOUS POULTRY PRODUCTS USING HYGIENA'S REAL-TIME PCR SOLUTIONS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The new regulatory framework for *Salmonella* in raw poultry products proposed by the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) will focus on alternative approaches to control and reduce *Salmonella* contamination throughout the production chain. These initiatives are a comprehensive strategy that will analyze pre-harvest risk through final product.

Purpose: The purpose of this study was to evaluate various poultry products at 10 CFU per g/mL using 3 different real-time PCR methods, including one for the detection of *Salmonella* species, one for highly pathogenic *Salmonella* and one for *Salmonella* serotypes Enteritidis and Typhimurium.

Methods: Boot swabs, poultry carcass swabs (MicroTally®), and ground chicken were prepared separately in individual studies. Analytical test portions were inoculated with a cold-stressed culture of *Salmonella* Typhimurium at 10 CFU per g/mL to create 10 replicates. Following inoculation, samples were enriched according to their respective test

Methods and incubated at 42 °C for 5-24 hours. Aliquots were tested in quintuplicate for repeatability on 3 real-time PCR assays.

Results: Qualitative results were assessed for the shortest incubation time that returned 100% positive

Results. For boot swabs, all samples were determined to be positive by real-time PCR at 8 hours. For carcass swabs and ground chicken, all samples were positive by real-time PCR at 6 hours. There were no differences between any assays tested. All samples were confirmed positive by culture.

Significance: The BAX® System Real-Time PCR assay for *Salmonella*, the BAX

System Real-Time Highly Pathogenic *Salmonella* assay and the foodproof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection Lyokit are sensitive and specific, meeting industry standards for poultry products contaminated at 10 CFU per g/mL, as well as providing valuable hazard information on serotype or pathogenicity.

P3-129: THERMAL INACTIVATION OF SALMONELLA AND THE SURROGATE ENTEROCOCCUS FAECIUM IN RECONSTRUCTED GROUND CHICKEN MEAT AFFECTED BY TEMPERATURE AND SALT CONCENTRATIONS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Chicken products reconstructed with salt and tripolyphosphate can be contaminated with *Salmonella*, which is responsible for approximately 35% of the foodborne illnesses associated with poultry products.

Purpose: To determine the thermal inactivation of *Salmonella* and the surrogate *Enterococcus faecium* in reconstructed ground chicken meat as affected by temperatures and salt concentrations

Methods: Fresh 500 chicken breast was grounded, inoculated with Nalidixic-acid (NaL-100 ppm) resistant *S. Typhimurium* followed by adding NaCl (0, 1.0, 3.0 and 5.0 %) + Na-tripolyphosphate (0.5%) solutions to achieve pump rates of 8%. Samples were then weighted for 10-Gram and added into filtered food sample bags vacuum packaged and followed by storing at 4°C for 42-h before heating in a circulated water bath set at 62, 66, 70, and 74C for 0 to 240 s, respectively. Counts of the pathogen cells were analyzed on tryptic soy agars plus NaL. Microbial survival populations and D-values [n=6, Global-Fit and United States Department of Agriculture (USDA)-integrated-Predictive-Modeling-program software] were analyzed by R-software.

Results: Heating temperature, salt concentrations and their interactions all affected ($p < 0.05$) the heat resistance of bacterial cells. Thermal dynamic data fit Weibull Model (RMSE=0.48 to 0.71 and AIC values=-21.45 to -56.80), but not the linear model (RMSE >1.000). Salt (3 and 5% vs 0%) added to chicken meat

increased ($p < 0.05$) the thermal resistance of *S. Typhimurium* (reduction: 1.02 vs 1.86 logCFU/g) when heated at 62°C whereas when the heating temperature increasing to 74°C the pathogen cells become more vulnerable (reduction: 3.04-4.44 vs 1.63 logCFU/g). *E. faecium* were resistant to heat compared to *Salmonella* as shown less reductions (1.02-4.44 vs 4.61-6.94 logCFU/g) and higher or similar D-values in all tested chickens.

Significance: Results suggest that thermal resistance of *Salmonella* was significantly affected by temperature, and salt concentrations, which will be useful by the poultry meat industry to develop proper thermal processes to eliminate the pathogen in chicken products. *E. faecium* is a promising pathogen surrogate could be used in thermal inactivation validation studies.

Award Application: Developing Scientist Award

P3-130: EFFICACY AND DECAY KINETICS OF PERACETIC ACID (PAA) AS A DISINFECTANT IN POULTRY PROCESSING

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: A major public health concern is pathogen contamination during poultry processing, which calls for creative solutions to reduce foodborne diseases. Enhancing sanitation procedures requires an understanding of the bactericidal effectiveness and degradation kinetics of disinfectants like peracetic acid (PAA).

Purpose: This study aims to assess PAA's efficacy and decay kinetics in simulated poultry processing environments, highlighting its interactions with organic matter and potential to improve pathogen control.

Methods: Whole chicken carcasses (~4 lbs) were immersed in 70 mg/L and 200 mg/L PAA solutions at 4°C in a bench-scale immersion chilling tank. Portable electrochemical sensors and digestion techniques were used to measure pH, PAA, total dissolved solids (TDS), and chemical oxygen demand (COD) levels. A five-strain *Salmonella* cocktail inoculum (high: 10^9 CFU/mL; low: 10^7 CFU/mL) was spread onto chicken thighs (100 ± 20 g), and their shedding and inactivation dynamics were measured over 0.5 to 10 minutes of exposure to 1–50 ppm PAA, with or without organic load, using a neutralizing buffered peptone water rinse. All studies were repeated independently at least three times.

Results: Results showed that store-bought samples decayed PAA faster than plant-sourced carcasses, and greater COD and TDS levels indicated higher organic load release in the chiller tank. Residual PAA at ≥ 10 ppm considerably reduced bacteria, and 5 ppm nearly eliminated low *Salmonella* loads immediately. PAA concentrations of 40–50 ppm with exposure durations of ≥ 2 minutes consistently achieved $>99.5\%$ bacterial inactivation at high bacterial loads. A strong correlation was observed between the organic matter exudate from chicken thighs (TDS) and PAA efficacy in *Salmonella* inactivation.

Significance: This study elucidated PAA effectiveness, decay kinetics, and

interactions with organic matter during lab-simulated poultry chilling conditions. These findings could help improve poultry safety and reduce the risk of foodborne illness associated with poultry processing by optimizing PAA disinfection dosage and exposure regimen.

Award Application: Developing Scientist Award

P3-131: TRANSFER OF SALMONELLA ENTERICA ADHERED ON DISH SPONGES TO PLASTIC SURFACES DURING SIMULATED DISHWASHING

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Salmonellosis is one of the most widespread foodborne diseases globally. A significant proportion of cases are attributed to cross-contamination from kitchen utensils, including dish sponges.

Purpose: This study aims to quantify the transfer rate of *Salmonella enterica* from dish sponges to plastic surfaces under a simulated dishwashing scenario.

Methods: The transfer of three *S. enterica* strains (S1, S2, and S3) was simulated using 1.5 x 1.5 cm dish sponges (Ds) and 3 x 3 cm plastic surfaces (Ps). Sponges were inoculated by placing them in Petri dishes containing a suspension of food waste (0.1% chicken, 0.1% egg-based, and 1% lettuce waste) with *S. enterica* (~4 log CFU/mL). After a 4-hour incubation at 25°C to allow bacterial attachment, the sponges were washed with isotonic saline to remove non-adherent cells. The dishwashing simulation involved wiping the plastic surfaces using a 2% soap solution with two horizontal and two vertical strokes. The transfer rate (TR) of *S. enterica* from Ds to Ps was calculated by $[(CFUPs) / (CFUPs + CFUDs)] \times 100$. ANOVA was used to assess strain-dependent effects on TR.

Results: The concentration of *S. enterica* in the inoculated sponges was 2.52 ± 0.28 , 3.0 ± 0.20 , and 2.7 ± 0 log CFU/mL for S1, S2, and S3, respectively. On plastic surfaces, the concentrations were 1.9 ± 0.30 , 1.4 ± 0.36 , and 1.5 ± 0.38 log CFU/mL. About 22.22% of samples—mostly S3—were below the detection limit (1.7 log CFU/surface). The transfer rates of *Salmonella* ranged from 23.95% to 44.29%, with no significant differences observed between strains ($p > 0.05$).

Significance: Although there were no statistically significant differences in TR among strains, *S. enterica* was consistently transferred to plastic surfaces, underscoring a health and hygiene risk in domestic dishwashing scenarios.

Award Application: Undergraduate Student Award

P3-132: IMPACT OF STARTER CULTURES ON LISTERIA MONOCYTOGENES REDUCTION DYNAMICS IN SALAMI

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Ready-to-eat (RTE) uncooked fermented sausages can be a source of foodborne outbreaks, emphasizing the critical need to understand pathogen inactivation processes to ensure their microbiological safety.

Purpose: This study evaluated pH change induced by three commercial starter cultures (Saga 200, Kerry; LHP, and F-LC, Chr. Hansen) and their impact on the reduction of a 5-strain *Listeria monocytogenes* cocktail ($>3 \times 10^7$ CFU/g) in salami.

Methods: Salami batters inoculated with *L. monocytogenes* were fermented to a target pH of 5.0 ± 0.1 . Saga 200 reached pH 5.0 in 12 h (sampled every 2 h), while F-LC and LHP required 18 h (sampled every 3 h). Samples were collected at seven time points in duplicate across 9 trials. Pathogen concentration, pH, and temperature were monitored in 19 and 32 mm diameter salami. The Gompertz model was applied to describe pathogen reduction dynamics, focusing on the total reduction, the rate of reduction, and time to achieve the maximum reduction rate.

Results: Differences in *L. monocytogenes* reduction dynamics were observed among the starter cultures. While the total reduction and reduction rates were not statistically significant ($p > 0.05$), the time to reach the maximum reduction rate varied significantly among the starter cultures ($p < 0.05$). Fermentation with Saga 200 had the fastest reduction rate (0.47 h^{-1}) and reached the point of maximum reduction rate earliest (4.2 h). F-LC achieved the greatest total reduction in *L. monocytogenes* levels (-1.53 CFU/g) but had the slowest reduction rate (0.16 h^{-1}). LHP fermentation balanced a high total reduction and moderate reduction rate (0.32 h^{-1}), while requiring the longest time to reach the maximum reduction rate (10.8 h).

Significance: This study highlights the critical role of starter culture selection in shaping low pH stress and ensuring food safety. It is part of a broader investigation into fermentation and drying processes in RTE sausages. The next

phase will evaluate drying kinetics and microbial behavior under worst-case scenarios.

Award Application: Developing Scientist Award

P3-133: IN-PLANT COMPARISON OF INOCULATION SITES ON BEEF CARCASSES USING SURROGATE STRAINS OF BACTERIA

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: This in-plant study conducted side-by-side comparisons of sampling between the inner foreshank and outer foreshank of a 100 cm² area on a beef shank, applying either a 25 ml BPW spongecicle or a MicroTally mitt for a total of 1 minute after a 20-minute period was allotted post-spray for attachment.

Purpose: This study was conducted to evaluate the effectiveness of two sampling methodologies of testing for pathogen surrogates in a beef production facility for validation of the effectiveness of interventions.

Methods: Samples were taken from carcasses over a two-day period. Dehided carcasses were inoculated on the foreshanks by spraying a diluted 109 concentrated culture of the surrogate onto the shanks. The carcasses were sampled at various stages of the production line process during the two-day period, testing before and after antimicrobial treatments were applied to the carcasses.

Results: A total of 250 samples were obtained and compared (125 inside and 125 outside foreshank). Statistical analysis was performed for day 1 and day 2 of the three repetitions. P-value for day one comparison was $p=0.866$. For day 2, results yielded a p value of 0.488. It can be safely deduced that there is no statistical difference between inoculation of the inner or outer foreshank. The two days gave an average p value of 0.677, nowhere near the 0.05 needed to be considered significant.

Significance: Carcass sampling is an important part of validation of antimicrobial interventions. As technology evolves, it is critical to verify that sampling devices are consistent with previous sampling and will give similar results and thus can easily be transitioned into day-to-day operations.

P3-134: ANTIMICROBIAL INTERVENTIONS REDUCE *SALMONELLA* SEROVAR COMPLEXITY IN CHICKEN PARTS AT PROCESSING

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: About 17% of human foodborne *Salmonella* illnesses in the US are linked to the consumption of contaminated chicken meat products. In recent years, salmonellosis cases have remained constant, despite a notable reduction of contamination in final products over the same timeframe. Previous studies have demonstrated that antimicrobial interventions effectively reduce *Salmonella* from post-defeathering to post-chilling; interestingly, *Salmonella* prevalence increases after the carcasses are cut into parts.

Purpose: This study sought to assess *Salmonella* prevalence and serovar complexity during broiler processing, specifically in parts with bone and skin and without. **Methods:** Tender and wing rinses were collected in four broiler plants, pre- and post-intervention, during the beginning and end of a shift (n = 480). CRISPR-SeroSeq was performed on *Salmonella*-positive samples.

Results: In breasts (tenders or whole), 51.7% (62/120) of pre-intervention samples were *Salmonella*-positive, which was reduced to 24.2% (29/120) post-intervention with significant difference between the two stages ($X^2(1, N = 240) = 18.13, p = 2.068e-05$). Wings were lower with 24.2% (29/120) pre-intervention and 7.5% (9/120) post-intervention ($X^2(1, N = 240) = 11.29, p = 0.0008$). *Salmonella* prevalence decreased by the end of the shift for both tenders (-0.8%) and wings (-6.3%). A subset of the deep serotyping results (tenders from Plant #1; n=42) revealed that 75% (18/24) of the pre-intervention tenders contained multiseroovar populations and that this was reduced to 11% (2/18) following the antimicrobial dip. Overall, three serovars were detected including two of the proposed KPIs; serovar Typhimurium was the most prevalent (93%; 39/42), followed by serovar

infantis (50%; 21/42), and serovar Enteritidis (4.8%; 2/42).

Significance: These data demonstrate that in-plant processing antimicrobial interventions reduce *Salmonella* prevalence in both tenders and wings. However, there is a difference in reduction between the two parts, suggesting that some component of tender processing poses an increased cross-contamination risk.

Award Application: Developing Scientist Award

P3-135: EFFECTS OF VARIOUS CHILLING METHODS WITH OR WITHOUT HOT WATER DIP AGAINST AEROBIC, ESCHERICHIA COLI, AND COLIFORM COUNTS ON BROILER CARCASSES

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Chicken is the most consumed meat in the US, making its safety paramount. The immersion chilling method is a cornerstone in the poultry industry's efforts to maintain food safety standards. It involves submerging freshly processed poultry carcasses in cold water, often with ice or subzero saline water, to reduce their temperature rapidly. This swift cooling inhibits bacterial growth, extending the product's shelf life and minimizing the food safety risk.

Purpose: This study aims to investigate bacterial populations present on broiler skin and in reused red water during immersion chilling and to compare these populations among different processing methods.

Methods: Sixty chickens were randomly selected from the barn for processing and the experiment was repeated three times. The four processing methods included Water Immersion Chilling (WIC), Water Immersion Chilling with Hot Water Spray (WIC/HWS), Sub-zero Saline Immersion Chilling (SSC), and Sub-zero Saline Immersion Chilling with Hot Water Spray (SSC/HWS). The design was completely randomized, with replications used as blocks. The microbial population within each replication from each sample type was averaged, and values were $\log_{10}$ (CFU/chicken breast) transformed prior to statistical analysis. Data were analyzed using SAS (v. 9.4, Cary, NC). PROC MIXED function was used with LSMEANS option and significance was considered at $\alpha = 0.05$.

Results: Mean aerobic counts across all processing methods were significantly or numerically higher on poultry carcasses before chilling than after chilling. Mean aerobic counts and coliforms were significantly reduced after chilling with WIC and SSC methods. However, no significant differences were found among processing methods for mean aerobic counts and coliforms in the reused red water. Likewise, *E. coli* was significantly lower in the SSC method but did not differ among the other treatments.

Significance: Water immersion chilling with or without salt additions reduced aerobes and coliforms on chicken carcasses. However, reused water did not differ significantly in aerobic, coliform, and *E. coli* counts.

P3-136: EVALUATION OF MEAT/POULTRY SURFACE TEMPERATURE MEASUREMENT APPROACHES FOR USE IN SURFACE LETHALITY VALIDATION TOOLS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: USDA FSIS Appendix A has identified *Salmonella* survival on fully-cooked meat surfaces as a scientific gap; however, it also requires processors to demonstrate sufficient surface lethality where surface drying may occur.

Purpose: The goal was to evaluate multiple meat surface temperature measurement methods for potential integration into surface lethality validation processes.

Methods: Beef patty surface temperatures were measured real-time during pilot-scale cooking trials, using three typical methods: (a) thin-wire or (b) barbed thermocouples inserted into the top surface of the product, or (c) rigid thermocouples inserted through the product until the tip was under the opposite surface. Commercial raw beef patties (~150 g) were obtained, held frozen, and thawed (24 h, ~4°C) prior to experimentation. Either six probes of a single type or a combination of two types were placed with the probe tip located immediately under the top surface of the patty. The patties were then cooked in a moist-air impingement oven using typical cooking parameters (218°C, 15% humidity by volume, 7 min). Immediately upon oven exit, an infrared camera captured top surface temperature to compare with probe-measured point temperatures.

Results: The mean midpoint/endpoint temperatures for methods (a), (b), and (c) were 76.9/80.6, 84.9/89.3, and 59.1/80.1°C, respectively. During this period, probe variability for methods (a) and (b) was similar, with a mean standard deviation of $\pm 6.4^\circ\text{C}$ ($P > 0.05$); method (c) was less variable, with a mean standard deviation of $\pm 2.4^\circ\text{C}$ ($P < 0.05$). At the moment of infrared imaging, probe temperature mean and variability differences were negligible ($P > 0.05$); however, probe temperatures were 7.3°C greater than infrared-measured temperatures ($P < 0.05$) at exit.

Significance: The surface of meats during cooking can be highly variable, which confounds each method's measurement accuracy. Overall, no temperature measurement method was universally better, and any lethality prediction tool

should align with a specific, explicitly described surface temperature measurement method.

Award Application: Undergraduate Student Award

P3-137: LACTIC ACID SPRAYING ON BOVINE CARCASSES MODIFIES BACTERIAL ECOLOGICAL SUCCESSION IN VACUUM-PACKED SIRLOIN STORED FOR A LONG SHELF LIFE

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The deterioration of vacuum-packed meat reduces its shelf life, causing economic losses, as well as promoting outbreaks of diseases transmitted through it.

Purpose: To evaluate whether the spraying of lactic acid on bovine carcasses influences the microbial ecological succession of vacuum-packed sirloin steaks stored for a long shelf life.

Methods: Thirty-six half carcasses were used in this study, with 18 subjected to spraying with 2.5% (w/v) lactic acid and the other 18 subjected to spraying with potable water. From these carcasses, sirloin steaks (n=60) were collected and stored at 0°C for 120 days. Microbiological analyses were performed on days 0, 30, 60, 90, and 120 from sample collection and packaging. From serial dilutions, six samples per group were plated onto PCA, VRBD, STAA, CTAS, and MRS agar for the quantification of mesophilic heterotrophic bacteria, psychrotrophs, enterobacteria, anaerobes, *Brochothrix*, and *Carnobacterium*. Shapiro-Wilk test was used to assess data distribution, and unpaired t-test was used for comparisons between groups, which were considered significant when the

Significance level was higher than 95% ($p < 0.05$).

Results: In steaks from carcasses treated with lactic acid, a significant reduction in the populations of mesophiles and psychrotrophs was observed until the 90th day of storage (mean reduction of 1.18 ± 0.03 and 1.24 ± 0.05 log CFU/g, respectively), as well as a reduction in enterobacteria until the 60th day and anaerobes until the 30th day (reduction of 1.40 ± 0.15 and 1.98 ± 0.12 log CFU/g, respectively). During

the same period, a quantitative increase in *Carnobacterium* was observed (0.72 ± 0.03 log CFU/g). A reduction in *Brochothrix* populations was also observed between the 60th and 90th days after storage (0.90 ± 0.01 log CFU/g).

Significance: This study demonstrated that the spraying of lactic acid on carcasses can modify the bacterial microbial succession in vacuum-packed sirloin steaks, potentially contributing to an extended shelf life of the product.

P3-138: REDUCTION OF SALMONELLA INFANTIS BY DAIRY-ORIGINATED PROPIONIBACTERIUM FREUDENREICHII AND SALMONELLA TYPHIMURIUM VACCINE IN MARKET-AGE BROILER CHICKENS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella infantis* is an important pathogen that circulates in broilers. Preharvest mitigation of *S. infantis* could be vital to reducing outbreaks implicated in chicken products.

Purpose: We evaluated the effect of *Propionibacterium freudenreichii* and an *S. Typhimurium* vaccine alone and in combination to (1) reduce cecal colonization, (2) reduce internal organ dissemination of *S. infantis*, and (3) assess the effect on the cecal microbiome in broilers challenged with *S. infantis*.

Methods: Thirty one-day-old chicks were distributed into five treatments (NC – negative control, PC – positive control, PF – probiotic (10^7 cfu/mL), VC – vaccinated, PV – vaccinated and probiotic (10^7 cfu/mL)). The experiment was repeated twice ($n=12$ /treatment). On week 4, birds in all treatments except NC were challenged with *S. infantis* at 10^7 cfu/bird. After 7 days, they were euthanized to collect ceca, livers, and spleens. Differences in cecal colonization and organ dissemination were tested using one-way ANOVA and chi-square tests. Metagenomic sequencing on DNA from the cecal contents was also conducted. α and β diversity differences and differential abundance of species within treatments were determined.

Results: A 1 log cfu/g reduction in *S. infantis* cecal colonization and a 50–66.67% reduction in dissemination to the liver, and a 41.67–66.67% reduction in dissemination to the spleen by *P. freudenreichii* and *Salmonella* vaccine alone and in combination compared to PC was observed ($p<0.05$). β diversity of PF–PC and PV–PC was significantly different ($p<0.05$), suggesting microbial modulation by probiotics. At the species level, a significant reduction in the abundance of multiple pathogenic bacteria was observed in PF ($p<0.05$) compared to PC.

Significance: *P. freudenreichii* and the *S. Typhimurium* vaccine could effectively mitigate *S. infantis* colonization in chickens to ensure meat safety (USDA NIFA #2020-67017-30787).

Award Application: Developing Scientist Award

P3-139: CAPACITY, REGULATORY OVERSIGHT, AND WORKER INCENTIVES IN SLAUGHTER HYGIENE: EXPERIMENTAL EVIDENCE FROM WESTERN KENYA

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: High levels of microbial contamination has previously been reported in meat processed by Kenyan slaughterhouses, which are typically basic structures lacking running water.

Purpose: This study tests whether hygiene training, provision of basic equipment, and regulatory oversight, and additionally worker incentives, can improve slaughter hygiene practices and outcomes in a low-income setting.

Methods: A randomized controlled trial with three approximately equally sized arms (one control, two treatment) was conducted in 140 slaughter facilities in western Kenya. The primary intervention, conducted in both treatment arms engaged government veterinary officers to train workers and leveraged the presence of meat inspectors to complete weekly hygiene checklists. Personal protective equipment and basic hygiene equipment and supplies were also provided. In the second treatment arm, teams of workers who collectively adhered to good hygiene practices were additionally provided with bonus payments equivalent to 25% of their wages.

Results: Regression analysis controlling pre-intervention outcomes and additional LASSO-selected controls indicates that observed hygiene practices improved significantly in treatment facilities relative to controls (0.84 SD, $p < 0.01$), and contamination of both water (-0.39 SD, $p < 0.05$) and equipment (-0.54 SD, $p < 0.01$) fell. Impacts on practices but not contamination persisted beyond the intervention period. However, microbial contamination of meat did not improve. Higher volume of business in treatment facilities (10%, $p < 0.05$) driven by improved consumer perceptions of cleanliness (29%, $p < 0.01$) may have counteracted the effects of improved practices through cross-contamination and crowding. Impacts of the intervention with worker incentives were generally similar to those without incentives.

Significance: The lack of impact on carcasses contamination implies that training, basic equipment, and improved regulatory oversight are insufficient to improve food safety outcomes in rudimentary slaughterhouses without piped water. The positive impact of these interventions on customer perceptions and business outcomes indicates market demand for better hygiene practices in this context.

P3-140: IMPACT OF COLD ADAPTATION ON THE GROWTH OF LISTERIA MONOCYTOGENES IN DELI TURKEY: A COMPARATIVE STUDY WITH NON-COLD ADAPTED STRAINS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Listeria monocytogenes* is a foodborne pathogen that poses a significant health risk, especially in refrigerated foods. Unlike other pathogens, *L. monocytogenes* can grow at low temperatures (0-4.4°C), making it a major food safety concern for refrigerated foods.

Purpose: To evaluate the impact of cold adaptation on the growth of *L. monocytogenes* in uncured, deli turkey.

