Symposium Abstracts

Symposium

S1 Microbiological Environmental Testing and Validation: Leading-edge Issues for Low-moisture Foods

JEAN-LOUIS CORDIER, Nestlé Nutrition, Operations/Quality Management, Vevey, Switzerland
DONALD L. ZINK, Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA
STEVEN J. GOODFELLOW, Deibel Laboratories, Inc., Gainesville, FL, USA
MARK A. MOORMAN, Kellogg Company, Battle Creek, MI, USA
ROBERT L. BUCHANAN, University of Maryland, Center for Food Safety and Security Systems, College Park, MD, USA

Recent outbreaks of foodborne illness in several low-moisture foods (i.e., peanuts, peanut butter, cookie dough, etc.) have brought a growing public awareness of the complexities involved with the processing of this category of foods where traditional sanitation practices may not be applied or practical. As a result, the food industry and FDA have rallied to bring together best available thinking and practices necessary to assure control over foodborne hazards in such processes. However, one leading edge area that still remains a subject that offers opportunities for improvement is how control measures are verified in these processing environments through microbiological testing. This symposium will "zero in" on the challenges that face both food processors and food regulators in the area of environmental monitoring for these low moisture continuous processes.

S2 Data Deluge, Interacting Players and Complex Networks in Food Sciences – Computational Tools to Tackle Food-related Complexities

RéKA ALBERT, Pennsylvania State University, University Park, PA, USA
MARK TAMPLIN, Food Safety Centre, Hobart, TAS, Australia
GARY BARKER, Institute of Food Research, Norwich, United Kingdom
JÓZSEF BARANYI, Institute of Food Research, Norwich, United Kingdom

Food Science is one of the most multi-disciplinary sciences, consequently a holistic approach is not only desirable but rather a necessity, in order to integrate various food-related complex systems. The systems approach appears at every level of food science from Systems Biology to Food Safety and Security, from Risk Assessment to HACCP and from Food Chains and Networks to the whole Agri-Food Economy. Food Science is unique in terms of dealing with increasing complexity: "By contrast to other prominent industrial sectors such as the car industry or pharmaceuticals, Food Industry is per se characterized by a complex value chain. This chain links the procurement of agricultural raw materials, through their processing up to their presentation for final human consumption and includes their economic distribution. As a result, this industry involves multiple players such as farmers, input suppliers, manufacturers, packagers, transporters, exporters, wholesalers, retailers and final customers with different and changing interests, cultural attitudes and dimensions." - see: Wijnands et al.: Competitiveness of the European Food Industry. EU Commission report, 2007. Tackling such issues, such as involving interacting players in a multidimensional space, and spreading through broad spectra and scales, requires the future food scientist to receive training in: (i) Multi-disciplinary approaches to food science. (ii) Skills in using mathematical models, especially describing complex systems. (iii) Skills to handle databases and data processing.

S3 Converging Industry Initiatives on Traceability

DENNIS HELDMAN, Heldman Associates, Mason, OH, USA
DAVID GOMBAS, United Fresh, Washington, D.C., USA
FAYE FELDSTEIN, FDA, Severna Park, MD, USA

Recall events in the food industry in 2009 caused regulatory, industry and retail groups to see the need for change in the current system in order to better protect and inform the consumers, protect the industry and give the regulatory agencies more control over the process. New legislation, new methodology and new enforcement have occurred in 2009 and into 2010. It is important that these changes are effectively communicated to all segments of the food industry, including the regulatory agencies. This symposium will bring together the different avenues of programs and investigations. It will look at effectiveness and efficiency of these different processes and will introduce commonality needed to improve the speed and completeness of recalls, so as to protect the public, sectors of the food industry and the organizations that distribute and retail the merchandise. Finding the source of contamination and finding it quickly is important. Finding where the source lot was used and distributed is important, finding the retail locations and the customers who purchased the products is important. The need for a uniform approach has never been more obvious. Identifying plant-based products to their source field, grove or farm is essential. Identifying livestock to the producer, pasture and feedlot may be important. Where do we begin and end? What can we afford and what can we not afford? What do our consumers expect and can we deliver on those expectations?