Methods: Deli-style turkey (30 g) formulated with no antimicrobial (negative control), 0.7% Verdad® Powder N6, and 0.8% Verdad® Powder N30 was inoculated with a 5-strain cocktail of either cold or non-cold adapted *L. monocytogenes*. Each strain was cold-adapted by transferring 0.1 mL of overnight broth culture in tryptic soy broth supplemented with 0.6% yeast extract and incubated at 7°C for 6 d. All samples were vacuum-packaged and stored at 4.4°C until enumeration. Samples were diluted with buffered peptone water, stomached, and spread-plated on Modified Oxford agar (37°C for 48 h). Minitab 20.2 was used to perform Tukey's test to determine any significant differences in growth rates and lag time between the inoculum types.

Results: The cold and non-cold adapted *L. monocytogenes* population in the negative control sample reached 1-log outgrowth on days 5 and 8, respectively. In samples with Verdad® Powder N6, this occurred on days 23 and 30, while for Verdad® Powder N30, it was on days 15 and 30. No significant difference ($p>0.05$) in growth rates was found between the cold and non-cold adapted strains. However, the samples inoculated with cold-adapted strains had a significantly ($p<0.05$) shorter lag time compared to the non-cold adapted strains across all the treatments.

Significance: This study highlights the importance of cold adaptation of *L. monocytogenes* for challenge studies, as it better mimics microorganisms' natural behavior in refrigerated environments. This approach enhances the relevance and reliability of the challenge study, helping to inform effective food safety practices and risk management strategies.

P3-141: FERRIC UPTAKE REGULATOR (FUR) PLAYS A SIGNIFICANT ROLE IN THE SURVIVAL OF SALMONELLA TYPHIMURIUM ON CHICKEN MEAT

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* is a contaminant of poultry meat and its products. There is a pressing need to understand the genetic factors underpinning the survival of *Salmonella* in poultry.

Purpose: The contamination of meat products by *Salmonella* Typhimurium remains a significant challenge due to its rapid adaptation to environmental changes at phenotypic and genotypic levels. Therefore, understanding microbial responses at a molecular level is crucial for effective control.

Methods: Overnight cultures of nine transcriptional factor deletion mutants of *Salmonella* Typhimurium ST4/74 were spotted and incubated on chicken meat pieces (breast fillets–1 gram each) at 37 °C. Periodically, the meat pieces were washed, and serially diluted aliquots were plated onto XLD agar plates. The bacterial counts in CFU/mL were plotted against time. RNA isolated from the bacteria grown on meat pieces was sequenced to identify their transcriptomic profile. All experiments were conducted in biological triplicates.

Results: Of the nine deletion mutants, ST4/74Δfur (Ferric Uptake Regulator) was highly inhibited compared to the wild type. During iron-replete conditions, Fur binds DNA upstream of genes encoding siderophores and represses their production. On exhaustion of environmental-free iron, Fur disengages from the DNA, activating the different siderophore genes and allowing the bacterium to chelate the protein-bound iron (Fe³⁺) from the environment. Fur, therefore, enables intricate calibration of iron accumulation in the *Salmonella* cytoplasm. To

better understand meat-associated bacterial iron regulation, we deleted the gene for enterochelin production (entC) in ST4/74 Δ fur, creating ST4/74 Δ fur Δ entC. The double mutant grew as the wild type, showing that the repression associated with the fur deletion was relaxed, probably due to the reduction in intracellular iron accumulation. Additionally, RNA sequencing data enabled us to characterize genes that are differentially expressed by Fur during chicken meat survival.

Significance: We show early insights into how iron metabolism becomes critical for *Salmonella* Typhimurium during survival on chicken meat surfaces.

Award Application: Developing Scientist Award

P3-142: DEEP SEROTYPING OF POST-HARVEST MEAT AND POULTRY PRODUCTS REVEALS DIVERSE SALMONELLA SEROVAR POPULATIONS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Meat and poultry products pose a considerable risk of foodborne salmonellosis, necessitating comprehensive pathogen surveillance to prevent future outbreaks. Standard *Salmonella* monitoring often relies on characterization of a single isolate, which may allow some, potentially pathogenic, serovars to evade detection.

Purpose: Deep serotyping (DST) was applied to characterize serovar profiles across the four major food animals in post-harvest, post-intervention samples and to compare serovar identification against conventional culture approaches.

Methods: In this study, we measured multiseroovar *Salmonella* populations in 1,000 post-harvest meat and poultry products (chicken: 448, pork: 313, beef: 180, turkey: 59) collected across the United States by the Food Safety Inspection Service (FSIS). We employed CRISPR-SeroSeq, a next-generation sequencing approach to quantify the relative serovar frequencies in mixed populations. Concurrently, up to two isolated colonies were serotyped using an alternative molecular method.

Results: CRISPR-SeroSeq identified a total of 56 serovars, including 9/10 most common clinical serovars. The average number of serovars per sample was 1.4, with 23% (226/1000) containing multiseroovar populations and a maximum of eight serovars detected in a single sample. Frequency of multiseroovar populations ranged between commodity types, from 13% (24/180) in beef to 36% (112/313) in pork. A Kruskal-Wallis test with post-hoc Dunn's test determined that multiseroovar frequency in both beef and chicken samples differed from pork, respectively ($p < 0.001$). Serovars Typhimurium, Infantis, Montevideo, and Senftenberg were most often found in pork (27%; 86/313), chicken (36%; 162/448), beef (15%; 27/180), and turkey (24%; 14/59) samples, respectively. Comparison of DST and culture work showed 100% concordance, with DST finding additional serovars in 18% (180/1000) of samples.

Significance: This study highlights the need to determine factors influencing serovar frequencies and introduction points in food animal production. The distribution of clinically relevant serovars across all four commodity groups underscores the importance of developing new or improved controls.

Award Application: Developing Scientist Award

P3-143: EFFECT OF ORGANIC ACID COMPONENTS ON THE INHIBITION OF CLOSTRIDIUM PERFRINGENS DURING EXTENDED COOLING OF UNCURED MEAT PRODUCTS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Organic acids, lactate, propionate, and acetate/diacetate, are active components in clean label antimicrobials. Little is known about the effective concentrations or interactions of these organic acids on *Clostridium perfringens* growth inhibition in uncured meat products during extended Phase I cooling (48.8–27°C) in the 2021 FSIS Stabilization Guidelines for Meat and Poultry Products (Appendix B Option 1.2).

Purpose: To identify organic acid combinations and concentrations for *Clostridium perfringens* growth inhibition during extended Phase I cooling.

Methods: Ground turkey breast (75% moisture, pH 6.40, 1.40% NaCl) was formulated with organic acids Sodium Lactate (1.25%, 2.5%), Sodium Propionate (0.5%, 0.25%), and/or Sodium Diacetate (0.5%, 0.25%). Treatments were inoculated with three strains of *Clostridium perfringens* targeting 2–3 log spores/g, vacuum-packaged, and cooked to 73°C. Samples were cooled from 48.8–27°C (Phase I) in 3, 4, and 5 hours; Phase II (27–12.8°C) and Phase III (12.8–4°C) were standardized for 5 hours cooling each. *Clostridium perfringens* populations were enumerated from triplicate samples on Tryptose Sulfite Cycloserine Agar (TSC) at pre-cook, post-cook, and the end of each phase. Two trials were conducted.

Results: Formulations with 2.5% lactate inhibited *Clostridium perfringens* growth (<1 log CFU/g) for Phase I cooling of up to 5 hours with final population changes of -0.01 ± 0.24 log CFU/g. The highest concentrations tested of diacetate and propionate (0.5%), and the lowest concentration tested of lactate (1.25%), did not inhibit *Clostridium perfringens* growth during the 3-hour Phase I cool (1.18 ± 1.13 log CFU/g, 1.53 ± 0.21 log CFU/g, and 2.46 ± 0.22 log CFU/g, respectively).

Synergistic effects between the organic acids were observed, but no combinations at the highest tested levels for propionate and diacetate (0.5%) inhibited *Clostridium perfringens* growth.

Significance: Clean label preservatives containing $\geq 2.5\%$ lactate are effective against *Clostridium perfringens* during extended Phase I cooling of uncured turkey for up to 5 hours.

Award Application: Undergraduate Student Award

P3-144: VALIDATION OF SALAMI PROCESS FOR CONTROL OF SALMONELLA AND E.COLI O157:H7/STEC

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Low heat processing provides more desirable color to Genoa salami, however, due to the limited literature, the lethality of these processes needs to be validated.

Purpose: The objective of this study was to evaluate the lethality of selected Genoa Salami processing conditions on *Salmonella* and Shiga-toxin-producing *Escherichia coli* (including O157:H7).

Methods: Separate portions of raw pork Genoa salami batter were inoculated with 7-8 log₁₀ CFU/g of a five-strain mixture of either *Salmonella* or STEC (including O157:H7) and stuffed into 19mm or 127mm casings. Batter was fermented and cooked according to the test parameters of T1: 80°F for 12 hours and then 110°F for 24 hours or T2: 90°F for 4 hours, ramp-up to 110°F over 2 hours, hold until target pH is obtained, ramp to internal 128°F for 2 hours and maintain at 128°F for 1 hour. Genoa salami was dried at 55°F-60°F and 55-60% humidity for 6-8 days and 14-20 days (Aw<0.93) for smaller and larger diameter products, respectively. At each sampling period, total of 4 samples (25g) were taken from the center and one end of the 2 separate chubs. Samples were surface plated on XLD agar for *Salmonella* and Sorbital MacConkey agar for STEC. Uninoculated chubs were tested for pH and Aw.

Results: For T1 condition, large and small diameter products achieved >5-log and >6-log reduction for both pathogens, respectively. For T2, large diameter product fermented to pH 5.3 and cooked achieved <4-log reduction and drying to Aw <0.93 increased the log reduction to >5.5-log for both pathogens. Small diameter product needed to be fermented to a pH of <5.2 to ensure the >5-log reduction was achieved for both pathogens.

Significance: USDA requirement of >5-log reduction of *Salmonella* and *E. coli* (STEC) in Genoa Salami can be achieved by selected treatments.

P3-145: THE IMPACT OF FLIZ GENE IN THE SURVIVAL OF SALMONELLA TYPHIMURIUM IN EGG YOLK

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* can contaminate chicken eggs and cause foodborne outbreaks due to its adaptability to changing environments, aided by genetic factors.

Purpose: To identify genetic factors critical for the survival of *Salmonella* in egg yolk.

Methods: Single colonies of *Salmonella* Typhimurium ST4/74 and six isogenic deletion mutants were inoculated in 5 mL of LB broth and incubated overnight (37°C in static conditions). About $1-5 \times 10^5$ CFU/mL bacteria were inoculated into 50 mL of egg yolk and incubated at the same conditions. At every 2 hours during 14 h post-inoculation, 100 μ L aliquots of the *Salmonella* spiked yolk were serially diluted and plated on XLD agar. The growth curve of each mutant was compared with the wild type using one-way ANOVA ($p < 0.05$) (R-studio (V2.2)). All experiments were conducted in triplicate.

Results: ST4/74 Δ fliZ, regulating flagella gene expression, exhibited enhanced growth, while ST4/74 Δ psp, encoding phage shock proteins, showed inhibited growth compared to wild type in egg yolk. We restored fliZ into the mutant by cloning fliZ with its native promoter (fliZ-sp) in plasmid pBAD and transforming the pBADfliZ-sp into ST4/74 Δ fliZ (ST4/74 Δ fliZpBADfliZ-sp). The restored strain's growth resembled ST4/74, verifying that the effect of fliZ on egg yolk survival is specifically due to fliZ. We show that the loss of fliZ likely prevents motility and SPI-1 regulation, redirecting metabolic resources toward growth in the nutrient-rich yolk and resulting in enhanced growth compared to the wild type.

Additionally, we identified the genes regulated by fliZ during the survival of *Salmonella* in egg yolk. In the future, we aim to track the migration of ST4/74 Δ fliZ through the albumen to yolk by plating and fluorescent-based experiments.

Significance: These results highlight the role of *fliZ* in *Salmonella* motility and its adaptability to nutrient-rich environments like egg yolk, underscoring its significance in survival and colonization.

Award Application: Developing Scientist Award

P3-146: IN-WATER SUPPLEMENTATION OF TRANS-CINNAMALDEHYDE NANOEMULSION REDUCES SALMONELLA ENTERITIDIS CECAL AND OVIDUCT COLONIZATION AND EGG BORNE TRANSMISSION IN LAYER CHICKENS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* Enteritidis (SE) is a major cause of human gastroenteritis, often linked to the consumption of contaminated eggs. In layer hens, SE colonizes the ceca and oviduct, leading to contamination of eggs.

Purpose: This study investigated the efficacy of in-water supplementation of Trans-cinnamaldehyde nanoemulsion (TCNE) in reducing SE cecal and oviduct colonization and egg borne transmission in layer chickens.

Methods: TCNE was prepared with gum arabic and lecithin emulsifiers. In two trials, 48 seventy-week-old laying hens (12 birds/treatment/trial) were randomly allocated to 4 groups: control, emulsifier control, TC-0.005 and TCNE-0.005. Birds in the TC and TCNE groups were supplemented with TC oil or TCNE at 0.005% in drinking water for 9 weeks. One week after starting treatments, birds were orally challenged with a four-strain cocktail of SE (~9 log CFU/bird). Eggs were collected daily, and cloacal swabs weekly to test for the presence of SE. After 9 weeks, all the birds were sacrificed and SE colonization in the ceca and oviduct was determined. In addition, body weight gain, feed and water intake, and egg production was measured. Data from the two trials were pooled and analysed using Fisher's exact test with statistical

Significance at $P < 0.05$.

Results: Supplementing birds with 0.005% TCNE in drinking water significantly reduced SE on eggshells and in cloacal swabs by 15% as compared to control ($P <$

0.05). SE colonization in the cecum and oviduct decreased by 66% and 34%, respectively ($P < 0.05$). No effect in body weight gain, feed and water consumption, or egg production were observed by treatments as compared to controls ($p > 0.05$). The impact of treatments on egg quality and consumer acceptability is currently under investigation.

Significance: TCNE supplementation shows potential as an effective strategy for controlling SE transmission through eggs.

Award Application: Developing Scientist Award

P3-147: EFFECT OF USING ANTIMICROBIALS IN OZONATED WATER AS A POST CHILL DIP AGAINST SALMONELLA ON CHICKEN MEAT

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Combining antimicrobial treatments in a multi-hurdle approach offers an effective postharvest strategy to reduce *Salmonella* contamination on chicken carcasses.

Purpose: This study evaluated the efficacy of antimicrobials in ozonated water as a post-chill dip treatment to reduce *Salmonella* contamination on chicken meat and wings.

Methods: The post-chill dip treatments in this study included 10 ppm ozone (OZ), 500 ppm peracetic acid (PA), 2% cetylpyridinium chloride (CP), 0.5% carvacrol (CV), OZ+PA, OZ+CP, and OZ+CV. Chicken wings and breast meat samples (n = 5) were inoculated with 100 μ L of *Salmonella* Enteritidis (10^5 CFU/sample) and subsequently dipped in the respective treatment solutions for 60 seconds. Following treatment, samples were transferred to buffered peptone water for *Salmonella* recovery on XLT4 agar. Samples inoculated with *Salmonella* and dipped in distilled water served as the positive control (PC). Additionally, samples treated with antimicrobial solutions and refrigerated for 7 days were evaluated for the treatment effects on meat color and pH. Statistical analysis was conducted using PROC ANOVA in SAS.

Results: Treatment with PA, OZ+PA, CP, and OZ+CP reduced *Salmonella* levels on chicken meat by 1.63, 1.55, 2.43, and 1.68 $\log_{10}$ CFU, respectively, compared to the PC ($P < 0.05$). For chicken wings, reductions of 1.18, 1.49, 2.30, and 2.17 $\log_{10}$ CFU were observed with PA, OZ+PA, CP, and OZ+CP treatments, respectively,

compared to PC ($P < 0.05$). A significantly lower pH value was noticed in wing samples from the PA group compared to control on day 7 of storage ($P < 0.05$). No significant changes in chroma values were observed in both wing and meat samples from the antimicrobial treatment groups after dip treatment, compared to the control groups, on days 0 and 7 of storage ($P < 0.05$).

Significance: This study highlights the potential of using antimicrobials at the post-chill dip stage to reduce *Salmonella* outbreaks associated with chicken meat and meat products.

P3-148: DEVELOPMENT OF A WEIGHT-BASED EQUATION TO ESTIMATE SURFACE AREA IN CHICKEN PARTS TO STANDARDIZE PATHOGEN COUNTS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: In 2024, the USDA-FSIS introduced a revised regulatory framework for *Salmonella* control in poultry. These updated standards require precise methodologies to evaluate microbial loads in chicken parts, including expression by surface area (cm²), by weight (g), and by milliliters of composite rinse. This shift aims to standardize microbial load expression across different chicken parts for regulatory consistency.

Purpose: To calculate accurate equations for four chicken parts (Tenders, Drumsticks, Breast, and Wings) to predict surface area (cm²) based on weight (g), and integrate these into microbial load quantification methods for expression in CFU/cm², CFU/mL, and CFU/g.

Methods: One hundred samples of tenders, drumsticks, breasts, and wings were collected from a commercial processing line. 3D scans were recorded using Polycam™ software, and surface areas were calculated using AutoCAD. Manual length, width, and height measurements were taken for validation, and weight was recorded for each sample.

Results: Surface areas were calculated using regression models based on 3D scans, with R² values ranging from 0.72 to 0.99. Tenders weighed 63.0–83.0 g (average: 71.3 g) with surface areas of 28.7–160.0 cm² (average: 59.7 cm²). Drumsticks weighed 116.0–181.0 g (average: 147.2 g) with surface areas of 136.8–239.5 cm² (average: 189.4 cm²). Breasts weighed 285.0–369.0 g (average: 318.1 g) with surface areas of 136.2–444.6 cm² (average: 360.0 cm²). Wings weighed 71.0–285.0 g (average: 136.1 g) with surface areas of 176.7–291.5 cm² (average: 238.6 cm²). These results demonstrate high variability in both surface area and weight across parts, which significantly influences microbial load expression and highlights the spatial heterogeneity of *Salmonella* distribution.

Significance: Integrating surface area measurements with composite rinse results improves the accuracy of pathogen load expression in poultry products. These

findings support USDA's 2024 *Salmonella* regulatory standards and enable the development of more robust and consistent microbial load quantification methods for regulatory compliance.

P3-149: A METAGENOMICS APPROACH TO STUDY THE EFFECT OF TEMPERATURE ABUSE OF RAW POULTRY MEAT DURING SUPPLY CHAIN

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Metagenomics uses high-throughput sequencing on genetic material directly recovered from samples, providing the structure of entire microbial communities without the need for culturing.

Purpose: Application of metagenomics to determine temperature abuse influenced spoilage microbiome dynamics.

Methods: Raw chicken breast fillet tray-packs procured from a commercial poultry processor were subjected to temperature abuse conditions of 4°C for 30 minutes and then at 30°C or 37°C for 1 hour, repeated for six cycles. Control samples were maintained at a constant 4°C during this period. All samples were then stored at 4°C for eight days and sampled on days 0, 2, 4, 6, and 8. individual fillets were rinsed in 50 mL of buffered peptone water, and the rinsate was plated to determine aerobic, anaerobic, and lactic acid bacteria counts. An aliquot of the rinsate was stored at -80°C and later transferred for metagenomic analysis. Chicken rinsates were processed to extract DNA. Library preparation was performed using an Illumina DNA Prep Kit according to the manufacturer's instructions. Sequencing was conducted using an Illumina MiSeq instrument. The resulting FASTQ files were analyzed using the COSMOS ID-HUB Microbiome (CMBIO) metagenomic analysis pipeline. Organism identification and relative abundance were determined using the Taxa-Kepler Domain (1.1.0) workflow. The presence of antimicrobial resistance genes was identified using the AMR and

Virulence Markers (1.1.0) workflow.

Results: Metagenomic analysis indicated that by day 8, the samples abused at 37°C and 30°C had 80–100% of their microflora composed of *Pseudomonas* spp., while the 4°C samples had a combination of *Pseudomonas* spp. and *Acinetobacter* spp. Beyond the bacteriome, three avian viruses and protists were also detected. Antibiotic resistance genes for tetracycline and aminoglycosides were present in the genetic material, along with several virulence factors.

Significance: The metagenomics approach provides valuable insight into microbiome changes due to temperature abuse during the supply chain and can support data-driven decisions regarding product disposition.

Award Application: Developing Scientist Award

P3-150: ADVANCED OXIDATION PROCESSES EFFECTIVELY ATTENUATE SALMONELLA AND CAMPYLOBACTER IN SIMULATED WASTEWATER

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Poultry processing, a water-intensive sector, generates pathogen-rich wastewater. Conventional disinfection methods, although effective, impose considerable capital and operational costs while integrated recycling systems present a promising opportunity for enhancing resources and sustainability.

Purpose: This study investigated the use of Advanced Oxidation Processes (AOPs) generating reactive oxygen species like hydroxyl radicals and singlet oxygen that kills bacteria, specifically electrochemical (EC) and photochemical (PC) treatments to disinfect *Salmonella* and *Campylobacter* in simulated contaminated wastewater.

Methods: in replicated experiments, *Salmonella* Kentucky (SK), infantis (SI), and Typhimurium (ST), *Campylobacter jejuni* (Cj) and coli (Cc), and a mixture of Cj and ST were cultured in tryptic soy broth and diluted to 10⁶⁻⁷ CFU/mL in 7L contaminated water. The wastewater was subjected to EC voltage load of 30V, 40V, and 50V alongside PC using curcumin-coated spheres at concentrations of 0.4% (1X) and 0.8% (2X) for the *Salmonella* study. For *Campylobacter*, the EC was 50V and 70V with PC of 0.8% (2X) and 1.6% (4X), while optimal EC (70V) and PC (4X) was used for mixed cultures. Samples were collected every 20 (*Salmonella*) or 30 minutes (*Campylobacter*) during the disinfection process until 180 or 300 minutes, respectively and plated on XLD and *Campylobacter* agar that was incubated appropriately for bacterial enumeration. Data was analyzed using ANOVA in JMP Pro 15.

Results: *Salmonella* was effectively killed at 50V with 2X PC within 140 minutes and SI had the most reduction. *Campylobacter* was killed at both EC and PC levels

tested within 210 minutes with a 30-min reduction in time-to-complete-disinfection at the higher EC level (70V). The mixed cultures were completely attenuated in 120 minutes, establishing an optimal condition for effectively removing both pathogens in wastewater.

Significance: These findings suggest that combined EC and PC treatments effectively killed *Campylobacter* and *Salmonella* in contaminated water, highlighting the potential of AOPs as a sustainable poultry wastewater disinfection strategy, promoting microbial safety, water recycling, and reuse.

P3-151: FLUORESCENCE IMAGING AND MACHINE LEARNING FOR THE DETECTION OF RESIDUAL FECAL CONTAMINATION ON WASHED SHELL EGGS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* contamination on eggs can occur through the pathogen's presence in fecal matter wherein it can transcend the porous shell, allowing its proliferation and growth in the albumen and yolk of the egg. Detection of fecal contamination on shell eggs can be significant to the egg safety industry. Moreover, fecal contamination invisible to the naked eye can be a source of *Salmonella* contamination, but it has not been well studied. Through data analysis and fluorescent spectral imaging systems, detection of undesirable fecal residue not visible to the naked eye is revealed to potentially help reduce pathogen levels found on egg products.

Methods: Eggs (n=30) were purchased to carry out this research. Chicken feces were collected from a local chicken house to produce a slurry, which was applied in assorted sectors on the eggshell for detection. The slurry was positioned and allowed to dry. The CSI-D+ system was used for detection of contamination, sanitation, inspection, and disinfection residues through multispectral image production. Images were taken before fecal inoculation, after inoculation, and after egg washing.

Purpose: Utilizing fluorescence and the CSI-D+ system, this study aimed to evaluate the system's ability to detect fecal contamination on shell eggs and assess the performance of image analysis algorithms in classifying contaminated zones.

Results: Two Convolutional Neural Network (CNN) configurations evaluated images for classification. The Google Colab algorithm used four data augmentations for testing and validating the CSI-D+ system. Fluorescence successfully exposed the contaminated sectors of the egg during inoculation and after washing. Image analysis on Microsoft Azure at 80% threshold achieved 100% precision, 66.7% recall, and 100% Average Precision (AP). Each image was accurately classified into its correct label 80% of the time in the CNN configuration. Google Colab showed a differentiation accuracy of 50% for the validation model.

Significance: Image analysis of shell eggs presents a promising outlook for integration of multispectral systems into egg processing plants to improve egg safety.

Award Application: Developing Scientist Award

P3-152: MULTIDRUG-RESISTANT SALMONELLA INFANTIS: GENOMIC EVIDENCE FROM RETAIL CHICKEN MEAT

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* spp. is one of the leading causes of foodborne illnesses, with poultry meat serving as a primary source of transmission. Furthermore, there is a growing incidence of multidrug-resistant strains of *S. infantis* harboring a megaplasmid (pESI), posing a significant global public health concern.

Purpose: To investigate the prevalence and genomic characterization of *Salmonella* spp. isolated from retail-sold chicken meat in Chile.

Methods: Over a period of 6 months, over 100 retail stores in Santiago, Chile were surveyed. In each visit, 24 chicken meat samples were collected. For the isolation of *Salmonella* spp., a modified BAM-FDA protocol was used, and presumptive *Salmonella* colonies were confirmed using invA-PCR. Serogroup determination was performed with multiplex PCR. Strains were sequenced using the ILLUMINA platform and analyzed to identify genomic features, structure, and relationship to previously reported isolates. Plasmids from each isolate were also reconstructed and characterized.

Results: *Salmonella* was detected in 45% (65/144) of the chicken meat samples. A total of 192 *Salmonella* isolates were obtained, all belonging to serogroup C1. Genomic analysis revealed that 100% of strains were *S. infantis* from clonal complex HC20 343 and carried a pESI-like megaplasmid. Additionally, 45% of the strains encoded the ESBL gene *bla*_{CTX-M-65}, and 100% harbored clinically relevant resistance genes including *sul1*, *aadA1*, and *tet(A)*.

Significance: This study highlights the extensive prevalence of *S. infantis* in retail chicken meat in Chile, especially the HC20 343 clonal complex. The widespread occurrence of the pESI-like megaplasmid with frequent antibiotic resistance genes suggests a potential risk to human health. These findings underscore the

importance of implementing effective control measures to ensure food safety.

Award Application: Developing Scientist Award

P3-153: OUTGROWTH OF CLOSTRIDIUM PERFRINGENS DURING THE THERMAL STABILIZATION OF COOKED, UNCURED MEAT SAUSAGES FORMULATED WITH NITRITE REMOVERS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Improper thermal stabilization (chilling) of meat products is a risk factor often associated with *C. perfringens* outbreaks, posing significant public health risk. Nitrite removers and replacers are becoming increasingly popular to formulate meat products with clean-label ingredients.

Purpose: To determine the outgrowth of *C. perfringens* during thermal stabilization of the cooked, uncured meat sausages with nitrite removers.

Methods: Ground pork samples (10 g) were inoculated with *C. perfringens* B-23847, vacuum-sealed, and hand-massaged for even spore distribution. Samples underwent a 20-min heat shock in a water bath at 75°C to inactivate vegetative cells and activate spores, followed by dynamic cooling over 6.5 h. Eight treatments were evaluated, with each timepoint consisting of six samples (Nitrite removers: NR1, NR2, NR3, and NR4, and positive and negative controls) were enumerated at 48-minute intervals, with two independent trials.

Results: Initial *C. perfringens* levels for NR1, NR2, NR3, NR4, positive control, and negative control were 4.08 ± 0.25 , 4.39 ± 0.02 , 4.31 ± 0.02 , 3.31 ± 0.08 , 3.20 ± 0.16 , 3.52 ± 0.06 log CFU/g, respectively. After the 6.5-hour cooling process, NR1 exhibited a growth of 4.01 ± 0.07 log CFU/g, with a population reduction of 0.07 log CFU/g. NR2 showed a population of 4.18 ± 0.05 log CFU/g, with a microbial reduction of 0.21 log CFU/g. Meanwhile, NR3 and NR4 displayed growths of 4.15 ± 0.11 and 3.15 ± 0.07 log CFU/g, with microbial reductions of 0.162 and 0.161 log CFU/g, respectively. The positive and negative controls exhibited growths of 2.77 ± 0.20 and 3.48 ± 0.10 log CFU/g, with reductions of 0.43 and 0.04 log CFU/g, respectively. All nitrite remover treatments effectively prevented the outgrowth of *C. perfringens* and reduced the microbial population.

Significance: The results indicate that nitrite removers and replacers evaluated effectively prevent the outgrowth of *C. perfringens* to less than 1 log CFU/g during cooling, making them suitable as clean-label ingredients in meat products.

Award Application: Developing Scientist Award

P3-154: INVESTIGATING THE ANTIMICROBIAL PROPERTIES OF CYANOBACTERIA-DERIVED ORGANIC ACIDS AGAINST COMMON FOODBORNE PATHOGENS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Justification: The rising demand for sustainable and natural food preservatives for meat industries drives the need for innovative alternatives to synthetic additives. Cyanobacteria, when autofermented, can produce organic acids that serve as effective natural food preservatives.

Objective: To investigate the potential of autofermenting cyanobacteria to produce organic acids as natural food preservatives and evaluate their antimicrobial efficacy.

Methods: Bacterial cultures of *Salmonella enterica* (ATCC: 10398) and *Campylobacter jejuni* (ATCC: BAA_1062) were purchased from ATCC and cyanobacterial strain *Nostoc muscorum* (UTEX: 2209) from UTEX. The biomass concentration was maintained at 10 grams per liter. Autofermentation experiments were conducted in triplicate (N=3) using 160 mL serum vials under anoxic conditions. Vials were wrapped in foil to exclude light and incubated at 30 °C with agitation at 180 rpm. Organic acid production was analyzed via HPLC with a PDA detector at 210 nm. The MIC of cyanobacteria was determined. A bacterial suspension ($\sim 5 \times 10^5$ CFU/mL) prepared from a 24-hour culture and cyanobacterial crude extract at pH 4.9 were inoculated (each 100 μ L/well). After a 24-hour incubation at 37 °C, 40 μ L of 0.4 mg/mL INT solution was added as a growth indicator, and plates were further incubated for 30 minutes at 37 °C to visually determine the MIC values.

Results: Butyric and lactic acids were identified as major metabolites in autofermented *Nostoc muscorum* aqueous crude extracts. The minimum inhibitory concentration (MIC) of the cyanobioactives/crude extracts was 1:7 dilution for *Salmonella enterica*, indicating strong antimicrobial activity at low concentrations. For *Campylobacter jejuni*, the MIC was 1:6 dilution, reflecting moderate effectiveness. No antimicrobial activity was observed with fresh *Nostoc muscorum* aqueous crude extracts without autofermentation.

Conclusion: These findings underscore the potential of organic acids derived from

the autofermentation of cyanobacteria as promising antimicrobial agents against foodborne pathogens.

Award Application: Developing Scientist Award

P3-155: SALMONELLA PREVALENCE IN TWO TYPES OF LYMPH NODES COLLECTED FROM MARKET HOGS HARVESTED AT COMMERCIAL PROCESSING FACILITIES IN THE U.S.

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Data about *Salmonella* prevalence in the market hogs value chain, and its loads in lymph nodes (LNs) is important for risk-based decision-making in processing operations when strategies to reduce contamination in pork products are considered.

Purpose: This study aimed to detect *Salmonella* in mesenteric and superficial inguinal lymph nodes (LNs) from market hogs harvested at two U.S. plants (A and B) in the Midwest and Great Plains.

Methods: Two types of LNs (mesenteric and peripheral) were sampled from hogs at two facilities. Hogs were housed at four farms (F1, F2, F3, F4) near the plants, with three truckloads per farm sampled over two days. A total of 840 LNs were collected (420 per type, $n=35$ per truck, $N=105$ per day) and tested for *Salmonella* using the BAX® System Real-Time PCR Assay. Prevalence data was analyzed using Fisher's Exact test with a 95% confidence interval. Animals from F1 and F2 were sent to Plant A, while animals from F3 and F4 to Plant B.

Results: Out of 820 LN samples, 272 tested positive for *Salmonella*. in Plant A, mesenteric LNs had the highest prevalence in animals from F1 (57%) and the lowest in F2 (15%) ($p<0.05$). For peripheral LNs, animals from F1 had a 12% prevalence, while F2 had a 5% prevalence ($p<0.05$). in Plant B, mesenteric LNs from F4 had a 40% prevalence, significantly higher than F3 (14%) ($p<0.05$). For peripheral LNs, hogs from F3 had a 10% prevalence, while F4 had a 4% prevalence ($p<0.05$). Fisher's Exact Test revealed significant differences in *Salmonella* prevalence across the four farms ($p<0.05$). LN samples of hogs from

Farms F1 and F4 had notably higher contamination, suggesting potential *Salmonella* contamination from on-farm sources or cross-contamination in lairage, with lower translocation to peripheral LNs. No correlation was found between mesenteric and peripheral LNs prevalence ($p>0.05$).

Significance: These findings highlight that pork lymph nodes are a significant source of *Salmonella*, with farm practices potentially influencing contamination, underscoring the need for targeted mitigation strategies at pre- and post-harvest locations.

P3-156: EMERGING TRENDS IN AI AND FOOD SAFETY RESEARCH: A CONTENT ANALYSIS

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: There has been a growing interest in applying AI to solve food safety challenges, with researchers using various data sources for predictive analysis of foodborne illness outbreaks

Purpose: This study aimed to identify emerging trends in the existing literature on AI applications in food safety. Specifically, the research questions were as follows related to published research: 1) what AI applications are being used to make food safety predictions, 2) how is AI application performance measured, and 3) what fields are accepting the use of AI to make food safety predictions

Methods: Researchers collected peer-reviewed articles published between January 2019 and December 2024. Keywords used included: food safety, prediction, online reviews, artificial intelligence, restaurant, and risk. Articles were reviewed to ensure only relevant peer-reviewed research articles remained

Results: After applying exclusion criteria, 30 peer-reviewed articles were identified. Emerging themes were found, including the use of machine learning models, the internet of Things, and deep learning models in AI-related food safety research. Random Forest (13 articles) was the most common machine learning model used for food safety predictions, the internet of Things (1 article) was applied in digital technology for health monitoring and food safety, and Artificial Neural Networks (5 articles) were frequently used for complex analyses mimicking human brain functions. R-square was commonly used to validate Random Forest and Artificial Neural Networks models. The predictions made in these articles covered areas such as food freshness, food safety, food fraud, water quality, pathogens, and restaurant food safety violations. The published research spanned various fields, including microbiology, animal science, civil engineering, chemical and biomolecular engineering, public health, and food science

Significance: This study provides researchers with insights on published research and trends about AI technologies and food safety topic areas

P3-157: ONE-STEP KINETIC ANALYSIS OF BACILLUS CEREUS GROWTH IN RICE WITH CHICKEN

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: *Bacillus cereus* continues to be a cause of food poisoning outbreaks associated with the consumption of starch-based multicomponent products such as cooked rice with chicken. The main cause is primarily attributed to inadequate chilling of foods after cooking or their storage at room temperature for an extended period before consumption.

Purpose: The objective of this study was to quantify and model *B. cereus* growth from spores in a cooked rice with chicken product between 10 to 49 °C.

Methods: A 4-strain spore cocktail of *B. cereus* was heat-shocked and subsequently used to inoculate the cooked rice combination product. Samples were stored at isothermal temperatures, pulled periodically, and enumerated. The growth curves were analyzed using the USDA IPMP-Global Fit software's one-step kinetic analysis, with the Huang growth model as the primary model in combination with one of three secondary models: Ratkowsky square-root, Huang square-root, and Cardinal parameter models.

Results: *B. cereus* growth was observed between 13 and 46 °C, but not at 10 and 49 °C. The maximum growth temperatures estimated by all three models were similar, i.e., 48.6, 48.2, and 47°C for the Ratkowsky, Huang, and Cardinal models, respectively. The Huang square-root model accurately described the minimum growth temperature (9.34 °C), in contrast to the Ratkowsky (7.17 °C) and Cardinal models (7.42 °C). However, all three models exhibited an accurate growth prediction (RMSE ~ 0.4) with a similar Akaike information criterion (AIC) between them. These models can assist the food retail industry to predict *B. cereus* growth and determine its risk in a cooked rice combination product.

Significance: The kinetic models developed will assist processors to predict the growth of *B. cereus* growth in cooked multicomponent product during chilling or storage at improper temperatures as well as to determine compliance with

regulatory performance standards.

P3-158: EVALUATING SELECTED PREDICTIVE MODEL FOR VALIDATING BACTERIAL GROWTH CURVE IN BROTH CULTURE AT VARIOUS LEVELS OF ANTIMICROBIAL ENVIRONMENT

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Modeling microbiological data is essential for enhancing the statistical analysis of experimental findings. It facilitates the estimation and prediction of microorganisms' behaviors, such as growth, survival, and death, under given environmental conditions.

Purpose: This project aims to check the performance of a selected model in predicting the growth curve of various *Listeria monocytogenes* (LM) strains in an antimicrobial environment with various usage rates of buffered dry vinegar.

Methods: A set of tryptic soy broth (TSB) growth media was treated with 0.5%, 0.75%, and 1.0% of dry vinegar (DV). The growth media were inoculated with LM (ATCC 19111, 19112, 19115, 19118, 13932) individually at a concentration of 5–6 log CFU/mL. Optical density (OD) growth curves were measured using 96-well microliter plates. The data served as a training set to validate and fit the Baranyi growth model, which was used to calculate the model parameters. These parameters were used to predict the growth curve at various usage rates of buffered dry vinegar.

Results: The predictive model was validated with an R^2 of 0.99 for all selected dry vinegar usage rates across all LM strains. Model parameters derived from training data were used to predict the OD curve of DV at 0.65%, 0.85%, 0.25%, and 0.15%. Curve fittings were achieved with an average R^2 of 0.98 and a p-value ≥ 0.05 .

Significance: Our findings demonstrated that the Baranyi growth curve model effectively fit the selected LM strains. The model parameters obtained through validation accurately predicted the growth curve of LM at varying levels of antimicrobial environment.

P3-159: MACHINE LEARNING TO PREDICT HOST SPECIFICITY AND GEOGRAPHIC ORIGIN OF *SALMONELLA* KENTUCKY

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: *Salmonella* Kentucky, a polyphyletic serovar, is frequently isolated from food-producing animals in the United States. Though human infections are infrequent domestically, *S. Kentucky* remains a significant cause of salmonellosis worldwide, and cases within the U.S. are typically associated with travel abroad. Rapid methods for identifying epidemiologically relevant characteristics, such as animal host and provenance, remain underdeveloped.

Purpose: This study aimed to evaluate the efficacy of machine learning (ML) algorithms to predict the host and geographic origin of *S. Kentucky* isolates from genomic sequencing data.

Methods: Genome assemblies of 1,341 *S. Kentucky* isolates with sufficient metadata were obtained from Enterobase. Isolates varied by host (bovine or poultry), continent of origin (North America or other), and sequence type (152, 198, or 314). Core-genome SNPs, gene presence/absence, and intergenic features were extracted and used to train ML algorithms. Algorithms tested included Random Forest, Support Vector Machine, Logistic Regression, Naive Bayes, and K-Nearest Neighbor. Evaluation was based on F1 score and area under the receiver operating characteristic curve (AUROC).

Results: For distinguishing between poultry (n=890) and bovine (n=451) hosts, the highest performing model was Random Forest. The F1 scores for poultry and bovine were 0.95 and 0.90, respectively, with an AUROC of 0.94. Random Forest also performed best for distinguishing between North American (n=193) and non-North American (n=321) origins, achieving F1 scores of 0.98 and 0.99 and an AUROC of 0.99.

Significance: This study demonstrates the high performance of machine learning models in predicting the host and geographic origin of *S. Kentucky* isolates. These models may be used to support and accelerate epidemiological investigations into pathogen origin and to help identify sources of human infections.

P3-160: RISK ASSESSMENT PREDICTS FINISHED PRODUCT STANDARDS MOST SPECIFICALLY TARGETING PRODUCTS WITH HIGHEST LEVEL OF SEROTYPES OF PUBLIC HEALTH CONCERN MOST EFFICIENTLY REDUCE PUBLIC HEALTH RISK FROM SALMONELLA IN COMMINUTED TURKEY

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: In July 2024, the USDA FSIS published a proposed determination including finished product standards that would consider *Salmonella* contamination >10 CFU/g with one of three Serotypes of Public Health Significance (SPHS: Typhimurium, Hadar, and Muenchen) as an adulterant in raw comminuted turkey products.

Purpose: Our team modified our risk assessment for chicken parts to explore implications of finished product standards with different levels and strain specificities for comminuted turkey, evaluating the tradeoff between implicating more or fewer finished products and effects on public health risk.

Methods: We modeled critical assumptions from the FSIS risk assessment. We used 2016–2021 USDA-FSIS HACCP data for comminuted turkey (n=1219) for *Salmonella* contamination level ($\log_{10}$ -normal $\mu = -4.86$, $\sigma = 2.33$) and serotype. The attenuation distribution ($\log_{10}$ -normal $\mu = -5.00$, $\sigma = 1.91$) was incorporated, along with a scaling factor of -3.60 log, to account for variability in *Salmonella* reduction from raw product to consumption. The serotype-specific dose-response model was updated to use the FSIS genomics-based classification of high-virulence serotypes.

Results: Modeling 2016–2021 contamination, 86% of illnesses were attributed to 0.61% of servings with all *Salmonella* serotypes >10 CFU/g. For the proposed level and serotype standard, 47% of illnesses were attributed to 0.13% of servings >10 CFU/g with a SPHS. In contrast, under the level- or serotype-only standard, more illnesses (94%) were attributed to 3.8% of servings with any serotype >10 CFU/g

or any detectable SPHS (>0.0031 CFU/g). Slightly more illnesses were concentrated in slightly more servings with >10 CFU/g of all high-virulence serotypes (59% of illnesses in 0.15% of servings) and all contamination of high-virulence strains as determined by genomics (74% of illnesses in 0.16% of servings).

Significance: Our study confirmed for comminuted turkey that most salmonellosis risk is concentrated in products contaminated with high levels of high-virulence strains. More specifically targeting those products can more efficiently protect public health while minimizing implicated products.

Award Application: Developing Scientist Award

P3-161: USING A FRESH PRODUCE SUPPLY CHAIN RISK MODEL TO ASSESS THE IMPACT OF DEVIATIONS FROM STANDARD FOOD SAFETY PROTOCOLS

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: The fresh produce industry uses pre- and post-harvest assessments to monitor food safety systems and identify deviations from standard food safety protocols, however, limited quantitative tools have modeled deviations to support decision-making around response.

Purpose: To evaluate the impact of industry-relevant scenarios representing small-scale failures that introduce contamination or reduced control over existing microbial contamination using a supply chain risk model (SCRM).

Methods: Four industry-relevant scenarios were parametrized to represent (i) a nonfunctioning irrigation treatment pump after field irrigation, (ii) ineffective harvester equipment sanitation, (iii) small animal fecal contamination in a field, and (iv) inadequate process wash control. Each scenario was reviewed by domain-specific experts to critically evaluate the input parameters for modeling. The scenarios were applied to leafy green contamination with Shiga-toxin-producing *Escherichia coli* ($\mu = -2.65 \log$ (CFU/g)), with high ($\sigma = 0.8 \log$ (CFU/g)) and low ($\sigma = 0.2 \log$ (CFU/g)) variability. Lots were categorized by the risk of producing a positive test at retail.

Results: Under high contamination variability, inadequate process wash control resulted in a 9-fold increase in the overall risk of a positive test at retail compared to the baseline, and a meaningful increase in the number of highest public health risk lots (from 16 to 433). Under low variability, inadequate process wash control likewise resulted in a 10-fold increase in overall risk, but no highest public health risk lots were identified. Other scenarios that simulated a nonfunctioning irrigation treatment pump, ineffective harvester equipment sanitation, and small animal fecal contamination each resulted in less than 2-fold increases in overall risk and negligible changes in the number of highest public health risk lots across both

contamination variability scenarios.

Significance: Where good data and reasonable simplifying assumptions support scenario parametrization, our findings can help the produce industry with risk-based decisions on response, such as product disposition, following deviations from standard food safety protocols.

Award Application: Developing Scientist Award

P3-162: CHARACTERIZATION OF DIETARY EXPOSURES TO ELEMENTS IN MILK FROM COWS FED A SEAWEED DIET

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Certain types of seaweed supplements are used, or have been proposed, as amendments in the diet of dairy cows to improve feed nutrition benefits and minimize ruminant methane emissions. Seaweeds are also known to contain a variety of beneficial and harmful elements.

Purpose: The purpose of this research is to understand the extent to which these elements accumulate in milk from cows fed these amendments and characterize exposures and corresponding potential health risks for milk consumers. **Methods:** We analyzed milk samples from prior trials in which cows were treated with *Chondrus crispus* (n=12), with *Ascophyllum nodosum* (n=20), or neither (controls; n=16) for 8 potentially beneficial and 16 potentially harmful elements using ICP-MS. We used a Monte Carlo simulation to model exposures to these metals under hypothetical scenarios in which infants directly consume milk from treated cows as their diet. We compared infants' modeled elemental exposures under this hypothetical to four alternative scenarios: a diet of cow's milk infant formula, a diet of breastmilk, exposure at regulatory limits for drinking water, and exposure at regulatory limits or guidance for foods.

Results: Milk from cows treated with either seaweed species had significantly higher ($p < 0.05$) concentrations of arsenic and iodine than milk from untreated cows. Our simulation implied that dietary exposures to elements from the consumption of milk from cows treated with seaweed supplements were generally below or similar to exposures from cow's milk infant formula and breastmilk, and in compliance with regulatory limits or guidance.

Significance: While larger scale studies are needed, our pilot study suggests that the use of seaweed supplements for dairy cows will not result in element exposures above those expected from similar foods. Additional investigation is

needed to ensure that arsenic and iodine exposures are controlled.

Award Application: Developing Scientist Award

P3-163: INCORRECTLY ASSUMING A SALMONELLA SEROTYPE IS LOW-VIRULENCE COULD MEANINGFULLY LOWER THE PUBLIC HEALTH BENEFIT OF PROPOSED FINISHED PRODUCT STANDARDS FOR SALMONELLA IN CHICKEN PARTS

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: USDA-FSIS proposed finished product standards for *Salmonella* in raw chicken parts consider infantis a low-virulence serotype, despite a 2016–2022 spike in infantis-related illness that subsided in 2023, suggesting an ambiguous virulence status.

Purpose: To model the interaction between perceived and actual virulence status of *Salmonella* infantis on the potential effect of finished product standards on reducing salmonellosis.

Methods: We added to our peer-reviewed risk assessment model variable post-packing reduction of *Salmonella* matching the attenuation distribution in the FSIS' 2024 chicken parts risk assessment. The model was used to estimate the proportion of the overall risk of illness attributable to finished products with >10 CFU/g of one of three serotypes of concern (Enteritidis, Typhimurium, and infantis), our high-virulence/high-concern scenario. We modified the dose-response to treat infantis like other low-virulence serotypes (Anatum) as a low-virulence/high-concern scenario, replaced infantis with 4,[5],12:i:- as a serotype of concern for a high-virulence/low-concern scenario, and combined both changes for a low-virulence/low-concern scenario.

Results: Accounting for variability in *Salmonella* reduction post-packing, 87.9% of overall risk of salmonellosis was attributed to finished products with high levels of any *Salmonella*. In this high-virulence/high-concern scenario, 64.8% of risk was from products with high levels of one of the top three high-virulence serotypes, including infantis, accounting for 73.8% of the total risk in high-level product. When infantis was incorrectly assumed as high-concern (low-virulence/high-

concern), the fraction of high-level product risk from serotypes identified in the standard only modestly dropped to 60.7%. Similarly, 60.6% of the high-level product risk was from serotypes in the standard when infantis was correctly assumed low-concern (low-virulence/low-concern). Critically, only 39.6% of high-level product risk was from serotypes in the standard when infantis was incorrectly assumed low-concern (high-virulence/low-concern).

Significance: Product standards including a subset of high-virulence serotypes are most effective when the inclusion list is accurate, and incorrectly assuming low-virulence is worse than incorrectly assuming high-virulence.

Award Application: Developing Scientist Award

P3-164: DEVELOPMENT OF A SEMI-QUANTITATIVE RISK MODEL TO REDUCE FOOD SAFETY BARRIERS IN COMPOST APPLICATION FOR ORGANIC LEAFY GREENS PRODUCTION

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Organic farming promotes sustainability and reduces synthetic inputs, often using compost as a biological soil amendment. However, improperly treated compost can introduce foodborne pathogens, posing food safety risks. Therefore, effective risk assessment tools are essential for identifying contamination risks and supporting safe compost application in organic production.

Purpose: This study aimed to develop a user-friendly, semi-quantitative tool to reduce food safety barriers in compost applications for organic production of leafy greens.

Methods: A literature review of 250 publications, including research articles, reports, and production guidelines was conducted, with 33 publications focused on compost. Key risk factors, such as feedstock (animal waste, green waste, or mixed), composting process, storage condition, and compost application, were identified for parameterization and assigned likelihood risk scores from low to high (1-5). Contamination pathways were mapped based on the parametrization

assumptions. Expert input is elicited to address data limitations and model validation. A risk assessment model developed in R using quantrra package will be validated through global sensitivity analysis (GSA).

Results: The risk assessment model evaluated pathogen risks in harvested crops from farms using on-farm processed and commercially produced compost, considering feedstock contamination, pathogen die-off during the composting process, regrowth during storage, and compost application. A risk score was calculated by combining individual scores to assess food safety risks under varying composting management practices and environmental conditions. In one scenario of the model, dairy static compost, stored with low moisture content and high-temperature conditions, and applied as unfinished compost to field crops, results in a medium-high risk, indicating a potential food safety concern.

Significance: The findings contribute to evidence-based decision-support tools that will help organic growers, farm advisors, and food safety professionals manage compost safely and effectively in organic production, supporting food safety, regulatory compliance, and sustainable agricultural practices.

Award Application: Developing Scientist Award

P3-165: MODELING THE EFFECT OF PRE-INOCULATION TEMPERATURE HISTORY ON LAG PHASE DURATION OF DIFFERENT STRAINS OF LISTERIA MONOCYTOGENES

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: The lag phase, despite a period of no bacterial growth, is critical for food safety. Numerous studies have shown that exponential growth rate can be modeled accurately. However, inaccurate estimations of the lag phase duration (LPD) are made as it also depends on previous growth conditions. Temperature history is one of the critical factors impacting microbial growth.