Symposium

S4 Human Pathogens Associated with Edible Plants

DONALD L. ZINK, FDA–CFSAN, College Park, MD, USA
JACQUELINE FLETCHER, Oklahoma State University, National Institute for Microbial Forensics and Food and Agricultural Biosecurity, Stillwater, OK, USA
JERI BARAK, University of Wisconsin, Plant Pathology Dept., Madison, WI, USA
MARIA BRANDL, USDA – ARS – WRRC, Produce Safety and Microbiology Research Unit, Albany, CA, USA
MELANIE IVEY, The Ohio State University, Plant Pathology Dept., Wooster, OH, USA
STEVEN KOIKE, University of California, Cooperative Extension Service, Salinas, CA, USA

The past decade has seen a significant increase in the frequency of produce-associated outbreaks of human diseases, negating the paradigm that foodborne human pathogens are associated primarily with animal products. Scientists recognize that crop plants can be contaminated with human bacterial pathogens in the field. For example, preliminary research on *Salmonella enterica* in association with plants shows (1) preferential bacterial colonization in the rhizoplane; (2) bacterial growth on root surfaces; (3) extended survival in soil and population increases when crops are planted subsequently; and (4) complex surfaces, such as the cantaloupe rind, harbor and protect cells from decontamination methods. Despite these indications, most efforts have focused on potential postharvest solutions rather than addressing microbial-plant interactions in the field. Effective risk reduction and prevention strategies require knowledge of the interactions of food borne pathogens with one another, with plants, and with nonpathogenic microflora. Plant pathologists have extensive expertise on the complex relationships between microbes and plants and can be a valuable scientific resource to enhance fundamental knowledge of, and design effective solutions to, microbial contamination of food plants. The training and experience routinely applied by plant pathologists to understand the mechanisms of pathogen colonization and translocation within hosts, mechanisms of environmental dispersal, plant responses and strategies to defend themselves, and possible intervention strategies will be critical elements of a balanced program to minimize foodborne illnesses. Plant pathologists are contributing to needed research on human pathogen-plant interactions by applying modern plant pathology strategies for understanding host-pathogen interactions. Effective solutions will require the application of emerging research tools and strategies, as well as creative cross-disciplinary research efforts focused on the food production chain. This symposium will provide a view from the FDA on the need for multi-disciplinary approaches to ensure food safety, an overview of recent and proposed contributions of plant pathology to our understanding of fundamental plant-human pathogen interactions, attitudes of growers on proposed production guidances/regulatory requirements, and the role of extension and education in implementing safe production practices. The proposed symposium is unique in that it will combine the fundamental science of plant-pathogen interactions with extension and education targeted to growers. It will be focused on the increasing need to implement multidisciplinary solutions to food safety — from plant pathologists with expertise in host-pathogen interactions to educators and extension professionals to regulators to the broad range of food safety professionals.

S5 Global Water Shortages – Their Impact on Water Safety and Quality

JIM THEBAUT, The Chronicles Group, Inc., Los Angeles, CA, USA
OCTAVIA CONERLY, USEPA, Science and Technology, Washington, D.C., USA
RICHARD W. ATWATER, Inland Empire Utilities Agency, Fontana, CA, USA

Water Shortages: Their impact on Water Safety and Quality: What is being done about this problem? Water shortages and their impact on ingredient water quality is a growing concern for many countries globally. As traditional water supplies become depleted obtaining ingredient water that is free from microbiogical, chemical and heavy metal contamination makes water quality more difficult to manage in the food chain from farm to table. Today one of the leading causes of water shortage is the rising demand for fresh water for industries and agriculture. There is no more fresh water on Earth today than there was 2,000 years ago, when the population was about 3% of what it is today. This short symposium session will have a speaker from the Chronicles Group who produced the documentary "Running Dry" that can bring us up-to-date information on the global water shortage and where it impacts the food chain. Water re-use is a reality and being used in agriculture and food processing. A speaker from the Water Reuse Association will discuss new sources and provide insight of high quality water for consumption in Agriculture and industry. Lastly, a speaker will discuss the intrusion of pharmaceuticals into our water supply and how that may impact the food chain. The impacts of global water shortages have not been discussed before at the IAFP or in 2008 or 2009.

S6 Ripple or Tsunami? Riding the Regulatory Wave to Safer Bottled Water and Water Beverages

HENRY KIM, U.S. Food and Drug Administration, College Park, MD, USA
BOB HIRST, International Bottled Water Association (IBWA), Alexandria, VA, USA
WILFREDO OCASIO, The National Food Laboratory, Inc., Livermore, CA, USA

Bottled water and water beverages constitute one of the fastest-growing segments of the global beverage market. Ensuring the safety of these products – amid concerns about *E. coli*, DEHP, heavy metals, pesticides, disinfection by-products, and other contaminants – is an on-going challenge for bottlers and regulators alike. This short symposium will address the who, what, when, where, and how of water regulation, labeling, testing, and inspection; explore the technical, logistical, and economic impact of newly-promulgated FDA water regulations on the bottled water and water beverage industry; and provide guidance on formulation and processing strategies to ensure the safety and stability of flavored and nutritionally-enhanced water beverages.