Purpose: To model the impact of temperature shifts on growth kinetics and predict the physiological parameter, h_0 under varying temperature histories for *Listeria monocytogenes*.

Methods: Five strains of *Listeria monocytogenes* were used: 390-2, 390-6, MDD642, MDD640, MDD64. The effect of the pre-and post-inoculation isothermal temperatures (8 to 40°C) on bacterial growth was measured as optical density (OD) using Bio-Tek Cytation3 image reader. Each set of temperature upshift, and downshift experiments was conducted three times, separately for each of the five *Listeria* strains. During the pre-inoculation stage, 96- multiwell plates were incubated at all pre-inoculation temperatures, followed by transfer of exponentially growing cells to all post-inoculation temperatures. The Baranyi model estimated h_0 and μ_{max} . Further, the dependence of $\sqrt{h_0}$ on the temperature downshift and upshift conditions was modeled.

Results: The LPD, quantified using h_0 , increased with the magnitude of temperature shift. During the temperature downshift average value of $\sqrt{h_0}$ ranged from 5.07 to 5.70 going from 40 to 8°C and 3.66 to 4.23 going from 40 to 30°C. While, in the temperature upshift experiments average value of $\sqrt{h_0}$ ranged from 2.89 to 3.90 going from 8 to 40°C and from 4.19 to 5.30 going from 8 to 15°C, across strains. The minimum value of h_0 was zero, depicting no temperature shift. Growth rate was not influenced by the pre-inoculation conditions.

Significance: This study highlights the need to account for pre-inoculation temperature history to improve LPD predictions for accurate pathogen growth modeling.

Award Application: Developing Scientist Award

P3-166: IMPACT OF ADAPTIVE MULTIPADDOCK (AMP) GRAZING OVER TIME ON PECAN ORCHARDS SOIL MICROBIOMES USING AMPLICON SEQUENCING AND SHOTGUN METAGENOMICS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Adaptive-multipaddock (AMP) grazing has been gaining strong attention in the U.S. for its potential benefits including increases in pasture forage and animal production, and environmental sustainability. Soil microbiomes may play a significant role in these positive effects of AMP.

Purpose: The present study aimed to investigate the impact of AMP grazing over time on the soil microbiomes of pecan orchards.

Methods: Twelve soil samples were collected from twelve distinct plots in October each year (2022 & 2023). Total soil genomic DNA was extracted using DNeasy PowerSoil Pro Kits. Six hundred cycles of paired-end 16S rRNA gene and ITS-region targeted amplicon sequencing, and shotgun metagenomics sequencing were performed using the same DNA on NovaSeq 6000 platform at Novogene. Raw sequence reads quality was checked using FastQC. 16S rRNA gene and ITS-region amplicon sequencing raw tags were compared with the *Silva* and *UNITE* databases, respectively. Metagenome clean reads were assembled using *MEGAHIT*.

Results: *Actinobacteriota* was identified as the most dominant phylum in both years, followed by *Proteobacteria*. In amplicon sequencing, the mean relative abundance of *Actinobacteriota* increased significantly ($p < 0.05$) from Year-1 (0.44 ± 0.02) to Year-2 (0.50 ± 0.01). *Proteobacteria* showed no significant ($p > 0.05$) changes over time. *Firmicutes* abundance declined significantly ($p < 0.05$) with values dropping from 0.104 ± 0.017 (Year-1) to 0.029 ± 0.003 (Year-2), while *Acidobacteriota* increased significantly ($p < 0.05$) to 0.99 ± 0.01 , surpassing *Firmicutes* in Year-2. *Ascomycota* consistently dominated the fungal community in both years, followed by *Mortierellomycota* and *Basidiomycota* in Year-1, and *Basidiomycota* and *Chytridiomycota* in Year-2. Shotgun metagenomics also revealed greater taxonomic richness across bacteria, archaea, eukaryotes, fungi,

and viruses, alongside dynamic shifts in the functional capabilities including carbohydrate-active enzymes, orthologous groups, and metabolic pathways of soil microbiome over time.

Significance: The findings of this study highlight the impact of AMP grazing on soil microbiomes in pecan orchards over time, contributing to a more comprehensive understanding of regenerative agriculture practice.

P3-167: DYNAMIC CHANGES IN ANTIBIOTIC RESISTOMES OF PECAN ORCHARD SOIL UNDER ADAPTIVE MULTIPADDOCK (AMP) GRAZING MANAGEMENT

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Adaptive-multipaddock (AMP) grazing is an emerging regenerative agricultural practice which has been shown to enhance soil health, improve animal and forage production on the land, and simultaneously reduce soil greenhouse gas emissions. It may also play an important role in shaping the antibiotic resistomes in soil.

Purpose: To assess the impact of AMP grazing on the antibiotic resistome in pecan orchard soils over time.

Methods: Twelve soil samples were collected from twelve distinct plots in October each year (2022 & 2023). Total soil genomic DNA was extracted using DNeasy PowerSoil Pro Kits. Shotgun metagenomic sequencing was performed on the NovaSeq 6000 platform at the Novogene Sequencing Center. Raw reads quality was checked using FastQC, and clean reads were assembled using MEGAHIT. Antibiotic resistome screening was conducted by aligning sequences to the Comprehensive Antibiotic Resistance Database (CARD) (<https://card.mcmaster.ca/>) using the Resistance Gene Identifier (RGI) tool.

Results: Notable prevalence of vancomycin resistance gene clusters (vanA, vanB, vanG, vanL, vanM, and vanO) was observed in the soils of AMP-grazed pecan orchards. Resistance genes for oxytetracycline (otrA) and streptomycin (rpsL), and the multidrug-resistant membrane fusion gene (adeF), were also detected. A comparative analysis of the antibiotic resistome revealed significant shifts in resistance gene abundance over the two years. The vanW gene in the vanL cluster showed a significant decrease ($p < 0.0001$) in the second year of AMP grazing. Conversely, the vanT gene abundance in the vanG cluster increased significantly ($p < 0.0001$) in Year-2 (3.39 ± 0.27) compared to Year-1 (1.22 ± 0.16). Alarming, the multidrug-resistant adeF gene was newly detected (1.45 ± 0.21) in all soil samples in the second year of AMP grazing.

Significance: Our findings highlight the dynamic nature of antibiotic resistance gene shifts under AMP grazing.

P3-168: RAPID SEROTYPING OF SALMONELLA USING OXFORD NANOPORE TECHNOLOGY: A COST-EFFECTIVE APPROACH FOR PUBLIC HEALTH SURVEILLANCE

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Salmonella* outbreaks often involve multiple serovars, complicating public health responses. Traditional serotyping methods, such as serology and the Kauffmann-White scheme, are costly and time-consuming. The isolation of *Salmonella* during outbreaks is lengthy, taking several days and resulting in many colonies from which only one or two are selected for further characterization. A solution is to rapidly analyze multiple colonies from a single plate. Whole-genome sequencing (WGS) offers a promising alternative; however, its cost for testing multiple colonies can be prohibitive. We developed an affordable protocol using Oxford Nanopore Technology (ONT) combined with in silico analysis tools to quickly identify *Salmonella* serotypes from a single colony, with a total processing time of only 15 hours.

Purpose: This study aims to create a faster and more efficient method for serotyping *Salmonella* using ONT, improving outbreak response times.

Methods: Overnight cultures of *Salmonella* were prepared, and individual colonies were used to isolate DNA with the Maxwell RSC Cultured Cell DNA kit. DNA library preparation utilized the rapid barcoding sequencing kit, multiplexing up to 12 isolates per run. Sequencing was conducted on an R10.4.1 flow cell for 10 hours, which was successfully washed and reused up to four times. An in-house pipeline analyzed the data, employing de novo assembly with Flye v2.9.9, followed by taxa identification, serotyping (SeqSero Version 2.0), and analysis of antibiotic resistance and virulence genes (AMR Finder Plus). A total of 96 strains representing 37 serovars were tested, including 12 replicates.

Results: We successfully characterized and serotyped all 96 *Salmonella* strains. A single flow cell allowed characterization of up to 48 strains, demonstrating the efficiency of this method. In addition to accurate serotype identification, we performed multi-locus sequence typing (MLST), detection of antibiotic resistance genes, and identification of virulence genes. This comprehensive analysis

provided rapid serotyping along with critical insights into *Salmonella* pathogenicity and resistance.

Significance: This ONT-based method delivers rapid and cost-effective *Salmonella* serotyping within just 15 hours, enhancing public health responses and enabling timely interventions for salmonellosis.

P3-169: A SHOTGUN METAGENOMIC APPROACH TO EVALUATE THE IMPACT OF SCAVENGING MATERIALS ON THE MICROBIOME IN THREE DIFFERENT AQUAPONIC SYSTEMS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Climate change and urbanization have reduced land for soil-based agriculture, driving interest in aquaponic systems to meet growing food demands.

Purpose: This study compared the microbiomes of “Outredgeous” lettuce (*Lactuca sativa*) from three differently composed biofiltered aquaponic systems using shotgun sequencing for food safety risk characterization.

Methods: Lab-scale (12-replicate, 4-goldfish/37 L tank) biofiltered, recirculating aquaponic systems with Styrofoam rafts (separate 19 L tank) supported four lettuce plants. Three biofilter scavenger assemblage comparisons included: [1] Zeolite (Z), Sodium Zeolite (NaZ), Silica (S), or Control (C); [2] Sodium Arcillite (NaAr), Activated Carbon (AC), Arcillite (Ar), and C; [3] Z, AC, Ar, and C; with 3 replicate tanks/treatment assemblage. Parameters such as fish health, water temperature, dissolved oxygen, pH, nutrients (total nitrogen, nitrate, nitrite), and plant growth were recorded. Samples collected for metagenomic analysis included: Water (750 mL weekly/plant tank), roots, sponge, sediment, leaves, and rockwool with roots (at harvest). DNA extracted from samples was analyzed using shotgun metagenomic sequencing on an Illumina NextSeq-2000. Taxonomic profiling was performed using the EzBioCloudpro.

Results: Microbial communities in the aquaponic systems were predominantly composed of Bacteria (98.31%), followed by Viruses (0.78%) and Fungi (0.014%), with Metazoa, Protists, and Viridiplantae contributing <0.007%. Proteobacteria dominated the bacterial community (82.38%), followed by Actinobacteria (12.04%), Bacteroidetes (1.95%), Firmicutes (1.57%), and Fusobacteria (0.73%).

Alpha diversity showed no significant differences among experiments ($p > 0.05$). Microbial diversity peaked during the final harvesting week, with Bray-Curtis dissimilarity indicating significant variation among the microbial community in experiment-3 ($p < 0.05$). The aquaponic systems did not contain *Salmonella*, *Escherichia*, *Clostridium*, *Listeria* or Norovirus. However, *Vibrio* and *Shewanella* were present, along with *Salmonella* and *Escherichia* phages. Different biofilter scavengers did not have any recorded/observable effect on plant growth, physico-chemical parameters or microbes.

Significance: This study highlights the microbial composition and safety of aquaponic systems for sustainable food production.

P3-170: COMPARATIVE MICROBIOME ANALYSIS OF SOIL-BASED AND SOILLESS AGRICULTURE SYSTEMS USING SHOTGUN SEQUENCING FOR ENHANCED FOOD SAFETY AND SUSTAINABILITY

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Controlled environment agriculture (CEA) production of fresh leafy greens/herbs continues to expand to meet consumer demand and food security, whereas open-field production is increasingly challenged by water and arable land shortages, and environmental contaminants.

Purpose: Characterize and compare by shotgun sequencing prokaryote and eukaryote microbial communities of 'Outredgeous' lettuce (*Lactuca sativa*) grown in soilless (hydroponic) and soil-based systems.

Methods: In a CEA greenhouse, lettuce was grown in two, 36-plant nutrient-film hydroponic systems, and in 12, 6" pots containing organically-managed field soil. Samples [soilless: water (weekly), rockwool with seedling (at transplant), rockwool with shoot, and roots (at harvest); soil-based: soil (at transplant and harvest), roots, rockwool with seedling (at transplant), and leaves (at harvest)] were collected to evaluate microbiome diversity differences on lettuce over time. Extracted DNA was analyzed using shotgun metagenomic sequencing on an Illumina NextSeq-2000. Taxonomic profiling was performed using the EzBioCloudpro.

Results: The soilless system contained 1,343 species, including bacteria (98.92%), viruses (0.85%), fungi (0.21%), metazoa (0.003%), and protists (0.02%). Conversely, soil-based had 2,333 species, comprising bacteria (98.44%), viruses (1.33%), fungi (0.19%), and archaea (0.04%) indicating this soil-based system provided greater microbial diversity and substrate for microbial growth compared to this soilless system. Neither system harbored major foodborne pathogens genera (*Salmonella*, *Escherichia*, *Listeria*, or norovirus). However, presence of

pathogenic *Pseudomonas*, *Clostridium*, and members of the family Enterobacteriaceae (*Citrobacter*, *Klebsiella*, *Enterobacter*) were prevalent in soil-based samples. Alpha diversity analysis revealed significant differences across systems and sample types, while beta diversity revealed a significant difference in microbial community composition between the two systems ($p < 0.05$). Soilless system water and soil from the soil-based system demonstrated the greatest diversity, while leaves had the least ($p < 0.05$).

Significance: This study reveals a higher abundance of pathogens from Enterobacteriaceae and Clostridiaceae in this soil-based system, thereby providing insights to enhance microbial risk characterization and food safety.

P3-171: EVALUATION OF SEQUENCING DEPTH REQUIREMENTS FOR ACCURATE TAXONOMIC ASSIGNMENT BY LONG READ SEQUENCING

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Deep shotgun metagenomics is a powerful tool that enables the detection of all organisms present in a sample, even those that cannot be cultivated. While this approach provides previously unobtainable insights, it can be cost prohibitive, especially for large-scale studies or routine surveillance applications. Shallow shotgun sequencing has been validated for other sequencing technologies (e.g., short read sequencing) and shown to generate highly accurate taxonomic profiles using much lower sequencing depths.

Purpose: This work evaluates the information content of varying depths of long read shotgun metagenomic sequencing to determine minimal yields required for accurate taxonomic assignment.

Methods: The microbial communities of 13 samples, comprising food matrices, pet food, an environmental swab, and two mock communities, were DNA extracted using the Qiagen DNeasy PowerFood Microbial Kit. The DNA was sequenced using the Oxford Nanopore GridION. The resulting sequences were then quality filtered using Nanofilt and subjected to diversity scoring and taxonomic assignment using Kraken 2 at varying depths.

Results: Shannon diversity scores ranged from 0.0055 to 4.0659 after 72 h of sequencing. For 100% of samples, maximal diversity scores were achieved within 24 h of sequencing, amounting to an average 486 Mb of total yield. As expected, higher diversity samples required more time/yield to reach maximal diversity. Many samples (60%) were able to achieve maximal diversity with as little as 6 h of sequencing, amounting to as little as 250 Mb of total yield on average.

Significance: These findings indicate that species-level taxonomic assignment can be achieved successfully within 24 hours of sequencing using the Oxford Nanopore GridION, and with far less sequencing resources than needed for deep shotgun metagenomics. This affords the possibility to reduce sequencing costs by being able to include more samples per run, or decreasing time to result by

performing less sequencing per sample.

P3-172: COMPARATIVE ANALYSIS OF SEQUENCING AND BIOINFORMATIC APPROACHES FOR SHOTGUN METAGENOMICS OF FOOD-ASSOCIATED SAMPLES

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Shotgun metagenomics affords the food safety sector the ability to address many key issues by screening ingredients, products, and environmental samples for multiple pathogens and spoilage organisms at once, even those that cannot be cultivated. Though this technology is promising, it is essential to select the most appropriate form of sequencing and analytical approaches to effectively utilize this testing.

Purpose: This work evaluates the quality of shotgun metagenomics by comparing two sequencing platforms and different bioinformatics approaches for the analysis of food matrices.

Methods: DNA was extracted from food samples (blueberries, lettuce, blue cheese) and a known mock community using the Qiagen DNeasy Powerfood kit. NGS libraries were prepared and sequenced on both the Illumina MiSeq (short reads, SRS, 200 bp/read) and the Oxford Nanopore MinION platforms (long reads, LRS, >1000 bp/read). Resulting sequence reads were then subject to quality filtering before taxonomic assignment using Kraken 2 for both raw reads and assembled metagenomes. Assignment rate was determined for both reads and assemblies. Metagenomes were evaluated for their quality and completeness using Quast and CheckM.

Results: LRS resulted in a higher proportion of characterized reads, either when the sample data were evaluated as raw reads (52.84% for SRS; 71.74% for LRS on average) or as genome assemblies (45.67% for SRS; 80.94% for LRS on average). Metagenome assembly improved characterization for LRS for all but one sample. However SRS assemblies resulted in lower percentage of characterization for all samples compared to SRS raw reads. LRS resulted in less fragmented, more complete metagenome assemblies, characterized by fewer contigs and higher N50 values, compared to SRS.

Significance: This work demonstrates that LRS with metagenome assembly yields

the highest quality shotgun metagenomic

Results, and can be used to effectively define the microbial community in different types of food matrices.

P3-173: DEVELOPING AND IMPLEMENTING FSIS-SPECIFIC CAMPYLOBACTER ALLELE CODES

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: In 2019 the Centers for Disease Control and Prevention's PulseNet and bioMérieux deployed an allele calling scheme using core genome multilocus sequence typing for *Campylobacter* isolates submitted to the PulseNet National Database. However, the scheme had limitations for food animal isolates; *Campylobacter coli* isolates were ineligible for allele code assignment and only 60% of the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) *Campylobacter jejuni* isolates met the 95% core genome alignment requirement for allele code assignment.

Purpose: FSIS developed an allele code naming scheme to encompass a wider variety of *Campylobacter* genomes that previously were not eligible for allele code assignment.

Methods: In 2023, FSIS partnered with bioMérieux to develop and implement a customized allele code scheme using 1,011 *Campylobacter* core genes.

Results: The newly developed scheme encompasses ~99% of FSIS's *C. jejuni* and *C. coli* isolates and has successfully generated 9,389 (4,524 for *C. coli* and 4,865 for *C. jejuni*) unique allele codes for over 35,000 isolates. Additionally, preliminary analyses of the new scheme revealed that ~5.0% (n=814) of *C. jejuni* and 4.4% (n=755) of *C. coli* isolates are distinct subtypes in FSIS's database.

Significance: FSIS plans on using the allele codes to identify repetitive subtypes within establishments or corporations. This information will help FSIS and regulated establishments better understand both the diversity and ubiquity of subtypes and the effectiveness of process control strategies in establishments. FSIS also intends to use the allele codes for reporting within internal data systems and publicly available establishment-specific datasets.

P3-174: PLASMIDS AND PHAGES: LINKS IN THE 2024 CUCUMBER SALMONELLA OUTBREAK

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The multi-strain 2024 *Salmonella enterica* cucumber outbreak resulted in 551 reported illnesses and 155 hospitalizations. The outbreak was linked to 4 strains of serovar Braenderup and 1 strain of serovar Africana. None of the outbreak strains were recovered from the cucumbers, but one outbreak Braenderup strain was recovered from the implicated Florida farms. 30 non-outbreak-strain *Salmonella* serovars were also recovered from the farm regions and cucumbers during on-site sampling.

Purpose: This study aimed to determine whether horizontally transferred mobile elements—specifically plasmids and phages—could genetically link outbreak *Salmonella* strains to each other, to non-outbreak strains found on implicated farms, and to cucumber samples, thereby supporting potential source-tracing hypotheses.

Methods: Platon and phigaro were used to identify plasmids and phages in the genomic data, respectively. The plasmids and phages were clustered using Mash. The CFSAN SNP Pipeline was used to align clustered sequences and find variants. Garli was used to build phylogenies. BLAST and Mash were used to find the most similar sequences in the Pathogen Detection database.

Results: Our analysis identified 3 plasmid and 5 phage clusters that supported a link between the multiple outbreak strains, the farm regions, and the cucumber product. Amongst these was an incFIB phage-plasmid cluster whose phylogeny showed tight clustering of the clinical and non-outbreak-strain cucumber isolates. The other plasmid and phage phylogenies showed clustering of the various outbreak strains with each other and, sometimes, with non-outbreak-strain farm environment isolates. Analysis of the metadata associated with the top hits in the NCBI Pathogen Detection database did not reveal any potential animal sources of contamination due to the promiscuity of the phages and plasmids.

Significance: Our analysis supports that mobile elements like phages and plasmids can be a powerful source of information for source tracing investigations, providing evidence for connections between outbreak and non-outbreak strains

thought to originate from the same microbiome.

P3-175: GENOMIC EVIDENCE FOR ADAPTIVE EVOLUTION OF *SALMONELLA* TYPHIMURIUM

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Salmonella* Typhimurium, a significant foodborne pathogen, has a known capacity to infect a broad host range, but its mechanisms to genetically adapt to different hosts are underexplored.

Purpose: This study aims to uncover genomic variations that may drive the adaptive evolution of *S. Typhimurium* across various hosts, facilitating source attribution.

Methods: We analyzed 745 genomes of *S. Typhimurium* isolated from five sources, including bovine, swine, poultry, wild birds, and the environment. Pangenomes, mobile genetic elements (plasmids and prophages), and antibiotic resistance genes (ARGs) were characterized and compared across sources. BUSTED models followed by functional enrichment analysis were used to identify genes and functions under positive selection (PS). Machine learning (ML) models were trained to attribute isolation sources based on gene presence/absence and SNP variants.

Results: *S. Typhimurium* showed large genomic variance across isolation sources, as evidenced by source-associated subclades defined by single nucleotide polymorphisms (SNPs), variations in pangenome size and openness, and significant differences in the prevalence of plasmids, prophages, and ARGs. Of note, *S. Typhimurium* from swine exhibited the largest and most open pangenome and the highest prevalence of ARGs, likely mediated by horizontal gene transfer via transduction. While positive selection on core genes was weaker than on accessory genes, its frequency significantly varied across isolation sources. Isolates from the environment experienced the highest degree of PS, particularly among genes encoding cell cycle control and coenzyme and nucleotide transport and metabolism. Using ML, we further showed that isolation sources could be

predicted with high accuracy (mean auROC of 0.98 for both gene presence/absence and SNP-based models).

Significance: These findings suggest that *S. Typhimurium* undergoes adaptive evolution under different host and environmental conditions, with pangenome fluidity supporting a broad host range. This plasticity, combined with accurate ML-based source prediction, strengthens foodborne pathogen attribution and informs more effective food safety decision-making.

P3-176: GENETIC DIVERSITY OF SALMONELLA ENTERICA SEROVAR RUBISLAW ISOLATED IN THE U.S.

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Salmonella enterica* serovar Rubislaw (*S. Rubislaw*) is an important cause of human salmonellosis, with the majority of human cases being reported in the southeastern US. In contrast to other serovars that are commonly isolated from agricultural animals relatively little is known about common reservoirs of *S. Rubislaw*.

Purpose: This study aimed to characterize the population structure of *S. Rubislaw* isolates collected in the US from human clinical and environmental sources.

Methods: *S. Rubislaw* (2503 genome assemblies) with location 'USA' were downloaded from Pathogen Detection database. Representative isolates of *S. Rubislaw* for each SNP cluster, and those representing singletons with predicted type "Rubislaw" were included; assemblies with >300 contigs and an N50 <50,000 were discarded. Phylogenies based on core SNPs identified with kSNP4 were inferred with IQ-TREE with 1000 bootstrap repetitions for (i) 716 assemblies of *S. Rubislaw*, including 328 with state-level isolation data, and (ii) representative isolates of *S. Rubislaw* phylogenetic clades and 269 assemblies of 215 *S. enterica* serovars.

Results: *S. Rubislaw* isolates clustered into 6 major phylogenetic clades, with ~90% of isolates clustering into two primary clades. These two clades included predominantly isolates from the Southern US, but also environmental isolates from Western states, suggesting broad geographic distribution of these genetic subtypes in the US. Inclusion of representative assemblies for 215 additional serovars, suggested that *S. Rubislaw* shares a common ancestor with *S. Abaetetuba*. Based on tree topology, *S. Rubislaw* (I 11:r:e,n,x) is paraphyletic with *S. Abaetetuba* (I 11:k:1,5), suggesting that differential acquisition of H1 and H2 antigens led to the emergence of these two serovars from a common O11 expressing ancestor.

Significance: Our analyses support that *S. Rubislaw* isolated from environmental and clinical samples across the US are genetically similar, highlighting an important role of environmental sources (water and food) as potential reservoirs for *S. Rubislaw*.

Award Application: Developing Scientist Award

P3-177: POPULATION DYNAMICS OF CRONOBACTER SAKAZAKII AND NATIVE MICROBIOTA IN POWDERED INFANT FORMULA DURING ENRICHMENT

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Cronobacter sakazakii* has previously been linked to powdered infant formula (PIF) and PIF processing facilities. *C. sakazakii* contamination of foods generally occurs at low levels; therefore, enrichment is utilized for detection. Various enrichment methods are currently available for the detection of *C. sakazakii* from PIF.

Purpose: To determine the population dynamics of *C. sakazakii* and native microbiota during different enrichment procedures using metagenomic sequencing.

Methods: PIF was inoculated with *C. sakazakii* at 1 log CFU/g and enriched using the BAM, ISO, or R&F methods using a sample size of 100 g. Samples were removed throughout enrichment and DNA extracted using the MagMAX CORE Nucleic Acid Purification Kit on a KingFisher Flex. Samples were prepared using the Illumina DNA Prep Kit and sequenced on a NextSeq 2000 (P1, 600 cycles). Reads were quality checked, taxonomically classified using Kraken2, and taxa relative abundance (RA) estimated using Bracken. The Kruskal-Wallis test was used to compare differences in RA; $p < 0.05$ was considered significant.

Results: The PIF un-enriched microbiome was dominated by *Bacillus*, *Lactococcus*, *Streptococcus*, and *Actinoalloteichus*. After inoculation, *C. sakazakii* RA was $< 1\%$. For BAM enrichment, *C. sakazakii* RAs were 3.6% and 31.5% after 8 and 24 h, respectively, and 8.8% after centrifuging; *Bacillus* dominated after 24 h (55.3%) and after centrifuging (78.9%). For R&F enrichment, the RA of *C. sakazakii* significantly increased from 1.3% to 94.4% after 8 and 24 h, respectively. For ISO, the RA after 18 h in primary enrichment was 16.1%; after 8 and 24 h of secondary enrichment, the RAs were 82.9% and 92.7%, respectively. Overall, significantly higher final *C. sakazakii* RAs were observed for both the ISO and R&F methods compared to BAM.

Significance: Results of this study may help elucidate how the various enrichment

methods influence downstream detection of *C. sakazakii*.

Award Application: Developing Scientist Award

P3-178: COMPARATIVE TRANSCRIPTOMICS OF SALMONELLA HEIDELBERG, TYPHIMURIUM AND MONOPHASIC TYPHIMURIUM REVEALS DIFFERENTIAL EXPRESSION OF STRESS TOLERANCE, VIRULENCE, AND BIOFILM-ASSOCIATED GENES

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Some serovars and strains of *S. enterica* survive the stresses of food processing environments unusually well, potentially increasing their ability to cause an outbreak; however, mechanisms of stress tolerance may differ among serovars and strains

Purpose: Comparative transcriptomic analyses offer insight into the adaptations different serovars and strains develop to survive in processing environments

Methods: *Salmonella* Heidelberg (chicken outbreak, 2013-14; n=1, and chicken liver outbreak 2012 (n=2)); Typhimurium (peanut butter outbreak 2008; n=1); and monophasic Typhimurium (pork outbreak 2015; n=2) were selected for RNA sequencing. RNA was extracted from stationary-phase cultures on three separate days for each isolate using Purelink RNA mini kit, rRNA depleted with NEB Next rRNA depletion, and prepared into libraries using the Zymo RiboFree Total RNA Library Kit. mRNA was sequenced on the Illumina HiSeq. FastQC assessed quality and Trimalore removed reads < Q20. Serovar-specific alignments (minimap2) were performed with Heidelberg: GCF_000020705.1; monophasic Typhimurium: GCF_000170255.1; and Typhimurium: GCF_000006945.2. HTSeq defined gene counts and DESeq2 assessed differential expression (padj < 0.05 and |log2FoldChange| > 1). Genes were grouped and characterized by GO processes and manual curation.

Results: *Salmonella* Heidelberg showed higher expression of virulence genes *sopE*, *sopD*, *sopD2*, *sopF*, and *hfq* compared to *Salmonella* Typhimurium samples, and virulence genes *spaK* and *prgH* versus monophasic Typhimurium samples. Additionally, curli formation genes *csgAE* and stress response genes *dnaJ*, *soxR*, *bhsA*, *aaeA* and *uspF* were upregulated in Heidelberg samples vs monophasic Typhimurium samples, while *uspCF*, *cspE*, and *hslO* were upregulated in Heidelberg vs Typhimurium samples. Conversely, stress-response regulator *rpoS*,

acid-response hdeB, and iron uptake gene fur were upregulated in monophasic Typhimurium versus Heidelberg, and stress response genes ibpA and hdeB, and biofilm-associated adrA were upregulated in Typhimurium compared to Heidelberg.

Significance: Virulence, biofilm-formation, and stress-response genes were differentially expressed between serovars, supporting serovar-specific stress adaptations and risks to consumers.

P3-179: GENOTYPES OF MULTIDRUG RESISTANT PHENOTYPE BACTERIA ISOLATED FROM URBAN FARMS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Urban farms are an innovative way to support community engagement and local food production. However, food safety concerns may arise due to organic amendment inputs that are used to overcome depleted nutrient availability and potential heavy metal contamination to urban soils, which may select for bacteria carrying antimicrobial resistance genes (ARGs).

Purpose: The objective of this study is to (1) determine which microorganisms and ARGs are found in multi-drug resistant (MDR) phenotype bacteria on urban farms and (2) understand the transmissibility of ARGs in urban food systems.

Methods: In this study, we performed whole genome sequencing on 88 isolates of bacteria with MDR phenotypes that were recovered from leafy greens and soil from urban farms across the DC area (n = 7 sites). The genotypes of each isolate were characterized by a variety of bioinformatics tools.

Results: The majority of the MDR bacteria were identified as members of *Pseudomonas* (33%), *Serratia* (25%), *Providencia* (12.5%), and *Bacillus* (12.5%). A total of 251 ARGs were found among the isolates, the lot of them being efflux pumps and genes within the *bla* and *tet* groups.

Significance: The lack of ARGs found in the MDR *Providencia* genomes may reflect potential novel mechanisms of resistance. Moreover, heavy metal tolerance genes were found in 18% of the isolates, suggesting that soil contaminants may co-select for drug resistance. Determining whether the ARGs are located on mobile genetic elements would be a critical future direction to determine how antimicrobial resistance spreads through these emerging food systems, including to potential foodborne pathogens.

Award Application: Developing Scientist Award

P3-180: EXPLORING THE MICROBIAL QUALITY AND GEOGRAPHIC INFLUENCE ON THE ETHIOPIAN RAW MILK MICROBIOME

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The microbiome of raw milk is influenced by various factors, including temperature, pH, and geographic location, which can affect the quality and safety of milk.

Purpose: This study aimed to explore relationships between the microbiota composition, quality parameters, pathogen presence, and geographic location of Ethiopian raw milk.

Methods: Ninety raw milk samples were collected from farms and collection sites from two regions in Oromia, Ethiopia (i.e., Debre Zeit and Holeta) during the dry season of 2020. The pH and temperature were measured, and the presence of *Listeria monocytogenes*, *Salmonella* spp., and *Campylobacter* spp. were detected using the ISO 11290-1:2018, ISO 6579-1, and ISO 10272e1:2017-B, respectively. The microbiota composition was characterized using 16S rRNA amplicon sequencing. Statistical analyses included PERMANOVA to evaluate geographic effects on microbiota composition and redundancy analysis (RDA) to identify drivers such as geographic location, milk quality parameters, and pathogen presence.

Results: Raw milk from Debre Zeit and Holeta had a mean pH of 5.8 ± 0.7 and 5.9 ± 0.7 , respectively, and a temperature of 22.3 ± 6.5 and 19.6 ± 6.4 , respectively. In Debre Zeit, 6.5% (n=3), 54.3% (n=25), and 10.9% (n=5) of raw milk tested positive for the presence of *L. monocytogenes*, *Salmonella* spp., and *Campylobacter* spp., respectively. In Holeta, the prevalence of pathogens was 2.3% (n=1), 13.6% (n=6), and 6.8% (n=3), respectively. The geographic location significantly influenced microbiota composition ($p=0.001$). Raw milk from Debre Zeit was dominated by *Acinetobacter* ($34.3 \pm 3.6\%$, n=46) and *Aeromonas* ($28.7 \pm 3.8\%$, n=46), while milk from Holeta was dominated by *Lactococcus* ($52.5 \pm 3.7\%$, n=44) and *Acinetobacter* ($12.9 \pm 2.2\%$, n=44). RDA revealed that

geographic location, collection site, and pH were significant drivers of the microbiota composition of Ethiopian raw milk ($p=0.001$, $R^2=0.32$).

Significance: Our findings highlight the impact of geographic location on the microbiota composition of Ethiopian raw milk.

P3-181: PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF AEROTOLERANCE IN CAMPYLOBACTER SPECIES ISOLATED FROM COMMERCIAL BROILER CHICKENS AT SLAUGHTER

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The *Campylobacter* genus includes human foodborne pathogens commonly transmitted by the consumption of contaminated and/or undercooked poultry meat. Generally, thermophilic *Campylobacter* species require microaerophilic conditions to survive and grow. However, poultry meat processing likely disrupts these conditions in the micro-habitat.

Purpose: We therefore hypothesized that *Campylobacter* species isolated from broiler chickens at slaughter may be likely to exhibit aerotolerant characteristics.

Methods: Briefly, *Campylobacter* were isolated from broilers in a slaughter facility, weekly, over seven years. These isolates were screened for the ability to grow under aerobic versus microaerophilic conditions. A subset of these isolates (n = 46) were subjected to two additional aerobic growth trials and whole genome sequencing. Whole genome sequences were assembled with Spades and annotated with Prokka. Annotated sequences were analyzed with Roary and Scoary to establish the pan-genome and to identify genes which may influence the ability of *Campylobacter* to grow aerobically.

Results: Of 269 isolates, 127 grew aerobically (47%). In the subset of 46, 11 grew aerobically in all three trials (high), 18 grew in two of three (moderate), 10 grew in only one of three (low), and seven never grew aerobically. In the genomic analysis, 50 genes were significantly associated with aerotolerance (Fisher's Exact Test; $p < 0.05$). However, only one gene, a hypothetical protein, was present in more than 70% of isolates which grew aerobically and less than 30% of those which did not. Conversely, 24 genes (16 of which were hypothetical proteins) were present in more than 70% of non-aerotolerant isolates but absent from more than 70% of aerotolerant isolates.

Significance: This analysis identified *Campylobacter* genes which could be related to the ability to grow aerobically. These genes could be evaluated for phenotypic functionality, which may enable the development of novel, targeted interventions to reduce *Campylobacter* contamination during poultry meat processing.

P3-182: METAGENOMIC ANALYSIS OF THE MICROBIAL COMMUNITY OF AN EXPERIMENTAL HYDROPONIC SYSTEM GROWING LEAFY GREENS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Hydroponics is gaining popularity for the growth of fresh produce due, in part, to the idea that hydroponic growth improves food safety outcomes. However, recent recalls of hydroponically grown lettuce due to contamination with foodborne bacterial pathogens highlight knowledge gaps regarding the microbiology of hydroponic systems.

Purpose: This study explored the diversity and development of hydroponic microbiomes from inputs (seeds, nutrient solution, water) to harvest, and sought to identify food safety risks.

Methods: Romaine and butterhead lettuce were sprouted in rockwool and then grown in a benchtop deep water culture hydroponic system. Metagenomic sequencing was performed on samples from the hydroponic system and its inputs to characterize the microbiome and to identify potential food safety hazards. Samples were collected from the hydroponic system from the root zone, leaves, nutrient solution, and the interior surfaces during the 85-day growth period, as well as from the seeds (pelleted and naked), sprouts, rockwool, and nutrient solution inputs. Samples underwent shotgun metagenomic sequencing.

Results: The microbial communities were taxonomically distinct, demonstrating variation by sampling location, lettuce type, and timepoint. The hydroponic system retained over 90% of the original seed genera, with half of all genera originating from the seeds. No foodborne pathogens were detected in the metagenomes; however, *Legionella*, a human pathogen often found in water, was amongst the top ten most abundant genera. Functional profiling of the metagenomes revealed a high abundance of metal resistance genes for copper, mercury, and nickel, suggesting that heavy metals might build up in such closed loop systems and need to be monitored in future work.

Significance: This study highlights that the inputs to a hydroponic system have a large and lasting impact on its microbiome. Future work will assess how the inputs might introduce and lead to the persistence of pathogens in hydroponic systems.

P3-183: SALMONELLA SEROVAR INFANTIS REPJFX01 ISOLATES HARBOR A PESI PLASMID CONTAINING ADDITIONAL GENES NOT FOUND IN PESI PRESENT IN CLOSELY RELATED NON-REP STRAINS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Salmonella enterica* subsp. *enterica* serovar *infantis* strain REPJFX01 is a multidrug-resistant strain associated with poultry and international travel to Latin America that has been designated as a reoccurring, emerging, and persistent (REP) strain by the CDC due to the many foodborne illnesses and outbreaks associated with this strain.

Purpose: This study aimed to assess whether the presence of certain genes is associated with *S. infantis* REPJFX01 in comparison to closely related *S. infantis* isolates.

Methods: Publicly available genomes of *S. infantis* REPJFX01 (n=100), non-REP isolates from the same SNP cluster (n=100) and non-REP isolates from four other SNP clusters within the *S. infantis* group (n=100) were downloaded from NCBI Pathogen Detection. Genomes were annotated with Prokka and differences in the pangenome of the three groups was assessed with Panaroo and Scoary. pESI plasmid presence was assessed with Blast, and the location of REP-associated genes was assessed through mapping to a closed assembly. kNSP4 was used to create a phylogenetic tree of all genomes (n=300).

Results: When comparing REPJFX01 isolates to non-REP isolates from South America within the same SNP cluster as REPJFX01, no significant association was observed. However, when comparing REPJFX01 isolates to non-REP isolates from four closely related SNP clusters, 29 genes were significantly associated with REPJFX01 isolates ($p < 0.001$ and proportion difference ≤ 0.35). REPJFX01-associated genes included arsenic and antimicrobial resistance genes (AMR), including β -lactamase blaCTX-M, and genes involved in genetic modulation and recombination. Most of these genes (25/29) were located on the pESI plasmid, which was also found in most non-REP isolates from closely related SNP clusters (75/100), although without these genes.

Significance: The presence of AMR genes suggests that resistance to antimicrobials may facilitate persistence of REPJFX01 in poultry flocks. Identifying genetic characteristics specific to REP strains may help us implement additional intervention and control strategies.

P3-184: GENOMIC ANALYSIS OF STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM A DAIRY FARM IN TEXAS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Staphylococcus aureus* is the leading cause of contagious mastitis among dairy cattle, resulting in reduced milk production.

Purpose: To better understand specific genotypes of *S. aureus* associated with cattle mastitis and its relatedness to SA from foods and humans, we aimed to investigate virulence profiling for *Staphylococcus aureus* strains isolated from a dairy farm in Texas.

Methods: A surveillance study was conducted from July 2022 to August 2023. A total of 282 culture samples were collected on the dairy farm from cow's milk, feed, udder, feces, and environment. All samples were processed and analyzed for *Staphylococcus* spp. The *Staphylococcus* spp. were speciated and submitted for further evaluation using whole-genome sequencing with the Illumina NextSeq 2000 system. GTDB-Tk R207_v2 was applied to determine taxonomic classification and VirulenceFinder 2.0 was used to identify virulence genes in *Staphylococcus aureus* strains.

Results: There were 13 different *Staphylococcus* species identified, including *S. aureus* (n=150), *S. simulans* (n=28), *S. chromogenes* (n=14), *S. muscae* (n=12), *S. haemolyticus* (n=8), and *S. saprophyticus* (n=7), in addition to *Mammaliicoccus sciuri* (n=23) and *Aerococcus urinaeequi* (n=10). Virulence genes identified in the *S. aureus* strains included *aur*, *splABE*, *hlgABCD*, and *lukDE*. Genes encoding staphylococcal enterotoxins included *sea*, *sec*, *sed*, *see*, *seh*, *sei*, *sel*, *sem*, *sen*, *seo*, and *seu*.

Significance: The study provides some of the most comprehensive genomic data and analysis to date for *Staphylococcus aureus* and other types of *Staphylococcus* isolates from a dairy farm, offering novel and deep insights for contagious mastitis management and potential linkages of isolates from the dairy farm to the food and feed supply, particularly human toxin poisoning.

P3-185: PANGENOME ANALYSIS OF LISTERIA: BASIS FOR IDENTIFICATION OF SPECIES AND SEROTYPE-SPECIFIC GENETIC MARKERS FOR MOLECULAR DIAGNOSTICS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Listeria*, a Gram-positive bacterium and one of the four major foodborne pathogens posing global public health risks, requires precise detection methods like PCR, with gene-specific targets essential for effective diagnostics and species identification in food.

Purpose: To conduct a pan-genomic and core genomic analysis of *Listeria* species and *Listeria monocytogenes* serotypes to identify genetic variation to exploit for gene-specific diagnostics.

Methods: Thirty-three and fifteen high-quality genomes representing 10 *Listeria* species and 8 *Listeria monocytogenes* serotypes, respectively, were retrieved from the NCBI database and curated using the CheckM tool. These genomes were annotated for pangenome analysis through the Roary ILP Bacterial Annotation Pipeline (RIBAP). Targets were identified through the gene report generated by the pipeline and validated using NCBI MegaBLAST.

Results: The analysis revealed significant genomic diversity in the core gene content across various *Listeria* species, with 856, 665, 362, 89, and 16 core genes identified at 60%, 70%, 80%, 90%, and 95% nucleotide identities, respectively. At the 95% identity level, core genes such as *rpsR*, *spoVG_2*, *spxA*, *prs_1*, *pstC1*, *rpoA*, and *rpsS* were identified and are involved in molecular functions, biological processes, and cellular components. The total accessory genes were 10,400 at 60% and 25,486 at 95% nucleotide identity. However, *Listeria monocytogenes* serotypes displayed lower genetic diversity, with 2,458 core genes identified at a 60% identity level and 2,145 core genes at a 95% identity level. The gene-specific targets identified were hypothetical proteins with unknown functions, yet they

exhibited 90% sensitivity and 98–100% specificity.

Significance: This study provides gene-specific targets for efficient diagnostics and differentiation of *Listeria* species and *Listeria monocytogenes* serotypes. This can be further validated and offers a foundation for improving public health responses, advancing molecular diagnostics, and exploring pathogen evolution.

Award Application: Developing Scientist Award

P3-186: DEVELOPMENT AND OPTIMIZATION OF A TARGETED AMPLICON SEQUENCING ASSAY FOR EARLY DETECTION AND RAPID FINGERPRINTING OF CRONOBACTER STRAINS FROM FOOD SAMPLES

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Hee Jin Kwon, University of Maryland

Rachel Binet, U.S. Food and Drug Administration

Eric Brown, FDA-Human Foods Program

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Cronobacter sakazakii* strains are implicated worldwide in dangerous infections in infants under 2 months of age after ingestion of contaminated powdered infant formula (PIF).

Purpose: To develop critical robust sequence-based methods for accurate taxonomic calls and rapid fingerprinting of contaminating *Cronobacter* strains in PIF to ensure the safety of PIF supply.

Methods: A *Cronobacter* Targeted Amplicon Sequencing (TAS) panel with 192 optimized targets was developed and tested in the laboratory as follows. About 500 representative whole genome sequences of seven *Cronobacter* species and near-neighbor strains known to co-contaminate PIF samples were used to determine taxon-specific unique targets. A pre-defined set of 100+ *Cronobacter* strains isolated from clinical, environmental, PIF-manufacturing and food samples belonging to 35+ sequence types (ST) were used for optimization. Total DNA was generated from nutrient and PIF-enriched cultures for constructing custom libraries that were sequenced on Illumina MiSeq using Nano flowcells. A new bioinformatic workflow was built using a custom allelic database with 500+ strains of *Cronobacter* and other genera for sequence analysis.

Results: All of the 146 TAS panel targets for *Cronobacter* were identified from mixed samples containing respective species. The variable sequences of the 56 C.

sakazakii target amplicons enabled strain-level discrimination among different *C. sakazakii* strains. About 20 targets had conserved primers across different *Cronobacter* species and amplified additional targets that were genotyped bioinformatically. 18 strains of *Franconibacter*, *Siccibacter*, *Salmonella*, and *Escherichia coli* were correctly identified using the reads from the 17, 11, 10, and 8 unique targets respectively. High quality reads from PIF-enriched and pure cultures of 50 clinical and environmental strains belonging to 24 *C. sakazakii* strains with known genomes resulted in accurate allele calls.

Significance: These results highlight the potential of the TAS application for routine surveillance of PIF samples and manufacturing environments, providing rapid identification and high-resolution genotyping of *Cronobacter* species critical for PIF safety.

P3-187: DEVELOPMENT AND SUCCESSION OF MICROBIAL COMMUNITIES ON THE LEAF SURFACE OF LEAFY GREENS IN RESPONSE TO POSTHARVEST PROCESSING TREATMENTS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The effect of postharvest processing on the native microbiota of leafy greens has not been well established and requires further investigation.

Purpose: The study examined the effect of postharvest processing steps including washing and storage on the microbiome of leafy greens.

Methods: Baby lettuce and spinach were grown in a controlled environment chamber. At harvest, the plants were divided into four treatments: (1) untreated, (2) chlorine wash, (3) chlorine wash with storage at 4°C for 7 days, and (4) chlorine wash with storage at 24°C for 7 days. After treatment, leaves were sonicated in 1X Tris-EDTA buffer with 2% Tween 80; negative controls were used. DNA was extracted using Qiagen DNeasy PowerMax Soil Kit. Illumina MiSeq was used to perform 16S amplicon sequencing.

Results: In both baby lettuce and spinach, the untreated samples showed more microbial variation than the chlorine wash and storage samples. Results indicate postharvest treatment of leafy greens is an important driver of bacterial community composition ($p < 0.05$). Spinach samples subjected to chlorine rinse and 24°C storage had more dominant *Enterobacter* species than the untreated samples. *Pseudomonas* species were dominant on baby lettuce samples subjected to 4°C storage after chlorine rinse. *Enterobacter* species were dominant on baby lettuce samples subjected to 24°C storage after chlorine rinse as compared to untreated samples.

Significance: Postharvest treatment of leafy greens affects microbial community composition. Chlorine rinse can amplify a few genera of bacteria that can affect food safety.

P3-188: MICROBIOME ANALYSIS OF NUTRIENT SOLUTION AND BOK CHOY IN FIVE DIFFERENT HYDROPONIC SYSTEMS ACROSS SEASONS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Hydroponic farming is a growing field with limited data on microbiome differences between systems and seasons. This study aimed to understand these differences by growing bok choy in five systems – Kratky (KR), Nutrient Film Technique (NFT), Deep Water Culture (DWC), Ebb and Flow (EF), and Drip Irrigation (DI) - over 7 weeks.

Methods: Nutrient solution was sampled weekly and the outer leaves of the harvested bok choy were collected from each system. Microbiota composition was characterized through 16S rRNA V4 sequencing. Experiments were conducted in fall (November-December) and spring (February-March). Data were analyzed in RStudio using DADA2, ALDEx2, and PERMANOVA.

Results: A total of 7,325 amplicon sequence variants (ASVs) were identified, with DI having the most (1,509) and DWC having the least (180). *Pseudomonas* species were present in 94.2% of samples with relative abundance ranging from 0.03 - 91%. Microbiota composition significantly differed between systems, except for DWC – NFT and DWC – KR. Seasonal differences were only significant for the NF and DWC systems ($p < 0.001$). While pairwise comparisons of systems revealed 252 significantly differentially abundant ASVs, KR and DWC (two systems closely related in terms of operation) did not have any differentially abundant ASVs.

Significance: Microbiota composition varied significantly among most systems, and seasonal differences were found in two systems. The KR and DWC systems had similar bacterial composition, likely due to their similarities in interactions between nutrient solution and plant roots. *Pseudomonas* spp. were abundant in all studied systems in both seasons, highlighting their importance in future hydroponic farming research. The DI and EF systems had the highest number of unique ASVs, likely due to these systems' use of soilless substrates in addition to nutrient solution. These findings highlight a need for more research focused on the role of *Pseudomonas* spp. and soilless substrate microbiomes in hydroponic

systems.

Award Application: Developing Scientist Award

P3-189: TRANSCRIPTOMIC ANALYSIS OF SALMONELLA ENTERICA SEROVAR TYPHIMURIUM IN RESPONSE BLUE LIGHT WITH A 405 NM WAVELENGTH

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Antimicrobial blue light (aBL; 405 nm) reduces the viability of foodborne pathogens, including *Salmonella*. It is hypothesized that the killing mechanism of aBL is similar to general bacterial response to visible light mediated by photosensitive proteins; however, limited information exists on *Salmonella*'s aBL stress response.

Purpose: This study was undertaken to characterize and identify gene expression differences in *Salmonella* Typhimurium strain 14028s in response to sub-lethal exposure to aBL.

Methods: Tryptic soy agar cultures were suspended in phosphate-buffered saline (8 log CFU/mL), and liquid suspensions were exposed to 405 nm aBL (405 J/cm²) at room temperature. Total RNA was extracted from exposed and control bacterial suspensions, followed by quantity (Qubit) and quality (Bioanalyzer and gel-electrophoresis) checks. Experiments were repeated three times. Libraries were constructed, and RNA-seq was performed using the Illumina NextSeq 2000 platform with paired-end sequencing. Differential gene expression was analyzed using edgeR, with fold change (FC) ≥ 2 and $P < 0.05$ considered significant.