S7 Government, Academic and Industry Collaborations to Advance the Development and Use of Microbiological Risk Assessments

RÈGIS POUILLOT, Food and Drug Administration, College Park, MD, USA
DAVID ORYANG, Food and Drug Administration, College Park, MD, USA
STEVEN M.GENDEL, Food and Drug Administration, College Park, MD, USA
JULIANA M.RUZANTE, Joint Institute for Food Safety and Applied Nutrition, College Park, MD, USA
DENISE EBLEN, USDA, Food Safety and Inspection Service, Washington, D.C., USA
RICHARD WHITING, Exponent, Inc., Bowie, MD, USA

Collaborations among U.S. federal agencies, international regulatory agencies, industry, and academic institutions are critical to advance the development and use of microbiological risk assessment. Risk assessment is a useful tool for analyzing the complexities of a food supply system. It provides an objective interpretation of scientific knowledge to support decision-making, including the evaluation of options for risk mitigation and control and for resource allocation. A risk assessment can help to focus future research and other activities to answer public health questions. The complexity of risk assessments often necessitate a need to obtain input from a variety of sources including experts from regulatory institutions, industry, academic, and consumer groups. This symposium will highlight successful government, academic, and industry collaborations in the development of microbiological risk assessment.

Symposium

S8 Less Recognized and Presumptive Pathogens: What Now, What Next?

PURNENDU VASAVADA, University of Wisconsin, River Falls, River Falls, WI, USA
KALMIA KNIEL, University of Delaware, College of Agriculture and Natural Resources, Newark, DE, USA
YNES ORTEGA, University of Georgia, Center for Food Safety, Griffin, GA, USA
MARY TRUCKSESS, FDA, College Park, MD, USA
SCOTT WELLS, University of Minnesota, St. Paul, MN, USA
ROBERT DICKEY, FDA, Division of Seafood Science, and Technology/Chemical Hazards Branch, Dauphin Island, AL, USA

This symposium is being submitted with support of the IAFP Applied Lab Methods Professional Development Group. The food industry is constantly vigilant against established foodborne pathogens such as *Salmonella*, enterohemorrhagic *E. coli* and *Listeria monocytogenes*. Periodically, however, we face challenges from heretofore unheard of or less recognized microbiological threats. Some of these organisms may already be on the horizon and suspected as possible threats to public health, (e.g., *Mycobacterium paratuberculosis*, and avian influenza). Others, such as rare viruses, parasites, or cancer-inducing microorganisms, have the potential to erupt as the next major pathogenic player, given the right set of conditions. This symposium will elucidate a number of proposed, potential, or presumptive foodborne and waterborne pathogens or their toxins. The speakers are highly credentialed and qualified individuals; experts on their assigned topic. From bacteria to viruses to molds to parasites to toxigenic microorganisms, this series of lectures will benefit the food industry, researchers, regulators and students in expanding their knowledge of "Less Recognized and Presumptive Pathogens."

S9 Buy Local? Addressing the Safety Issues Behind Green Food Trends

BENJAMIN CHAPMAN, North Carolina State University, Dept. of 4-H Youth Development and Family and Consumer Sciences, Raleigh, NC, USA
PATRICIA MILLNER, USDA-ARS, Beltsville, MD, USA
JOAN MENKE-SCHAENZER, ConAgra Foods, Inc., Omaha, NE, USA

In December 1998, Dennis Avery printed in the Wall Street Journal that the consumption of "organic" and "natural" foods resulted in an eight-fold higher risk of infection by *Escherichia coli* O157:H7. The CDC responded that studies comparing the risk of infection with *E. coli* O157:H7 through organic/natural foods as compared to conventionally grown have not been conduced. Over a decade later, buying local, the slow food movement and purchasing organic/natural foods have become a few of the new purchasing patterns of consumers. With the increasing incidents of foodborne outbreaks in commodities such as fresh produce, the question of food safety in these popular food trends still remains a question. Often consumers automatically assume that these local, slow and organic foods are also safer. This symposium addresses what these buzzwords mean to the food industry in regards to safety and in procurement strategies. Topics include consumer and media perspectives on the safety of these food trends, current research conducted on microbiology of local/slow/organic produce as compared to conventional produce and a comparison of local/slow/organic food safety versus commercial food manufacturing food safety.