Results: RNA-seq analysis revealed significant changes in gene expression following sub-lethal aBL exposure. A total of 231 genes were up-regulated, and 828 genes were significantly down-regulated (FC ≥ 2 , $P < 0.05$). Functional annotation showed that 24 differentially expressed genes (DEGs) were involved in catalytic activity. Notably, *katG*, which encodes for a catalase involved in hydrogen peroxide hydrolysis and oxidative stress defense, was highly up-regulated (FC=21.2). Genes related to protein folding (*dnaK*, *dnaJ*) and oxidative stress (*ahpF*, *ahpC*) were also significantly up-regulated. Interestingly, virulence-associated genes such as *sifA* and *spvR* also showed increased expression (FC ≥ 2).

Significance: This RNA-seq analysis generated valuable insights into the molecular mechanisms of *Salmonella*'s response to aBL exposure. Understanding these responses may aid the development of more effective aBL-based antimicrobial

strategies for food safety.

Award Application: Developing Scientist Award

P3-190: HIGH RESOLUTION AND USER-FRIENDLY MICROBIAL AND VIRAL GENOTYPING IN A SINGLE PCR-READY-TO-SEQUENCE FORMAT

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: A streamlined, comprehensive, affordable, and user-friendly method is needed to detect, genotype/serotype, subtype, monitor surveillance, and differentiate multiple serotypes, all within a single-tube PCR format. Current state-of-the-art methods are fragmented, requiring multiple procedures/techniques, which are expensive, complex, and time-intensive. Additionally, many laboratories lack the necessary infrastructure to perform comprehensive analyses in-house, relying instead on third-party service providers.

Purpose: Utilizing high-resolution, cost-effective single-tube-PCR, with simple streamlined workflow for microbial and viral pathogens, capable of detecting, serotyping, subtyping, surveillance, identifying multiple serotypes within a single sample as well as an integrating data analysis software without the need for bioinformatics expertise.

Methods: We have developed the MLSTnext NGS technology that amplifies and sequences up to 200 polymorphic loci in a single-tube-PCR reaction, which combines amplification and barcoding/indexing within the same reaction. After amplification, amplicons are pooled and sequenced using the Illumina NGS platform. MLSTnext has been applied to a wide range of food safety applications such as *Salmonella*, *Listeria*, *Legionella*, HAV, STEC/*E. coli*, *Campylobacter*, *Cyclospora*, and more.

Results: All assays generated high-resolution results capable of detecting and accurately serotyping each sample down to the subtype level. For instance, the *Salmonella* assay distinguished between two *S. typhimurium* strains by identifying sequence variations in four loci, revealing the same serotype but different strains. Additionally, the assay demonstrated the ability to detect the co-presence of multiple isolates, such as *Salmonella* or *Listeria* (up to nine serotypes), within a single sample. Furthermore, the unique loci profile generated for each species enabled effective traceability and source tracking, supporting robust contamination surveillance and outbreak management.

Significance: MLSTnext is versatile and user-friendly, generating comprehensive results cost-efficiently. The entire workflow takes 10–24 hours (depending on the sequencing platform) without the need for pure colonies/isolates. The workflow is standardized across all assays, enabling multiple assays to be sequenced together in the same sequencing run.

P3-191: IMPACT OF ANTIBIOTIC APPLICATION AND WATER TEMPERATURE ON THE RESISTOME AND MICROBIOME DYNAMICS IN CATFISH GUT

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Terramycin (oxytetracycline) is an FDA-approved antibiotic commonly used to treat enteric septicemia in catfish caused by *Edwardsiella ictalurid*. Despite its widespread use, its impact on the gut microbiota of catfish remains poorly understood. Additionally, water temperature, a key factor influencing disease outbreaks, fish health, and antibiotic degradation, has been underexplored in this context.

Purpose: This study evaluated the combined effects of antibiotic administration and water temperature on the catfish gut microbiome and resistome.

Methods: Thirty-six 35-gallon tanks were set up at the Alabama fish farming center, with four replicates per treatment. Tanks were filled with pond water and equipped with biofilters. Temperatures were maintained at 20, 25, and 30°C, with dissolved oxygen at ~6 mg/L. Each tank housed 25 channel catfish fingerlings pre-fed commercial feed for two weeks to stabilize microbial communities. Terramycin-medicated feeds were administered during treatment, followed by commercial feed during withdrawal. Samples were collected at three time points (start, end of treatment, and end of withdrawal). Intestinal contents were collected using FLOQSwabs®, pooled for plating or stored for DNA extraction and metagenomic sequencing. ARGs were analyzed to assess their abundance and diversity.

Results: Temperature significantly influenced the fish gut microbiome. At higher temperatures (25°C and 30°C), *Plesiomonas* increased from 11.8% at 20°C to 30.0% and 25.0%, respectively, while *Cetobacterium* declined from 82.5% to 40.7% and 47.0%. Antibiotic application further altered the microbiome, increasing the relative abundance of *Mycobacterium*, *Enterobacter*, and *Klebsiella* at 20°C, 25°C, and 30°C. Total ARG abundance and richness rose significantly with temperature and antibiotic treatment, with tetracycline-resistant genes enriched at all temperatures except 20°C ($p < 0.05$).

Significance: This study examines how antibiotic application and water temperature shape the catfish gut microbiome and resistome, revealing their impact on AMR dynamics. The findings offer insights into strategies for sustainable aquaculture.

P3-192: PREVALENCE AND CHARACTERISTICS OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING ENTEROBACTERIACEAE IN POULTRY AND LEAFY GREEN FARM ENVIRONMENTAL SAMPLES

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Multidrug-resistant bacteria remain a challenge in both agriculture and healthcare, as antibiotic residues persist in the soil and water of leafy green farms using poultry manure, despite South Korea's ban on antibiotic use in animal feed.

Purpose: This study aimed to investigate the potential association of antibiotic resistance characteristics or gene transfer between the poultry and leafy green environments by isolating Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae from poultry and leafy green farms.

Methods: A total of 62 samples were collected from leafy green and layer farms located in Chungnam, Gyeongbuk, Gyeongnam, Korea. Among them, 42 were layer farms and 20 were from leafy green farms. Genomic DNA was extracted from each sample and subjected to polymerase chain reaction (PCR) screening for ESBL genes before bacterial isolation using culture methods. Primers targeting the following ESBL genes were used: OXA, DHA, SHV, CTX-M (groups 1 and 9), and TEM. CHROMagar™ Orientation with added ESBL supplement media was used for bacterial isolation and morphological inspection. Identification of isolates was performed using matrix-assisted laser desorption ionization time-of-flight (MALDI-

TOF) mass spectrometry.

Results: The total number of isolated ESBL-producing Enterobacteriaceae was 77, including 67 *Escherichia* spp., 3 *Enterobacter* spp., and the remaining *Klebsiella* spp. Antimicrobial susceptibility testing, performed using Sensititre, revealed that bacteria isolated from manure, leafy vegetables, and soil exhibited resistance to cefotaxime, ampicillin, and ceftriaxone. Notably, bacteria isolated from leafy vegetables and soil showed additional resistance to ceftiofur.

Significance: The findings highlight the prevalence of ESBL-producing Enterobacteriaceae in poultry and leafy green farm environments and underscore the potential for antibiotic resistance transfer through agricultural practices. These results emphasize the need for stringent monitoring and mitigation strategies to address the spread of antimicrobial resistance in agricultural ecosystems.

P3-193: OPTIMIZING OXFORD NANOPORE WORKFLOWS FOR ACCURATE ENTERIC DISEASE SURVEILLANCE

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Whole Genome Sequencing (WGS) technologies have transformed enteric disease surveillance, detection, and response activities in public health and regulatory settings. PulseNet USA, the national molecular surveillance network in the United States, fully implemented WGS in 2019 using Illumina sequencing. Oxford Nanopore Technology's (ONT) sequencing platform offers a small laboratory footprint, cost effectiveness, and longer reads that improve genome assembly, advances that are attractive for public health labs. Currently, however, bioinformatics tool development for ONT data is an area of ongoing research.

Purpose: To evaluate ONT's comparability with Illumina using an assembly workflow employing the newly released Medaka-v2 tool for consensus sequence generation.

Methods: A panel of 10 *Salmonella*, 10 *Campylobacter*, 6 *Shigella* spp., and 4 *E. coli* O157 were sequenced using both Illumina and ONT. Illumina sequencing followed Standard PulseNet MiSeq Protocols with analysis through the PulseNet 2.0 workflow. ONT libraries were generated using the Rapid Barcoding Kit, sequenced on a GridION, and basecalled with Dorado SUP 0.5.0. Assemblies were generated using a Flye-based workflow (<https://github.com/ncezid-narst/stylo>) with Medaka-

v1 or v2 for consensus generation. Allele calling was performed using the PulseNet 2.0 workflow.

Results: ONT *Salmonella* isolates analyzed with Medaka-v1 were 0–4 (median: 2) allele differences from their Illumina counterparts, which decreased to 0–2 (median: 0) with Medaka-v2. For ONT *E. coli*/Shigella, allele differences relative to Illumina were consistent across both Medaka 1 and Medaka 2 at 0–4 (median: 0) for both. *Campylobacter* showed a substantial improvement in comparability with Illumina using the Medaka-v2 workflow with 0–1 (median: 0) allele differences, compared to 0–32 (median: 10.5) with Medaka-v1.

Significance: These results demonstrate the comparability of ONT and Illumina data using cgMLST, PulseNet’s method for foodborne outbreak detection. Further evaluation is needed for other analyses, such as AMR, in-silico serotyping, and virulence profiling. The findings highlight advancements in ONT accuracy and its potential for foodborne disease surveillance.

P3-194: GENETIC DIVERSITY AND ANTIMICROBIAL RESISTANCE IN MASTITIS ASSOCIATED ESCHERICHIA COLI ISOLATES

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Mastitis is a major disease affecting dairy cattle worldwide, leading to substantial economic losses for farmers due to reduced milk production, veterinary costs, and culling. Among the bacterial pathogens responsible for mastitis, *Escherichia coli* (*E. coli*) is one of the most prevalent due to its environmental persistence, genetic adaptability, and ability to cause severe intramammary infections.

Purpose: This study aimed to assess the genetic diversity, phylogenetic relationships, and antimicrobial resistance profiles of mastitis-associated *E. coli* strains using whole-genome sequencing (WGS).

Methods: WGS was employed to characterize 695 *E. coli* isolates from dairy cows diagnosed with mastitis. The isolates, collected between 1976 and 2014 across 13 U.S. states (as well as from Canada and Spain), were archived at the *E. coli* Reference Center (ECRC) at The Pennsylvania State University. Genome sequencing allowed for in-depth phylogenetic and resistance gene analysis.

Results: Preliminary WGS analysis revealed substantial phylogenetic diversity among the isolates. The *mdfA* gene, commonly associated with multidrug resistance, was present in nearly all isolates examined, suggesting a widespread resistance mechanism. Additionally, genes linked to tetracycline and ampicillin resistance were identified in several isolates, highlighting the potential for multidrug resistance across multiple lineages.

Significance: These findings underscore the importance of monitoring the genetic evolution of *E. coli* in dairy herds to better understand mastitis outbreak dynamics. WGS analysis of historical isolates offers valuable insights into the development of virulence and antimicrobial resistance over time, supporting efforts to improve mastitis prevention and treatment strategies to protect public health.

Award Application: Developing Scientist Award

P3-195: DEVELOPMENT OF AN INNOVATIVE WORKFLOW FOR REAL-TIME PCR DETECTION OF FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: In light of evolving foodborne pathogens and increasing global food trade, novel methods in food safety testing are essential to ensure the protection of public health and the integrity of the food supply chain. An innovative workflow was designed to enhance the detection and analysis of pathogens in food samples by integrating advanced lysis and PCR technologies.

Purpose: The objective of this study was to demonstrate the reliability, performance, and ease of use of a new method for detecting pathogens. This study focused on results obtained with *Salmonella* spp., *Listeria* spp., and *Listeria monocytogenes* in various food matrices. The solution aimed to streamline workflows, reduce processing time, and achieve accuracy equal to or greater than traditional methods.

Methods: The EZ-Check Solution—comprising a universal lysis buffer, ready-to-use PCR strips, and user-friendly software—was evaluated for sensitivity (limit of detection, LOD), specificity (inclusivity/exclusivity), and time to results. Ease of use was assessed through user feedback and observation.

Results: All target/non-target strains tested yielded expected results: *Salmonella* spp. (40/36), *Listeria* spp. (33/36), and *L. monocytogenes* (30/34). The sensitivity study showed eLOD₅₀ values ranging from 0.48 to 2.89 CFU/sample depending on matrix and target. Integration of advanced lysis and PCR technologies resulted in a streamlined process, reducing processing time and improving overall efficiency.

Significance: The EZ-Check Solution offers significant advantages for food safety testing. Its ease of use and high performance make it an ideal choice for

laboratories and food processing facilities.

P3-196: EVALUATION OF MICROBIOMES AND PHYTONUTRIENT PROFILES OF OUTREdgeous LETTUCE (LACTUCA SATIVA) GROWN IN SOILLESS AND SOIL-BASED SYSTEMS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Soilless lettuce production in controlled environments, such as hydroponic and aquaponic systems, is expanding, although open-field production still dominates. The microbiome and phytonutrient profiles of these alternative systems remain largely uncharacterized.

Purpose: To characterize and compare microbiomes and phytonutrient profiles of soil- and soilless-grown lettuce.

Methods: 'Outredgeous' lettuce was grown in three systems in a controlled-environment greenhouse: (1) two 36-plant nutrient-film hydroponic systems, (2) twelve 6" pots containing organically managed field soil, and (3) an aquaponic lab-scale recirculating system with three replicates in 37.85 L deep-water tanks with biofilters. Physico-chemical parameters and plant growth were recorded weekly. Soil, lettuce (seedlings, roots, shoots, leaves), water, and fish feed were analyzed for Aerobic Plate Counts (APC), and microbiomes were characterized using 16S rRNA amplicon sequencing. Lettuce at harvest was analyzed for phenolic acids, anthocyanins, and flavonoids using standard methods.

Results: APC levels ranged from log 2.32–8.26 CFU/g/mL across all systems.

Soilless systems had higher APC levels (log 8.26 CFU/g) compared to soil-based systems (log 7.40 CFU/g). APCs were highest in roots (log 8.26 CFU/g), followed by soil (log 7.40 CFU/g) and leaves (log 3.05 CFU/g). Soil supported the greatest microbial diversity at all taxonomic levels. Soil microbiomes had higher relative abundances of pathogenic bacteria from Enterobacteriaceae, Clostridiaceae, and Listeriaceae. Principal Coordinate Analysis showed distinct clustering of microbial communities by production system. Biofilter scavenger type had no effect on plant growth, microbial diversity, or physico-chemical parameters. Phytonutrient contents were significantly greater ($p < 0.05$) in soilless systems than in soil-based systems.

Significance: Results provide unique comparative microbiome and phytonutrient profiles from soilless and soil-based systems, offering insights useful for food safety and quality.

P3-197: EXPLORING THE ANTIMICROBIAL RESISTANCE OF DIARRHEAGENIC ESCHERICHIA COLI FROM SOUTHERN AFRICA USING WHOLE GENOME DATA

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Whole-genome sequencing (WGS) has improved surveillance of pathogens in point-source outbreak investigation, tracking the spread of antimicrobial resistance genes and attribution of the illness to food and the environment.

Purpose: To characterize evolutionary relatedness and transmission dynamics of antimicrobial resistance genes of significance in food safety from the Southern African Development Community (SADC).

Methods: The non-interleaved paired-end whole genome FASTQ sequence files of eleven DEC isolates from food and water samples consumed by children under five years with diarrhoea in Maputo, Mozambique, with 125 others from food, animals and environment available in the Enterobase database (<http://enterobase.warwick.ac.uk>) were obtained. The assemblies of the DEC isolates were analyzed using cgMLSTfinder 1.2 and ResFinder 4.5.0 web-based pipelines. The alleles and annotations of the genomes were analyzed using R statistical environment (v 4.2.2); ape (v 5.6-2), phangorn (v 2.11.1), pvclust (v 2.2-0) and compGenomeRData (v 0.1.0) packages.

Results: From the 136 DEC isolates, 97 were resistant to at least one antimicrobial resistance gene, while 128 had at least one plasmid present with incFIB(AP001918) (23.06%), incFII (12.78%), and incFII(pHN7A8) (9.27%) being most abundant. Insightful evidence on horizontal resistance gene transfer was observed as the resistance profiles clustered based on the geographical location and source niche, with ST number, pathovar or O:H antigen playing a minor role in the clusters. Principal Coordinate Analysis revealed that evolutionary relatedness accounted for 27.55% of the genetic diversity. Resistance to folate pathway antagonists (sulfamethoxazole-55.9%), beta-lactamases (amoxicillin, ampicillin, and piperacillin-all 54.4%), and aminoglycosides (streptomycin-55.1%) were most abundant.

Significance: The resistance profiles create a need to develop a framework for

proactive response in addressing the transfer of antimicrobial genes for a regional effort to protect public health.

P3-198: IMPACT OF BIOLOGICAL SOIL AMENDMENTS OF ANIMAL ORIGIN, INCLUDING HEAT-TREATED POULTRY PELLETS AND SEABIRD GUANO ON THE SOIL MICROBIOME IN CALIFORNIA

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Biological soil amendments of animal origin (BSAAO) are commonly used as organic fertilizers to enhance soil fertility, but their application carries the risk of introducing foodborne pathogens into agricultural soils.

Purpose: This study aimed to investigate the effects of BSAAO on soil microbial diversity, community structure, and the proliferation of pathogenic bacteria within the soil microbiome.

Methods: A 42-day experiment was conducted in California to evaluate the soil microbiome amended with heat-treated poultry pellets (HTPP) and seabird guano (Guano). Raised-bed plots were left unamended (UA) or amended with HTPP or seabird guano. Soil samples were collected on days 0, 1, 7, 14, 21, 28, 35, and 42. Bacterial genomic DNA was extracted, and the V4 region of the 16S rRNA gene was amplified and sequenced using the Illumina Miseq platform. Data were analyzed with the QIIME2 pipeline. Differences in Shannon and Chao1 indices and microbial relative abundance were analyzed using ANOVA, while differences in Bray-Curtis distances were analyzed using PERMANOVA.

Results: HTPP-treated soils consistently exhibited significantly lower Shannon and Chao1 indices across all sampling days compared to other treatments. Additionally, the bacterial community composition of HTPP-treated soil was

significantly distinct from those of the Guano and UA treatment groups. Microbial relative abundance analysis showed that HTPP treatment significantly increased populations of the Proteobacteria phylum compared to other treatments throughout the experimental period. At the genus level, *Acinetobacter* dominated in the HTPP-treated group, whereas *Nicrosphaeraceae* became dominant in Guano-treated and UA soils. Opportunistic pathogens, including *Psychrobacter*, *Glutamicibacter*, and *Massilia*, were more abundant in both HTPP- and Guano-treated soils compared to UA soils.

Significance: These findings suggested that BSAAO amendments may increase the risk of soil contamination by promoting populations of pathogenic bacteria.

P3-199: A NEW *L. MONOCYTOGENES* STRAIN TYPING METHOD, GENE-UP® TYPER, CAN PROVIDE HIGH-RESOLUTION STRAIN TYPING IN AS LITTLE AS 24 H

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *L. monocytogenes* strain differentiation is essential for root cause investigations and effective corrective action plans. Notably, strain typing allows a facility to assess if an LM-positive sample is the result of a re-occurring (persistent) or new (transient) strain. While whole-genome sequencing (WGS) is the gold standard, it is currently not widely adopted by food producers for several reasons. Notably, WGS (i) is costly (~\$1-3,000 USD), (ii) has a long time-to-

Results (5 d to >1 week), (iii) may have regulatory implications, and (iv) requires complex computational analyses. Previous studies have highlighted that alternative approaches (e.g., RiboPrinter) have lower discriminatory power compared to WGS.

Purpose: The purpose of this study was to compare the discriminatory power of GENE-UP TYPER, a new real-time PCR-based typing method to whole-genome sequencing (WGS)-based methods and ribotyping for LM strain typing.

Methods: An LM strain set (n=51) representing four lineages (I, II, III, IV) and 12 serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, and 4e) was analyzed by three typing methods (WGS, RiboPrinter®, GENE-UP® Typer). WGS data was sourced from public databases (NCBI) or generated using Illumina's MiSeq. All GENE-UP Typer and Riboprinter typing was performed per the manufacturer's instructions.

Results: Overall, the GENE-UP Typer generated strain resolution comparable to WGS-based MLST and demonstrated higher discriminatory power than the RiboPrinter. Among the 51 strain tests, seven required further analysis by WGS; four of these strains represent either a serotype (3a) or lineage (III, IV) not commonly associated with human listeriosis.

Significance: The data from this study supports the GENE-UP Typer is a good alternative to WGS for strain typing. In addition to the discriminatory power, samples could be processed and analyzed within 2 h at < \$400 USD/isolate.

P3-200: DECODING TREATMENT AND ENVIRONMENTAL EFFECTS ON SOIL MICROBIOMES AND FOODBORNE PATHOGEN SURVIVING ACROSS GEOGRAPHIC REGIONS WITH MACHINE LEARNING

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Clinical laboratory tests for enteric disease form the basis of public health surveillance and outbreak detection, but only a fraction of patients with acute gastroenteritis submit specimens for testing.

Purpose: The objective of this study is to characterize the sociodemographic factors associated with laboratory test orders placed for patients with acute gastroenteritis.

Methods: Medical records from encounters of patients with acute gastroenteritis within one Minnesota-based healthcare system from 2017–2023 were reviewed. Encounters were included if they met any of the following criteria: diagnoses of enteric diseases or diarrhea of unknown cause, patient complaints of diarrhea, and orders of laboratory tests for enteric pathogens; patients were excluded if they had diagnosed chronic conditions that cause diarrhea. Encounters per patient were grouped into illness events, resulting in 254,629 illness events in this sample. A log binomial regression model was used to calculate relative risks of

orders placed across racial/ethnic groups, adjusting for age, sex, insurance coverage, and first encounter type.

Results: Of the 254,629 cases of acute gastroenteritis, 24.5% (n=62,282) had orders placed for enteric pathogen laboratory tests. Across all encounter types, Non-Hispanic (NH) White patients had orders placed 26.1% (47,679/182,551) of the time, which was higher than any other group. After adjusting for age, sex, insurance coverage, area-level poverty, and first encounter type, NH Asian, NH Black or African American, and Hispanic patients were significantly less likely (20%, 14%, and 5% respectively) to have laboratory test orders placed than NH White patients.

Significance: Laboratory test ordering practices directly affect public health surveillance and outbreak detection; when certain demographic groups are less likely to be tested for reportable enteric pathogens, it makes identifying and controlling outbreaks of foodborne disease more difficult within these communities.

P3-201: PROTEOSTASIS AND STRAIN-SPECIFIC VARIATION UNDERLIE ANTIBIOTIC TOLERANCE IN CAMPYLOBACTER JEJUNI IN THE FOOD SUPPLY CHAIN

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Antibiotics are vital for treating bacterial infections and play critical roles in food production. However, recurrent *Campylobacter jejuni* infections, despite antibiotic treatment, have been linked to adaptive resistance rather than genetic antimicrobial resistance. Adaptive resistance, particularly antibiotic persistence, allows a small subpopulation of susceptible bacteria, known as persisters, to survive antibiotic exposure through transient tolerance mechanisms. Persisters are increasingly associated with the recurrence of bacterial infections, including those caused by *C. jejuni*, a major foodborne pathogen.

Purpose: This study aimed to identify the genetic determinants and phenotypic traits associated with the formation of *C. jejuni* persisters in the food supply chain. Comparative genome analysis was performed to evaluate the potential role of identified determinants in driving differences in antibiotic tolerance among *C. jejuni* strains.

Methods: *C. jejuni* cultures were grown in Mueller-Hinton broth with or without antibiotics at final concentrations of 100 µg/mL ampicillin or 1 µg/mL ciprofloxacin. After a 1-hour exposure, total RNA was extracted from untreated *C. jejuni* cells and persister cells, followed by purification, library preparation, and sequencing. Functional annotation of genes was conducted using the KEGG database, with significance defined as an absolute log₂-fold change >1 and p-value <0.05. Comparative genome analysis was also conducted to identify genetic determinants contributing to variations in antibiotic tolerance across *C. jejuni* strains isolated from different sources.

Results: Gene set enrichment analysis revealed consistent overrepresentation of pathways related to protein catabolism, quality control, SOS/stringent stress responses, peptidoglycan synthesis, and RNA processing. Conversely, the TCA cycle and respiratory electron transport pathways were underrepresented. Comparative genome analysis indicated that strains with reduced ability to

acquire transient antibiotic tolerance lacked critical genes essential for proteostasis, emphasizing the critical role of protein quality control in persister formation.

Significance: Understanding the mechanisms underlying persistence in *C. jejuni* is crucial for addressing antibiotic treatment failures and mitigating public health risks caused by *C. jejuni*.

P3-202: CHARACTERIZATION OF ANTIBIOTIC RESISTANCE IN ESCHERICHIA COLI FROM MEAT PRODUCTS AND HUMAN SAMPLES IN BOTSWANA USING MOLECULAR AND WHOLE-GENOME APPROACHES

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Botswana experiences high diarrheal illness rates in rural areas like the Chobe region, where *E. coli* from meat products and food handlers may contribute to antibiotic resistance (ABR) transmission, posing a potential threat to public health.

Purpose: Characterize the ABR profiles of *E. coli* isolated from meat products and human feces to explore potential links between meat and human ABR transmission.

Methods: Meat (chicken and beef) were collected from local grocery stores, and human fecal samples were obtained from food handlers in Kasane, Botswana. *E. coli* isolates were obtained by selective enrichment and plating on EMB and MacConkey agars. ABR profiles were determined for 12 antibiotics using the CLSI disk diffusion method. Whole-genome sequencing was performed on multidrug-resistant isolates using the Illumina Miseq platform and genomes were analyzed using Galaxy and BV-BRC.

Results: *E. coli* was isolated from 66 of 124 meat samples and 230 of 507 human fecal samples, with 56.06% (37/66) of meat isolates and 43.04% (99/230) of human isolates showing resistance to at least one antibiotic. Multidrug resistance (MDR) was detected in 30.30% (20/66) of meat isolates and 11.74% (27/230) of human isolates. Disk diffusion testing revealed that tetracycline and trimethoprim/sulfamethoxazole were the most frequently detected resistances across both meat and human isolates. Whole-genome sequencing identified no

shared sequence types (STs) between *E. coli* isolates from meat and human samples. However, both sources carried the same ABR genes, including *sul2*, *blaTEM-1*, *tetA*, and *qnrS1*, along with shared plasmid types (*incFIB*, *incFII*, *incX1*), while integrons, detected only in meat isolates, suggest horizontal gene transfer through mobile genetic elements.

Significance: The detection of shared antibiotic resistance genes and plasmid types between meat and human isolates underscores the importance of monitoring meat products to inform strategies for mitigating the transmission of antibiotic resistance in Botswana.

P3-203: GLOBALLY INTEGRATED SALMONELLA SEROTYPE TRACKING USING OXFORD NANOPORE TECHNOLOGIES LONG-READ SEQUENCING

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The reduced cost and faster turnaround times of next-generation sequencing (NGS) have enabled its seamless integration into high-throughput microbiology service laboratories. Eurofins laboratories in Madison, Wisconsin, and Nantes, France, conducted a proof of concept (POC) to implement a *Salmonella* serotype tracking system using Oxford Nanopore Technologies (ONT) and internally developed bioinformatics pipelines.

Purpose: To demonstrate a POC for a reliable NGS sequencing method to serotype and globally track *Salmonella* isolates originating from the food industry, using ONT sequencing.

Methods: Three replicates of a reference serotype, *Salmonella enterica* subsp. *arizonae* DSM 9386, and three replicates of three wild-type *Salmonella* serovars (n=9) were grown overnight on XLD or TSA agar in Madison as well as Nantes. DNA from isolated colonies was extracted, purified, fragmented, and standardized according to internal/ONT protocols. The DNA was prepared for sequencing using the ONT Native Barcoding Kit 24 V14 and run on an R10.4.1 flow cell using the ONT GridION until each barcode generated at least 300 Mb of data. Both fastq data sets were then centralized for bioinformatic analyses using an internally developed and validated pipeline for *Salmonella* serotype identification and single nucleotide variation (SNV) analyses.

Results: The sequenced and *de novo* assembled genomes of the reference species *Salmonella enterica* subsp. *arizonae* DSM 9386 differed by less than 3 SNVs between replicates. The genomes of the wild-type *Salmonella* differed by less than 1 SNV between replicates. Less than 3 contigs were obtained per genome, and a coverage depth of more than 30X was obtained on 99.6% of the genomes. These results indicate that the method is accurate and reproducible.

Significance: The harmonized NGS sequencing system allows for the identification and tracking of *Salmonella* serotypes using Eurofins microbiology laboratories in Europe and North America without the need to ship pathogens internationally. This advancement has significant implications for global food safety and pathogen tracking.

P3-204: COMPARATIVE GENOMIC ANALYSIS REVEALS HOST-ASSOCIATED VARIATION OF CAMPYLOBACTER JEJUNI

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Campylobacter jejuni* is a zoonotic pathogen commonly found in the commensal microbiota of various hosts, with chicken being a major contributor to human *Campylobacteriosis*. Despite its public health

Significance, the host-associated evolutionary trajectories remain underexplored.

Purpose: The study investigates the genomic variation of *C. jejuni* between human and chicken hosts to identify potential host-specific adaptations.

Methods: A total of 365 publicly available *C. jejuni* complete genomes from human and chicken hosts were analyzed. After processing assemblies for quality control with CheckM, high-quality genomes (predicted completeness > 99%, contamination < 1%; n=344) were annotated using Prokka and a pangenome was built with Panaroo. Metabolic features were determined using eggNOG-mapper2, and AMRFinderPlus was utilized to detect antimicrobial resistance (AMR) and other stress response genes. Machine learning algorithms were employed to explore accessory gene enrichments based on host type.

Results: The *C. jejuni* pangenome is in an open state (power-law = 0.38), with similar total gene counts per genome across hosts ($P > 0.05$). Twelve accessory genes were homologous to AMR genes, such as those involved in efflux systems (e.g. *acr3*, *arsP*, *tetL*) and transferases (e.g. *aad9*, *catA13*). Several clusters of orthologous genes (COGs) enrichments were observed, with recombination, repair, translation, and signal transduction enriched in the genomes of human-derived isolates, while nutrients transport, metabolism, cell wall/membrane biogenesis, and defense mechanism were enriched in genomes associated with chicken (adjusted $p < 0.01$). A random forest model achieved high predictive accuracy for host classification (balanced accuracy = 0.88), uncovering key host-associated functions, including reductase (*ynfE*, *dmsA*, *dmsD*), synthase (*aroB*, *fabH*), kinase (*pknD*), protease (*pic*), transferase (*fmt*), chemotaxis protein (*pctC*, *mcpB*), and flagellin (*flaA*, *flaB*).

Significance: This study highlights the important host-associated genomic variation of *C. jejuni* and identifies genomic signatures that could potentially serve

as novel markers for pathogen surveillance.

Award Application: Developing Scientist Award

P3-205: HETEROGENEITY OF MICROBIAL AND VOLATILE PROFILES OF RETAIL KIMCHI SUSCEPTIBLE TO ANTIBIOTIC RESISTANCE

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Naturally fermented products are susceptible to various food safety and quality issues, such as antibiotic-resistant microorganisms (AR), opportunistic pathogens, and offensive odors, due to the traditional fermentation process.

Purpose: This study examined the prevalence of AR in kimchi and the microbial and chemical profiles of the products which contribute to food safety and quality issues.

Methods: Representative kimchi products from different brands and manufacturers were purchased from multiple retail stores in Northern California between March and Dec 2024. The samples were assessed for the prevalence of viable AR bacteria on BHI plates containing mold inhibitor and antibiotic (8µg/mL Tet or 16µg/mL Amp). The identity of representative AR isolates was determined by 16S rRNA gene sequencing, and the resistance profiles of AR isolates were determined by minimum inhibitory concentration assessment. The profiles of volatile compounds were determined by SIFT-MS.

Results: Phenotypes resistant to 8µg/mL of tetracycline suggested the presence of *tet* gene(s), and phenotypes resistant to ampicillin above breakpoint negate therapy by this common antibiotic, regardless of the presence of AR genes. AR isolates were identified in 7 out of 8 California kimchi samples. Multidrug-resistant isolates including opportunistic pathogens such as *Rahnella* sp., *Pseudomonas* sp., *Enterobacter ludwigii*, *Stenotrophomonas indicatrix*, and *Klebsiella* sp. were identified. Some isolates were resistant to most 4th generation cephalosporins, and even carbapenem antibiotics (*Stenotrophomonas indicatrix*). Moreover, multidrug-resistant lactic acid bacteria such as *Latilactobacillus sakei*, *Leuconostoc mesenteroides*, and *Latilactobacillus curvatus* were also identified from different kimchi samples. Volatile compounds also varied in abundance among samples.

Significance: Results illustrated that retail kimchi has diversified microbial profiles, yet is still high in the prevalence of AR bacteria. The randomness of natural fermentation and the broad spectrum of microbes involved likely contributed to the diverse profiles of volatile compounds and flavors of these products.

Award Application: Developing Scientist Award

P3-206: BACTERIAL ABUNDANCE AND TAXONOMIC PROFILING ANALYSIS OF BEEF LYMPH NODES AND DIGESTA USING WHOLE GENOME SHOTGUN SEQUENCING

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The United States and Canada have studied the microbiome of beef cattle lymph nodes using 16S rRNA gene amplification. However, this method faces limitations in capturing the complexity of microbial ecosystems, as it primarily reveals specific bacterial groups or species. Investigating bacterial biodiversity through metagenomic approaches by comparing bacterial abundance in lymph nodes and the gastrointestinal tract (GIT) could address these limitations, reduce bias in previous findings, and establish a baseline for bacterial profiling. We hypothesize that variations in lymph node bacterial abundance may signal the presence of foodborne pathogens translocated from the GIT to mesenteric and peripheral lymph nodes.

Purpose: This study examines bacterial abundance and taxonomy in lymph nodes using whole-genome shotgun sequencing, comparing results with bacterial translocation from digesta (fecal and cecal content).

Methods: Beef lymph node (LN) tissues were collected from a meat facility in the Midwestern USA. A total of 38 lymph nodes were sampled: inferior-ileocecal (IILNs, n=8), popliteal (PLNs, n=8), subiliac (SLNs, n=8), superior-ileocecal (SILNs, n=8), and mandibular (MLNs, n=6). Additionally, eight cecal and eight fecal digesta samples were included. DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit, and total genomic DNA was analyzed using whole-genome shotgun sequencing (WGS) on the Illumina platform. Library preparation, bioinformatics, and statistical analyses were conducted with Qiagen CLC Genomics Workbench (version 24.0).

Results: Metagenome sequencing revealed that *Escherichia* was predominant,

accounting for 20–33.8% of the bacterial community in popliteal lymph nodes (LNs), followed by *Clostridium* at 9.9–22.4% and *Staphylococcus* at 7.5–12%. Shotgun sequencing identified the dominant taxa at the species level, with *Escherichia coli* at 16.0%, *Shigella sonnei* at 5.1%, and *Staphylococcus aureus* at 4.0% in LNs. *Escherichia coli* was the most abundant in cecal content and fecal samples, constituting 25.0%. A low prevalence of *Salmonella* was observed.

Significance: Using WGS, this study highlights lymph nodes as reservoirs for microbial contamination in beef production, offering critical insights into pathogen persistence and translocation to improve food safety practices.

Award Application: Developing Scientist Award

P3-207: GENOME-WIDE ANALYSIS OF CADMIUM RESISTANCE GENES HARBORED BY CADMIUM-RESISTANT LISTERIA MONOCYTOGENES STRAINS FROM SWEDEN

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Listeria monocytogenes* causes listeriosis and often exhibits resistance to cadmium. While multiple cadmium resistance genes have been identified, our understanding of the full landscape of cadmium resistance determinants is far from complete.

Purpose: This study aims to investigate cadmium resistance genes via genome-wide screening of cadmium-resistant *L. monocytogenes* from human listeriosis cases in Sweden.

Methods: Thirty-two cadmium-resistant isolates were subjected to whole-genome sequencing. Plasmid-borne contigs were identified in silico, and cadmium resistance genes were found using BLAST2 against known cadmium resistance genes and pan-genome analysis. These genes were compared, and the deduced proteins were aligned with prototype proteins, bootstrapped, and visualized. To determine the location of the genes, the DNA sequences—including flanking regions—were compared with one another and, for chromosomal cadmium resistance genes, with the complete genome of strain F2365.

Results: We identified three known cadmium resistance operons (*cadA1C1*, *cadA2C2*, and *cadA4C4*) and two novel operons (*cadD8A8* and *cadA9C9*). *cadA1C1* was the most frequently found and was typically plasmid-borne, except in one CC101 strain, which bore a *cadA1C1*-harboring chromosomal island. Chromosomal islands with cadmium resistance genes also included the previously characterized LGI2 (*cadA4C4* and arsenic resistance genes) and a novel island harboring *cadA9C9* and an arsenic resistance gene. All *cadA9C9*-positive strains lacked

plasmids and other cadmium resistance operons. We also identified *cadA10*, which was chromosomally harbored by all strains in our panel and the cadmium-susceptible strain F2365, suggesting that it mediates innate baseline resistance of *L. monocytogenes* to cadmium.

Significance: Our findings highlight the complex distribution of cadmium resistance genes within *L. monocytogenes*, which will help us better understand the genes that facilitate ecological adaptations—potentially leading to persistence in the environment and increased risk of *L. monocytogenes*-contaminated foods and foodborne infections.

P3-208: DETERMINATION OF SPOILAGE MICROBIOTA OF ATLANTIC WHITE SHRIMP (AWS) USING 16S SHOTGUN SEQUENCING AS AN ALTERNATIVE METHOD TO THE STANDARD QUALITY EVALUATION DURING THE COLD CHAIN

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Bacterial contamination of aquatic products often occurs during processing and handling. Psychrotrophic bacteria, such as *Shewanella* spp. and *Pseudomonas* spp., thrive at low temperatures and are key contributors to spoilage. Traditional methods focus on selective media or 16S rRNA gene analysis. This study evaluates the efficacy of 16S shotgun sequencing in monitoring specific spoilage organisms (SSO).

Purpose: To assess the safety and spoilage microbiota of fresh Atlantic White Shrimp stored at 4°C using 16S shotgun sequencing.

Methods: Shrimp samples were stored at 4°C for 10 days, with psychrotrophic counts measured in triplicate. Physicochemical properties (pH, TVB-N, TMA, and Lab color values) were analyzed to monitor spoilage. Microbial communities were identified using 16S shotgun sequencing, providing a comprehensive overview of microbiota changes during storage.

Results: An inconsistent growth pattern was observed for psychrotrophic bacteria at 4°C. *Shewanella* spp. and *Pseudomonas* spp. were prominent in selective media. TVB-N levels increased from ≤ 25 mg/100 g N to 31 mg/100 g N over 10 days. Shotgun sequencing revealed dominant microbiota shifts, with *Acinetobacter* accounting for >60% abundance, followed by *Psychrobacter*, *Shewanella*, *Pseudomonas*, and *Vibrio*, contributing to spoilage.

Significance: 16S shotgun sequencing provides detailed insights into spoilage microbiota in AWS under cold storage. This method surpasses traditional culture-dependent techniques by uncovering key microbial dynamics, facilitating better control strategies for seafood safety and quality during the cold chain.

P3-209: A PORTABLE DEVICE FOR OFFLINE, EFFICIENT, AND SCALABLE PROFILING OF FOODBORNE PATHOGEN GENOMES

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: For more than a decade, whole genome sequencing (WGS) and WGS-enabled analytics of major foodborne pathogens has led to a paradigm shift in public health surveillance of foodborne infectious diseases. Despite rich genome resources and ample analytical tools, the success of genomic epidemiology remains disparately distributed across countries and largely scarce in the private sector of food and agriculture.

Purpose: We aim to decentralize and simplify the exhaustive search by integrating efficient query algorithms and miniaturized genome databases into Mashpit, a portable device based on a single-board computer system.

Methods: We developed Mashpit, a pathogen detection and query proGram, and deployed it on a portable Raspberry Pi device. Mashpit downloads and sketches the NCBI Pathogen Detection database using Min-Hash. To evaluate performance, we constructed databases for *Salmonella*, *Listeria*, *E. coli*, and *Campylobacter* and tested sensitivity using 1,000 randomly selected genomes from each species.

Results: Mashpit uses minimal storage and performs rapid queries on a Raspberry Pi. It identifies the most similar NCBI Pathogen Detection SNP clusters for a given query. For *Salmonella*, the database requires approximately 400 MB and completes a query in about 30 seconds. Mashpit achieves a 100% serotype prediction rate for *Salmonella*. Sensitivity and accuracy vary among species: *Salmonella* and *E. coli* include the true SNP cluster in the top 25 hits for 70% of queries compared to 81% for *Listeria* and 92% for *Campylobacter*.

Significance: Mashpit provides a local, portable, and efficient platform for foodborne pathogen surveillance, making it an ideal solution for laboratories with limited computational resources or privacy concerns. By addressing these challenges, Mashpit empowers diverse stakeholders, including those in low-resource and private-sector settings, to leverage genomic epidemiology for food safety.

Award Application: Developing Scientist Award

P3-210: IMPLICATIONS OF NANOSCALE AGROCHEMICALS ON MICROBIOME EVOLUTION IN AGRI-FOOD SYSTEMS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The agri-food microbiome, which shows promise in addressing agricultural challenges by facilitating nutrient cycling, nitrogen fixation, and more, is increasingly threatened by environmental stressors like nanomaterials (NMs), due to their migration and prolonged persistence, potentially leading to unforeseen consequences, as their use in agriculture grows.

Purpose: The current study investigates the effects of NMs on microbial structure and pathogen persistence in the agri-food environments.

Methods: Commonly used metallic NMs in agri-food systems, viz., Ag, CuO, CeO₂, Fe₂O₃, MgO, TiO₂, and ZnO were tested in soil collected from a site classified under the Apopka soil series. A total of eight soil pots for each treatment (7 NMs and a control) were maintained in a greenhouse facility for a period of 12 weeks. From each of these pots, cross sectional samples at two different heights were collected periodically at time points zero, 4 weeks, 8 weeks, and 12 weeks. Aerobic plate counts (APC) data was recorded to gather information on the effect of variables such as NM migration due to rainfall and time. The collected samples will be used to assess the impact of NMs on the native soil microflora and foodborne pathogens using multi-omics and machine learning strategies.

Results: Among the tested NMs, Ag treatment significantly reduced bacterial growth, as indicated by APC. This reduction was observed at both soil pot depths: middle ($p < 9.31 \times 10^{-10}$) and bottom ($p < 0.000128$) at a 95% confidence interval, compared to the controls. However, within the Ag treatment, bacterial growth was not significantly affected by time at either the middle ($p = 0.39$) or bottom ($p = 0.508$) depths.

Significance: This research could inform Ag NMs' long- and short-term effects on microbes, food safety and pathogen persistence in the environment, in addition to shedding light on their safe end-of-life disposal.

Award Application: Developing Scientist Award

P3-211: GENOMIC LANDSCAPE OF SALMONELLA ENTERICA REVEALS ADAPTIVE ASSOCIATIONS WITH FRESH PRODUCE

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Salmonella enterica* is a leading cause of foodborne bacterial illnesses in the United States, with an increasing prevalence in fresh produce-associated outbreaks. The pathogen's ability to survive and colonize fresh produce highlights its potential for adaptation and persistence within fresh produce matrices.

Purpose: This study leverages high-throughput genomic analyses to discover the adaptive genetic associations and phylogenetics of *S. enterica* serovars isolated from 12 distinct fresh produce matrices within the USA.

Methods: Whole-genome sequencing reads from 1,413 *S. enterica* samples were retrieved from the NCBI Pathogen Isolates Database and quality controlled, resulting in 1,403 high-quality genomes. The *Salmonella* genomes represented 12 fresh produce types, including alfalfa sprout (n = 34), avocado (n = 24), basil (n = 62), cantaloupe (n = 173), cilantro (n = 164), cucumber (n = 303), jalapeño pepper (n = 52), leafy greens (n = 105), oregano (n = 22), papaya (n = 271), serrano pepper (n = 43), and spinach (n = 150). A core genome was identified from 106 *S. enterica* serovars using the high-quality *Salmonella* Typhimurium LT2 reference genome, which served as the basis for read mapping and variant calling.

Results: Mapping short-read sequence data to the core genome (~2.94 Mb) identified 282,540 high-quality single nucleotide polymorphism sites (SNPs) among the 1,403 *S. enterica* genomes. Logistic regression analyses of 45,494 SNPs with a minor allele frequency $\geq 5\%$ revealed significant genetic associations with cantaloupe (13 SNPs), papaya (14 SNPs), cilantro (37 SNPs), cucumber (343 SNPs), and spinach (3,189 SNPs) at a high significance level ($p = 2.02 \times 10^{-98}$ – 1.10×10^{-6}). These SNPs represent putative loci underlying adaptation of *S. enterica* to different fresh produce matrices.

Significance: This study provides new insights into the adaptive genetic landscape of *S. enterica* in fresh produce and suggests produce-specific selective pressures shaping *S. enterica* adaptation. The research emphasizes the utility of high-throughput genomics in unraveling pathogen adaptation and guiding risk mitigation.

P3-212: GENOMIC CHARACTERIZATION OF H₂S-NEGATIVE SALMONELLA TYPHIMURIUM 4:I:1,2 ISOLATE FROM CALF FECES IN THE USA

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The circulation of hydrogen sulfide (H₂S) negative Salmonella serovars continues to be a public health threat. Recent studies have reported outbreak situations and clinical cases involving these atypical serovars. Purpose: In this study, we analyzed the genome of an isolated H₂S-negative Salmonella isolate for the presence of plasmids and antimicrobial-resistant genes. The phs, cys, and asr operons of the Salmonella genome essential for H₂S production were also analyzed. Methods: In a surveillance study conducted in the spring of 2024, 311 fecal samples were collected from cattle raised on cow-calf farms in rural Alabama in the United States. Among these, eleven samples were positive, and one isolate recovered from a calf exhibited an H₂S-negative phenotype. This isolate was sequenced, and the genome was analyzed for the sequence type, presence of plasmids, and antimicrobial resistance using PubMLST, Plasmidfinder, and the Comprehensive Antimicrobial Resistance Database (CARD). Bettercallsal was used to identify the closest neighbor to this isolate. Results: Genetic analysis revealed that the isolate was a Salmonella Typhimurium i-4-1,2 of the sequence type ST19. Also, except for a missense mutation of A>C on the phsC gene and a synonymous mutation of C>T on the cysI gene, no other mutations were detected on the other genes of the operons phs, cys, and asr reported to be involved in H₂S production by Salmonella. The closest neighbor to the isolate was a Salmonella enterica Typhimurium strain DM2018 which clustered with the Poulvac poultry Salmonella vaccine. The isolate also carried notable multi-drug efflux genes mdtBC, mdsABC, mdtK, and marA and the plasmids: IncFIB(S), IncFII(S), and IncFII(pAR0022). Significance Mutations in the phs, asr, and cys operons may not necessarily be consequential in producing H₂S-negative Salmonella isolates. This result also reiterates the need to look out for these isolates in routine isolation protocols

Award Application: Developing Scientist Award

P3-213: INVESTIGATING THE EFFECT OF DIFFERENT CONCENTRATIONS OF CITRIC ACID ON LISTERIA MONOCYTOGENES AND E. COLI O157:H7 IN FERMENTED SPI SYSTEMS WITH DAIRY AS A CONTROL

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Presentation type/Format: Poster

Category: Plant-Based Alternative Products

Abstract:

Introduction: The growing demand for plant-based foods highlights the urgent need for effective microbial safety strategies, as limited knowledge exists on food safety risks and process controls for alternative protein systems.

Purpose: This study investigated the effect of different concentrations of citric acid (CA) on *Listeria monocytogenes* and *Escherichia coli* O157:H7 in fermented soy protein isolate (SPI) yogurt, using dairy yogurt as a control.

Methods: SPI yogurts were prepared, fermented, chilled, and individually inoculated with *L. monocytogenes* and *E. coli* O157:H7. Samples were evaluated for pH, total titratable acidity (TTA), and microbial counts of *E. coli*, *L. monocytogenes*, and lactic acid bacteria (LAB) over 48 hours. The plant-based yogurt formulation included 6% (w/v) SPI, 5% (w/v) sucrose, and 0.12% (w/v) LAB, mixed with distilled water (25°C) and citric acid at either 0% or 1%. Mixtures were blended at 75–85 rpm for 10 minutes. Fermentation occurred at 45°C for 24 hours, followed by 24 hours of refrigeration at 4°C. Samples (200 mL) were inoculated individually with *L. monocytogenes* and *E. coli*. Measurements were taken at 0, 15, and 24 hours of fermentation, and after 24 hours of refrigeration.

Results: Samples with 1% CA had significantly lower pH and higher TTA than those without CA, indicating enhanced acidification. Addition of 1% CA improved *E. coli* reduction by approximately 2 log CFU/mL and *L. monocytogenes* reduction by approximately 4.0 log CFU/mL over 48 hours. LAB counts significantly decreased in dairy yogurt, while SPI systems—particularly those with 1% CA—demonstrated moderate inhibitory effects on LAB.

Significance: These findings demonstrate citric acid's potential to enhance microbial safety in plant-based fermented foods, supporting the development of safer and more sustainable dairy alternatives.

Award Application: Developing Scientist Award

P3-214: HIGH PRESSURE PROCESSING (HPP) EFFECTS ON MICROBIAL LOADS OF MEAT-BASED, PLANT-BASED AND HYBRID PATTIES

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Presentation type/Format: Technical

Category: Plant-Based Alternative Products

Abstract:

Introduction: Hybrid meat products are foods in which a portion of meat has been replaced by other plant-based ingredients and more sustainable protein sources.

Purpose: Determine the efficacy of HPP on microbial loads of meat-based, plant-based and hybrid patties.