S10 Good Agricultural Practices and the Small Scale Producer: What's Really Going on out There?

ELIZABETH A. BIHN, Cornell University, New York State Agricultural Experiment Station, Dept. of Food Science and Technology, Geneva, NY, USA
MELISSA MUNDO, Duda Farm Fresh Foods, Food Safety Coordinator, Oxnard, CA, USA
MARTHA ROBERTS, University of Florida, North Florida Research and Education Center, Qunicy, FL, USA

In 1998, the Food and Drug Administration (FDA) published the Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables which outlined good agricultural practices (GAPs). GAPs aim to prevent contamination of fresh produce during production and packing on the farm. Since the 1998 guide, many educational programs have been put into place to assist growers with understanding and implementing GAPs. However, foodborne illness associated with the consumption of fresh produce continues to occur. Most large scale producers have detailed GAPs plans in place because of purchaser requirements. Alternatively, small scale producers are less likely to have any implemented food safety plan because their buyers have never made this a requirement. With increased consumer demand for locally grown products, many retailers are now sourcing product from small, local producers. It is clear that extension programs throughout the U.S. are reaching growers of all sizes that sell in diverse markets including retail stores, farmers markets, community sponsored agriculture, and direct farm markets. With all the produce associated outbreaks, buyer requirements, and federal discussions, why are some small growers still not developing and implementing food safety plans on the farm? This symposium proposes to address questions about GAPs implementation with particular focus on the small scale producer. GAPs implementation has not been a symposium topic covered at IAFP in 2008 or 2009, and with the new trend toward sourcing product from small, local producers it is felt this is an area needing attention. With the upcoming general meeting being held in California, it is felt that small growers from the area may find this

symposium of particular interest and thus attract new members to IAFP. Symposium attendees will have a better understanding of challenges to GAPs implementation by smaller producers, the likely impact new marketing and regulations will have on growers who direct market their crops, how much their production is likely to impact consumption, and if this represents a significant risk.

S11 What's Been Keeping You up at Night – Selected Unanswered Food Safety Questions

RONALD H. SCHMIDT, University of Florida, Gainesville, FL, USA
LEE-ANN JAYKUS, North Carolina State University, Food, BioProcessing, and Nutrition Sciences, Raleigh, NC, USA
JOSEPH D. MEYER, Food Safety, Kellogg Company, Battle Creek, MI, USA
LORALYN LEDENBACH, Kraft Foods, Food Safety and Microbiology, Glenview, IL, USA
ROBERT V. TAUXE, CDC, Atlanta, GA, USA
ANDREW BENSON, University of Nebraska, Applied Genomics and Ecology, Lincoln, NE, USA

The symposium is a potpourri of food safety issues currently under debate in the food industry, plus some discussions on potential ways to discover solutions to emerging food safety issues before they happen. It is not intended to be another emerging pathogen symposium, but is intended to highlight several "burning" questions presently on the minds of food safety professionals.

S12 Flour Food Safety: The Changing Landscape – *Escherichia coli* O157:H7

JOE SHEBUSKI, Cargill, Plymouth, MN, USA
TIM JACKSON, Nestle, Glendale, CA, USA
BEN WARREN, ConAgra Foods, Omaha, NE, USA

Flour has been viewed as a raw agricultural product for years; but with a recent outbreak involving consumption of an uncooked product containing flour, regulators and industry are re-examining whether flour should be treated as a RTE ingredient in some foods that may be consumed uncooked by the consumer. This mini-symposium will examine the history of flour and what industry knows about the microbiology of this product during production, harvesting and milling; the regulatory perceptions of flour as a potential vehicle of pathogens; the transformation of microbiological criteria associated with flour and the verification testing required to gauge compliance with the new criteria; and one solution available to deliver RTE flour as an ingredient.

S13 "Ingredient" is a Ten-letter Word for Financial Disaster

BILL MARLER, Marler Clark LLP PS, Seattle, WA, USA
ALAN MAXWELL, Weinberg Wheeler Hudgins Gunn and Dial LLC, Atlanta, GA, USA
D. ALAN RUDLIN, Hunton and Williams, Richmond, VA, USA
MADELEINE MCDONOUGH, Shook Hardy and Bacon LLP, Washington, D.C., USA
PAUL KASSIRER, Lester Schwab Katz and Dwyer, LLP, New York, NY, USA
DAVID HERMAN, Grocery Manufacturers Association, Washington, D.C., USA

This symposium will address the liability of foodborne illnesses and product recalls when ingredients are used from other companies, some of which may be from other countries. One ingredient may pass through numerous hands, contaminate thousands of pounds of product, be responsible for illnesses or deaths and even cause a company to close its doors forever. This symposium will look at how to find the causes of the contamination, discuss the risks and threats to our food supply, how to apportion the liability, and the effect on industry when tainted ingredients are knowingly, or unknowingly, used in the production of food products. The symposium will include case studies and perspectives from top attorneys in the field of food law. They will discuss current concerns: peanuts, cookie dough, leafy greens, ground beef, melamine, etc.