Methods: Ground beef was mixed with a commercial plant-based recipe containing texturized soy protein concentrate, soy protein isolate, gluten, texturizing base, canola oil, and coconut oil at different ratios of 3:0, 2:1, 1:2 and 0:3 were subject HPP at 400 and 600 MPa for 3 min and then stored at 4°C and changes in total aerobic plate counts (TAC) monitored by plating appropriate dilutions on plate count agar every four days for up to 24 days.

Results: HPP at 600 MPa reduced the TAC irrespective of formulation. However, increase in concentration of plant-based ingredients resulted in a significantly lower TAC reduction compared to formulations with higher ground beef. For instance, following HPP, TAC in formulation with 100% beef reduced to below detection levels (1-log), while no significant reduction in TAC (Pre-HPP: 2.74 ± 0.25 ; Post-HPP: 2.57 ± 0.31 log CFU/g) was observed in 100% plant-based formulation, suggesting that plant-based ingredients afford a baro-protective effect. Storage of HPP patties at 4°C for 24 days showed no significant recovery in TAC in formulations with 100% beef where counts remained below detection levels, while in hybrid (2:1 and 1:2, beef:plant ratio) and 100% plant-based formulations TAC increased to 3.56 ± 0.65 , 4.25 ± 0.80 and 3.23 ± 0.45 log CFU/g, respectively. These results suggest that plant-based ingredients afford a baro-protective effect and support recovery and growth of pressure induced sublethally injured bacteria.

Significance: These results suggest that plant-based ingredients used to formulate hybrid meat products could affect the efficacy of HPP and ultimately shelf-life of pressure treated products.

P3-215: EFFECT OF EXTRUSION PROCESSING ON THE ALLERGENICITY AND ANTI-NUTRITIONAL FACTORS OF LIMA BEANS FLOUR (PHASEOLUS LUNATUS L.) PRODUCED IN DELAWARE, UNITED STATES

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Presentation type/Format: Poster

Category: Plant-Based Alternative Products

Abstract:

Introduction: Lima bean is the main vegetable crop cultivated in Delaware, United States. It is an emerging legume with a high protein content (21–26%), and it is also rich in essential minerals such as calcium, iron, and phosphorus, which makes it a nutritionally dense legume. However, studies on the allergenicity and anti-nutritional factors that could impede digestion are still limited.

Purpose: This research aims to elucidate the impact of extrusion processing on the allergenicity and anti-nutritional factors of lima bean flour.

Methods: In this study, lima beans of different maturities (i.e., small and large lima beans) were processed into flours by grinding and passing them through a 100-mesh sieve. The lima bean flours were processed using a co-rotating twin-screw extruder with the heating barrel temperature set from 120 to 140°C and the moisture content at 18 to 22% w/w. Studies on anti-nutritional factors, such as trypsin inhibition and chymotrypsin inhibition, as well as allergenicity analysis using enzyme-linked immunosorbent analysis (ELISA), were analyzed before and after extrusion processing.

Results: The high shear generated by the twin-screw and the high temperature employed during the process resulted in the reduction of trypsin and chymotrypsin inhibition factors. The reduction is temperature-dependent, where higher temperatures result in more inhibition of trypsin and chymotrypsin activities. The quantities of allergens present before and after extrusion were measured.

Significance: The findings provide insight into how the extrusion process could be modulated to reduce the amount of anti-nutritional factors and allergens present in lima bean flours. Thus, positioning extrusion as a viable approach to improve the digestibility and reduce the allergenic potential of lima bean flour.nutritional

factors and allergens present in lima bean flours. Thus, extrusion may serve as a viable approach to improve digestibility and reduce the allergenic potential of lima bean flour.

P3-216: PLANT-BASED MEAT PRODUCTS: DETERMINATION OF MICROBIAL QUALITY FOR CONSUMERS UNDER VARYING HANDLING CONDITIONS

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Presentation type/Format: Poster

Category: Plant-Based Alternative Products

Abstract:

Introduction: Plant-based meat products have gained popularity, however, the microbial quality and shelf-life of these products in consumers' hands are not understood. Products are sold to consumers in both frozen and refrigerated states, therefore, there is a need to better understand the microbial growth and survival of spoilage bacteria in these products under typical storage conditions.

Purpose: This study aimed to characterize and evaluate microbial growth of naturally occurring microorganisms in plant-based meats over time at different storage temperatures (refrigeration and mild temperature abuse).

Methods: Commercial pea-based (PBM) and soy-based meat (SBM) products were tested for microbial quality when purchased frozen, and when stored at 4°C and 10°C. Subsamples on Days 0, 3, 6, and 10 were analyzed for aerobic plate counts (APCs), lactic acid bacteria (LAB), yeast and mold, coliforms, and pH. Bacterial community structure was characterized by 16S rRNA sequencing.

Results: At 4°C, APCs for PBM and SBM increased from 3.21 to 7.45 log CFU/g and 6.66 to 8.72 log CFU/g, respectively, over 10 days. At 10°C, APCs for PBM and SBM rose from 3.21 to 9.24 log CFU/g and 6.66 to 9.45 log CFU/g, respectively. LAB counts for PBM at 4°C increased from 3.49 to 7.58 log CFU/g, and SBM from 2.51 to 7.36 log CFU/g. Coliform counts for PBM at 4°C ranged from 1.40 to 1.72 log CFU/g, while SBM ranged from 3.40 to 3.96 log CFU/g. The pH remained stable at 4°C but decreased significantly at 10°C ($p < 0.05$). Microbiome analysis revealed distinct bacterial communities influenced by storage temperature and duration.

Significance: These findings highlight the need for consumer guidance regarding handling of these products purchased at refrigerated temperatures. They emphasize the need for proper storage of plant-based meats to limit microbial growth and ensure safety in the hands of consumers.

MP-01: SMARTPHONE-BASED OPTICAL DETECTION: A SUPERIOR ALTERNATIVE TO ATP SWAB KITS FOR BACTERIAL DETECTION ON FOOD-CONTACT SURFACES

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Presentation type/Format: Marketplace

Category: Laboratory and Detection Methods

Abstract:

Introduction: Effective bacterial detection on food-contact surfaces is critical for preventing foodborne illness outbreaks and ensuring food safety. While adenosine triphosphate (ATP) swab kits are widely used in the food and beverage industries for their speed and ease of use, the reliability of bacterial detection remains a significant challenge.

Purpose: This study aimed to evaluate the bacterial detection performance of a newly developed smartphone-based optical detection method in comparison to a commercial ATP swab kit.

Methods: *Salmonella enterica* (SE1045) cells (log 0-log 7 cells) were applied to 10 × 10 cm² stainless steel surfaces, with and without 1% (w/v) food matrices (corn oil, starch, vegetables, orange juice, egg, and whole milk). Surfaces were sampled using the smartphone-based method and online-purchased ATP swab kit. For the smartphone method, surfaces were swabbed with ethanol-moistened foam swabs, and bacteria were released into 300 µL ethanol. A 5 µL suspension droplet was deposited onto a 3-mercaptophenylboronic acid (3-MPBA) coated gold chip, and single bacterial cells were visualized on the smartphone through a smartphone adapter and quantified using a customized smartphone app. ATP measurements followed the manufacturer's instructions.

Results: Both methods completed detection within 5 minutes. However, the smartphone-based method demonstrated superior quantification, with a 2-log wider detection range (log 3- log 7 cells/100 cm²) and a 1.6-log lower detection limit (2,000 cells/100 cm²) compared to the ATP swab kit. Furthermore, the smartphone method's results were unaffected by food matrices like corn oil, vegetables, egg, and whole milk, whereas ATP test results were significantly influenced ($p \leq 0.05$) by all matrices tested.

Significance: The smartphone-based optical detection method outperformed the tested ATP swab kit, offering broader applicability and greater reliability, exhibiting

commercialization potential. This technology holds promise as a transformative tool for bacterial surveillance on food-contact surfaces, enhancing food safety protocols.

Award Application: Developing Scientist Award

MP-02: PRODUCE SAFETY ALLIANCE EPA-LABELED SANITIZERS FOR PRODUCE WEBTOOL

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Presentation type/Format: Marketplace

Category: Communication, Outreach and Education*

Abstract:

Introduction: One option produce growers, packers, and handlers have to reduce pathogen spread on the farm is the use of chemical sanitizers to treat irrigation water, maintain the quality of produce wash water during use, and sanitize cleaned food contact surfaces. EPA-registered sanitizer labels contain instructions for specific uses (i.e., treating irrigation water, preventing cross-contamination in produce wash water, sanitizing food contact surfaces) as well as efficacy statements against target microorganisms. Users are expected to select a product, understand the label, and follow the instructions.

Purpose: Produce growers and packers have expressed frustration at the difficulty of finding and navigating label information to select a sanitizer appropriate for their needs. The Produce Safety Alliance (PSA) Sanitizer Webtool was developed in response to grower requests for support. It was designed to help produce growers, extension educators, and others find and compare available sanitizers regulated by the U.S. Environmental Protection Agency (EPA) as antimicrobial pesticides.

Methods: The PSA Sanitizer Webtool, available in English and Spanish, serves curated data for over 100 EPA-registered sanitizers. The tool allows users to search, sort, and compare products based on farm-relevant labeled uses, sanitizer chemistry, efficacy statements, and Organic Materials Review Institute (OMRI) status. It includes direct links to the EPA master label, related product labels (when publicly available), and manufacturer contact information.

Results: EPA-labeled sanitizers were selected for inclusion based on conversations with users during food safety outreach, input from collaborators, and recommendations from industry (e.g., educators, regulators, sanitizer companies). Input from stakeholders was used to update the tool into its current form during

2024, resulting in enhanced accuracy and clarity. Video tutorials and recorded webinars were created in English and Spanish to support users.

Significance: The PSA Sanitizer Webtool helps users find and compare EPA-registered sanitizers quickly and effectively. It is the most visited resource on the PSA website, underscoring its value in produce safety education and outreach.

MP-03: DIGITAL DAIRY HUB: AN ONLINE PLATFORM HOSTING SPOILAGE PREDICTION MODELS FOR DAIRY PRODUCTS

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Presentation type/Format: Marketplace

Category: Dairy

Abstract:

Introduction: Food waste due to microbial spoilage is a critical issue in dairy products as the dairy industry lacks predictive models to assess spoilage comprehensively.

Purpose: We developed and deployed a freely accessible web application that hosts spoilage models for three dairy products.

Methods: A user-friendly interface was developed in R Shiny to allow users access to three of our previously developed Monte-Carlo simulation models, which can predict (i) fluid milk spoilage due to psychrotolerant spore-formers and Gram-negative bacteria, (ii) late-blowing defects in semi-hard cheese, and (iii) heat resistant spore levels in powder, respectively. The users can submit supply-chain specific data to predict the percentage of spoiled products in a production batch. As a case study for the use of this platform, we conducted a trial to provide insights to dairy producers who want to transition from conventional to organic dairy by running the model with microbial data collected from conventional and organic milk in previous longitudinal studies.

Results: Using the psychrotolerant spore-former count (PSC) collected from conventional and organic raw milk, the milk spoilage model predicted that, on average, organic milk will have 26 days of shelf life while conventional milk will have 22 days. Given that organic milk, on average, has a slightly higher butyric acid bacteria count (BAB), the model simulation showed that cheese made from organic milk might lead to more spoilage. Specifically, a batch of semi-hard cheese aged at 13°C and made from organic milk was simulated to have 24.8% of cheese with late-blowing defects at day 60 compared to 9.2% for those made from conventional milk.

Significance: The digital dairy hub can be an important step towards precision dairy, through which different stakeholders in the dairy supply chain can quantitatively evaluate spoilage of various dairy products, leading to more rationalized decision-making.

Award Application: Developing Scientist Award

MP-04: THE ATCC GENOME PORTAL: THE GENOMICS DATABASE OF A 100-YEAR-OLD CULTURE COLLECTION

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The ATCC Genome Portal (AGP, <https://genomes.atcc.org/>) is a reference database of authenticated and traceable whole genome sequence assemblies generated directly from strain vials from one of the largest and most diverse culture collections in the world.

Purpose: The AGP initiative provides a resource to the scientific community as many genomes that are in public genomic databases may represent sequences from strains that have been passed between laboratories, are mislabeled, or have accumulated genetic drift arising from laboratory domestication.

Methods: AGP sequences are hybrid high quality de novo assemblies with data generated from Illumina and Oxford Nanopore platforms. These genomes are assembled under standardized conditions to ensure reproducibility for the research community. Genomic sequences are reviewed for post assembly quality control metrics and checked for contamination before being published to the AGP. These genomes are paired with curated metadata from depositors' accessioning records, historical internal records, and undergo taxogenomic checks.

Results: Thousands of foodborne pathogens and food related microbes have been deposited into the collection, with isolates representing over a century of research. Currently, there are over 5250 reference quality genomes of prokaryotes, fungi, viruses, and protists available with new additional assemblies each quarter.

Significance: A majority of assemblies in AGP are of higher quality than equivalent assemblies in public genomic databases. Additionally, some of these assemblies represent strains and/or species that have no representation in public genomic databases. The AGP is a unique and authenticated resource for reference genomes.

MP-05: UNDERSTANDING AND ADDRESSING NEEDS FOR ON-FARM PRODUCE SAFETY EDUCATION IN HYDROPONICS AND AQUAPONICS

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Sujata Sirsat, University of Houston

Chris Callahan, University of Vermont

Presentation type/Format: Marketplace

Category: Produce

Abstract:

Introduction: The Northeast Center to Advance Food Safety (NECAFS) has identified a gap in educational resources tailored to produce safety in hydroponic and aquaponic (HP/AP) operations. Targeted educational resources are crucial for various audiences including growers, extension educators, researchers, and regulatory personnel.

Purpose: The overall goal of this project is to facilitate coordinated development of educational resources and experiences for HP/AP stakeholders across the nation to support increased understanding and reduced risk.

Methods: We took a 5-pronged approach which included:

1. forming a nationwide advisory and collaborator group;
2. conducting a literature review and Delphi study to determine educational needs;
3. developing educational curricula, programs, and resources;
4. delivering educational resources and programs and evaluating outcomes and impact; and
5. coordinating research efforts to address identified knowledge gaps

Results: To address the identified gap in educational resources, we developed a range of new educational materials:

1. Introductory to Produce Safety 101 Guide Factsheet and Web Blog: An accessible overview of produce safety in HP/AP systems, highlighting

common risks and best practices for mitigating them. Available in an accessible online blog and as a printable PDF.

2. Recorded and Upcoming Webinars: Interactive sessions providing detailed insights into HP/AP safety practices, featuring expert speakers and Q&A sessions to address specific concerns.
3. Grower Case Studies: Real-world examples showcasing produce safety in HP/AP operations, including interviews with growers and practical tips for implementation. Available in an accessible online blog.
4. Modified Produce Safety Alliance Grower Training Curriculum: Tailored specifically for HP/AP operations, incorporating feedback from experts, and to be piloted in 2025 with plans to deliver both in person and remotely.

Additionally, a comprehensive research roadmap has been created to outline key knowledge gaps identified through stakeholder feedback, guiding future research initiatives.

Significance: Through these resources, NECAFS aims to improve food safety practices for HP/AP growers, reduce risks, and enhance support and understanding among educators and regulators.

MP-06: PRODUCE SAFETY POSTERS: EDUCATIONAL TOOL FOR NON-ENGLISH-SPEAKING FRESH PRODUCE GROWERS

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Katelynn Stull, Kansas State University

Presentation type/Format: Marketplace

Category: Produce

Abstract:

Introduction: Despite produce safety being an important training topic for growers, many non-English-speaking refugee growers in the Midwest U.S. struggle to adopt these practices due to language barriers and a lack of accessible educational resources. To address this problem, the project team developed produce safety posters using culturally relevant animated visuals with minimal text in the native languages of the refugee growers.

Purpose: This project aims to equip refugee growers with posters that communicate key produce safety practices using visual illustration and minimal text in their native languages.

Methods: Insights were gathered from a project advisory board and a focus group (n = 10) with refugee growers. Four posters were developed addressing key produce safety topics: sick worker management, visitor management, animal-associated germ control, and fertilizer safety. The design incorporated culturally relevant visuals and minimal text, translated into Burmese, Swahili, Nepali, and Kirundi.

Results: The posters explain the rationale behind specific produce safety practices relevant to refugee growers, how to implement these practices, and the potential benefits of adopting them. They serve as tools for increasing understanding and engagement through culturally aligned communication strategies.

Significance: These posters provide culturally and linguistically tailored educational resources for produce safety training, helping improve refugee growers' knowledge, engagement, and adoption of safe farming practices.

MP-07: FREEZE-DRYING AT HOME: RESEARCH-BASED RESOURCES FOR EXTENSION EDUCATORS

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Presentation type/Format: Marketplace

Category: Low-Water Activity Foods

Abstract:

Introduction: The National Center for Home Food Preservation (NCHFP) has developed a toolkit designed to assist Extension agents and educators in conducting community-based programming on freeze-dried foods for home food processors. This initiative addresses the growing public interest in home freeze-drying while providing reliable resources to promote safe practices.

Purpose: This program aims to equip Extension agents with the necessary tools to deliver educational programming on home freeze-drying. It enables participants to understand the science, methods, and best practices associated with freeze-drying food at home.

Methods: The toolkit includes various components to facilitate the implementation of home freeze-drying workshops and classes in local communities. It contains resources tailored for consumers, including a detailed decision tree that guides them through the home freeze-drying process with practical insights for various food matrixes. It also provides in-depth information on freeze-drying basics, equipment, and food safety. A PowerPoint presentation supports educational sessions, and a detailed FAQ fact sheet addresses best practices, product quality, and safety.

Results: An evaluation tool is included to assess program effectiveness. These materials ensure that agents can deliver consistent, evidence-based information while addressing consumers' common questions and challenges.

Significance: This toolkit addresses the growing demand for accessible, research-based resources on home freeze-drying, as identified by the National Center for Home Food Preservation. By bridging gaps in educational outreach, it equips participants with essential knowledge about safe freeze-drying methods, empowering them to preserve food at home with minimized risks.

MP-08: DEVELOPMENT OF PRODUCE SAFETY VIDEOS FOR NON-ENGLISH SPEAKING PRODUCE GROWERS IN THE MIDWEST

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Shannon Coleman, Louisiana State University

Presentation type/Format: Marketplace

Category: Produce

Abstract:

Introduction: The safety of fresh fruits and vegetables is crucial to public health. A significant portion of the farm workforce in the Midwest comprises refugee populations who bring valuable agricultural expertise from their home countries. However, language barriers and the lack of culturally relevant training materials present significant challenges to their ability to comply with U.S. produce safety standards. This project aims to bridge that gap by developing animated videos in the native languages of refugee growers, enhancing their understanding and implementation of produce safety practices.

Purpose: The project seeks to create culturally tailored educational resources to improve produce safety knowledge among non-English speaking refugee growers, promoting better compliance with standards and practices.

Methods: In collaboration with Scientific Animation Without Borders (SAWBO), a multidisciplinary team of food safety professionals, graduate students, and a program evaluator developed two short, animated videos. The videos were created in four languages—Swahili, Kirundi, Nepali, and Burmese. Topics included “How to Control Germs from Animals on the Farm” and “Understanding Fertilizers and Their Risks.”

Results: The videos provide information on sources of germs from animals, methods to control germs on the farm, types of fertilizers, the importance of proper fertilizer application intervals, and record keeping. These educational tools aim to improve understanding and promote the adoption of safe agricultural practices among refugee growers.

Significance: By enhancing the understanding of produce safety practices among refugee growers, this project will contribute to safer and healthier food production.

These practices will help reduce the risk of foodborne illnesses, minimize outbreaks, and protect public health.

Award Application: Developing Scientist Award

MP-09: DEVELOPMENT OF AN INSIGHTFUL FOOD SAFETY MANAGEMENT TOOL

Primary Author:

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Kathleen Wybourn, DNV

Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: The ability to analyze non-conformance data and identify risk through a user-friendly tool is critical to managing risk. Developing such a tool is essential for food safety professionals to monitor system performance and support continuous improvement.

Purpose: To develop a user-friendly tool for visualizing non-conformity data to manage risk within a company and enable benchmarking with external companies.

Methods: • **Findings per day of audit:** This metric reflects the ratio between the number of findings and the audit duration. It provides an intuitive and comparable indicator of system performance across audits.

• **Management System Performance Index (MS-pi):** An overall performance score that consolidates audit metrics such as the number and category of findings, audit duration, company size, and maturity of the food safety management system. The MS-pi ranges from 0 to 100, with 100 being the best.

Results: A user-friendly tool was developed that allows filtering and analyzing non-conformance data. The tool consolidates data into a visual report and provides an MS-pi score, enabling food safety professionals to identify and prioritize high-risk areas for action.

Significance: This tool offers a powerful decision-making resource for the food industry, enabling the tracking and reporting of performance based on risk. It supports smarter, data-driven management of food safety systems across various food products.

MP-10: THE METAVERSE AS AN INNOVATIVE TOOL FOR SUPPLY CHAIN CONTROL: OPPORTUNITIES AND CHALLENGES FOR TRACEABILITY AND FOOD SAFETY

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Noemi Trombetti, UK ITA Group Ltd

Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: The metaverse—a convergence of virtual and augmented realities—offers significant potential to revolutionize supply chain management in the food industry. Through immersive simulations and real-time data integration, it can enhance transparency, efficiency, and sustainability.

Purpose: This summary highlights the most impactful current and future applications of metaverse technologies in controlling food supply chains, focusing on traceability, quality assurance, and collaborative planning.

Methods: The review draws on academic articles, industry reports, and case studies from companies like Accenture, Maersk, and technology providers exploring metaverse solutions for supply chains.

Results

- 1.Enhanced Collaboration:** The metaverse enables virtual meeting spaces for supply chain stakeholders to plan and adjust production in real time, improving responsiveness and reducing errors.
- 2.Supply Chain Transparency:** 3D visualizations of processes provide stakeholders with insights into production and logistics, allowing real-time monitoring of delays and risks.
- 3.Virtual Facility Design:** Digital twins of warehouses and factories help companies optimize operations before physical implementation, saving costs and improving efficiency.
- 4.Traceability and Food Safety:** Combining blockchain and metaverse technologies enhances tracking of food products from farm to table, ensuring authenticity and compliance with food safety regulations.
- 5.Sustainability Monitoring:** The metaverse enables mapping of product life cycles to monitor environmental impacts, helping companies improve sustainability and meet regulatory requirements.

Significance: Metaverse technologies provide groundbreaking tools for improving food supply chain management by increasing transparency, collaboration, and safety. By leveraging these tools, companies can ensure better product traceability, minimize risks, and enhance consumer trust, positioning themselves

to address future market and regulatory challenges effectively.

MP-11: FOOD SAFETY CULTURE TOOLKIT: A FREE RESOURCE FOR SMALL AND MEDIUM-SIZED COMPANIES

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Presentation type/Format: Marketplace

Category: Communication, Outreach and Education*

Abstract:

Introduction: Over the past five years, a team of food safety leaders has worked to develop and operationalize the Alliance to Stop Foodborne Illness' free, multimedia Food Safety Culture Toolkit. This accessible set of low- and no-cost resources gives companies customizable options to advance their internal food safety culture.

Purpose: When the team started this initiative, there was a clear lack of actionable, science-based food safety culture resources. The Toolkit was created to fill this gap, particularly for resource-limited small and medium-sized companies that often lack the time, resources, and expertise to maintain pace with larger players in the supply chain.

Methods: Since its launch in June 2022, the Toolkit has reached 1,347 registrants from 95 countries. Data collected through February 2024 show these registrants represent 665 unique companies, including 153 very small (23%), 102 small (15%), and 306 medium-sized (46%) enterprises. The Toolkit content was developed using deep industry expertise, drawing on both food safety culture successes and failures to drive effective change across the supply chain.

Results: Resources provided include a 40-part video series featuring Stop Foodborne Illness advocates, gamified learning modules, whitepapers from webinars co-hosted with the U.S. FDA, communications tools, and more. In 2025, the Alliance is piloting a mentorship program with three smaller companies that will systematically implement the Toolkit in their operations and provide feedback. In 2024, the Alliance received the IAFP Food Safety Innovation Award for the development of the Toolkit.

Significance: This Toolkit represents a scalable and impactful resource for improving food safety culture across company sizes and regions. It supports more equitable access to best practices in food safety and offers a model for how collaborative development and mentorship can elevate safety standards across the global food supply chain. Access the Toolkit here:
<https://stopfoodborneillness.org/food-safety-culture-toolkit/>

Developing Scientist Competitors

Dhakal, Aakankshya, *Louisiana State University* (P1-162)

Sharma, Aakash, *Dairy Farmers of America* (P2-60)

Alwahaimed, Abdullah, *Virginia Tech* (P1-117)

Mensah, Abigail Aba, *The Ohio State University* (T17-02)

Adeyanju, Adenike, *University of Massachusetts Amherst* (P1-31)

Bandara, Adheesha, *1Food Science Program, University of Missouri* (P3-42)

Arshad, Adina, *University of Massachusetts* (P3-213)

Kabir, Ajran, *University of Kentucky* (P3-94)

Edmundson, Alexandra, *University of Minnesota* (T1-01)

Omar, Alexis, *University of Delaware* (P1-207)

Howell, Allison, *The Ohio State University* (P1-97)

Rosenbaum, Alyssa, *University of Arizona* (P1-203)

Richards, Amber, *University of Georgia* (P3-134)

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