S14 National Institute of Food and Agriculture Showcase

ISABEL WALLS, USDA, National Institute of Food and Agriculture, Washington, D.C., USA
LEE-ANN JAYKUS, North Carolina State University, Dept. of Food, Bioprocessing and Nutrition Sciences, Raleigh, NC, USA
LYDIA MEDEIROS, The Ohio State University, Dept. of Human Nutrition, Columbus, OH, USA
ROBERT GRAVANI, Cornell University, Dept. of Food Science, Ithaca, NY, USA

The National Institute of Food and Agriculture (NIFA) is a new agency within the U.S. Dept. of Agriculture (USDA). NIFA replaced the former Cooperative State Research, Education, and Extension Service (CSREES), which had been in existence since 1994. NIFA's unique mission is to advance knowledge for agriculture, the environment, human health and well-being, and communities by supporting research, education, and extension programs in the US Land-Grant University System and other partner organizations. NIFA doesn't perform actual research, education, and extension but rather helps fund it at the state and local level and provides program leadership in these areas. This symposium will provide an overview of NIFA, and showcase activities being funded in food safety research, education and extension.

S15 Risk-based Design of Microbiologically Safe Foods

TIM JACKSON, Nestlé North America, Glendale, CA, USA
ALEJANDRO AMEZQUITA, Unilever, Safety and Environmental Assurance Centre, Bedford, United Kingdom
NATHAN ANDERSON, NCFST–FDA, Division of Food Processing Science and Technology, Washington, D.C., USA

Traditional rules for the delivery of microbiologically safe food products, e.g., the 12D concept for low-acid canned foods are well established and have a good safety record. These traditional rules are set to be widely applicable and do not take into consideration specific factors relevant to risk, e.g., raw material contamination levels, and changes that may occur during production, distribution, storage, preparation and use of a product. Thus, by their nature, traditional rules are somewhat conservative. Designing products and processes based on a risk assessment approach can bring a better understanding of the factors relevant to risk. Through the application of such approaches it is possible to improve the safety of products and to optimise the quality of products (taste, texture, nutrition) and to optimise energy use to minimise the environmental impact of processes. Moving away from traditional rules requires co-operation between the key stakeholders e.g., industry, regulators, researchers, KOFs. The aim of this symposium is for the key stakeholders to present and discuss the latest thinking and developments in this area. IAFP attendees will be presented with the latest developments in the application of risk-based approaches in the design of microbiologically safe food products and processes.

S16 Significance and Detection of STEC or Non-O157 *Escherichia coli*

PINA FRATAMICO, USDA-ARS, Atlanta, GA, USA
CHRISTINE ROZAND, bioMérieux Industry, Global R&D Industry, Marcy L'Etoile, France
ALEX GILL, Health Canada, Bureau of Microbial Standards, Ottawa, ON, Canada
STEFANO MORABITO, Istituto Superiore di sanita, Dipartimento di Sanita Pubblica Veterinaria e Sicurezza Alimentare, Roma, Italy
MICHAEL COOLEY, USDA, Western Regional Research Center, Albany, CA, USA
JEFF FARBER, Health Canada, Bureau of Microbial Standards, Ottawa, ON, Canada

The focus of concern for hemorrhagic *Escherichia coli*, particularly in the USA, has been on O157:H7. This symposium will address the global significance of STEC or non-O157 *E. coli* as foodborne pathogens and will review methods for detection. Speakers from Canada, United States and Europe will discuss the epidemiology of STEC and specific concerns in their respective geographical locations. The symposium will conclude with a panel discussion to allow attendees to question and comment on the issues raised.

S17 The *Salmonella* Smorgasbord: The Problem with Too Many Choices

PAULA FEDORKA-CRAY, USDA/ARS/BEAR, Athens, GA, USA
JULIAN COX, The University of New South Wales, Sydney, NSW, Australia
JASON RICHARDSON, Coca Cola, Atlanta, GA, USA
BOB REINHARD, Sara Lee Corporation, Downer's Grove, IL, USA
SHAWN BEARSON, USDA-ARS-NADC, Ames, IA, USA
MARK BERRANG, USDA-ARS-BEAR, Athens, GA, USA

Although hundreds of articles are written about *Salmonella* each year, it continues to command top billing as a foodborne pathogen. We have revisited the same issues over a period of time without hitting upon a "eureka" moment. Often, we tend to travel in the comfort zone, bringing in the lateset and greatest technology which, while providing insight regarding specific gene functions, has not provided us with the magic control solution. This symposium is meant to take us out of the comfort zone, sometimes back to areas we long thought of as complete, of little significance or too hard to deal with. For "Niche Displacement: Can you Really Find a New Renter?" the talk with focus on issues surrounding control. There is a preponderance of evidence that if you control for one serotype another comes in. The notion that you can 'get rid' of a specific serotype may not solve the problem as the niche seems to require filling by *Salmonella*, regardless of serotype. This is meant to be a thought provoking talk without the intent to talk about competitive exclusion, prebiotics or probiotics. A compelling example of what has happened over the last 11 years with Kentucky, Enteritidis, Heidelberg, Typhimurium and Typhimurium 5- should make the audience take pause. Additionally, what may not be present in plant may appear at retail! In "Country Specific Serotypes: Why Some *Salmonella* Never Seem to Travel" the talk will compare and contrast serotypes between countries. The goal would be to postulate why some seem rooted in one country. The DT104 story in England or Sophia in Australia are good examples of a widespread problem that hasn't really travelled well. "The Slugfest – Why Some *Salmonella* Outcompete Others" explores the differences between *Salmonella* serotypes in recovery and competition during cultivation both in vivo and in vitro. Although some may ask why this has to be revisited, there are serious implications as regulations are being made on the data which can be impacted by cultivation methods. "*Salmonella* — A Regulatory Challenge for Industry?" will focus on the challenges associated with developing regulations around *Salmonella*. This could also be given by someone who can speak to trade issues. "A Blast from the Past? Why We Need to Build a Better Host" will focus on the host/bug interactions, immunity and what molecular biology at the gut level can do for us today. If we can't control *Salmonella* by conventional means, will control in the host really work? Finally, "Give It Your Best Shot: The Chemical Control Conundrum" will focus around the myriad of serotypes found in a plant and how one chemical intervention may not necessary provide universal control. Therefore, if you can control it on farm but not in the plant, how much do you really gain?

S18 European Concept on Hygiene Monitoring in the Food Supply Chain – 'Farm to Fork' Concept in Practice

HELMUT STEINKAMP, Food Safety, German Institute of Food Technology, Quakenbrück, Germany
EBERHARD HAUNHORST, Lower Saxony State Office for Consumer Protection and Food Safety, Oldenburg, Germany
GUENTER KLEIN, University of Veterinary Medicine Hannover, Institute for Food Quality and Food Safety, Hannover, Germany

The idea of the symposium is to present a transatlantic comparison on food hygiene concepts based on EU-regulations. Eberhard Haunhorst: Monitoring of Food Safety in Practice. The EU demand on food safety is directed towards a cross-stage cooperation for hygiene monitoring in food production. Based on the existing EU-Regulations the European way towards an improved food safety in Europe is shown. The EU-Regulations are transfered on national level of the EU-Member States. Following the German model on food safety control, the practical application of the hygienic concept is presented. Guenter Klein: Zoonosis Control in Primary Production and in the Food Chain. The implementation of the EU zoonosis-regulations requires the monitoring of *Salmonella* and *Campylobacter* in animal production. The application of these regulations is described and shown to be an effective method for zoonosis reduction in the food supply chain. Only combined measures in primary production and in the following steps of the food chain (harvesting, processing) are successful to reach the goal of the regulations. Helmut Steinkamp: European Food Safety Standards. The European regulation concerning food safety and consumers' health is implemented by introducing the self control principles and the prevention of risks for foodstuffs following the HACCP principles (Hazard Analysis Critical Control Points). Furthermore food retailers are induces the food industries to elevate their qualitative and sanitary-hygienic standards. To export in Europe and to produce trademark products, it is necessary to demonstrate the capacity of applying international acknowledged standards such as the BRC (British Retail Consortium), the IFS (International Food Standard) and the EFSIS (European Food Safety Inspection Service). The aims are to control risk factors for consumers through: traceability, preventions of food intolerances, continuous updating of microbiological and chemical-physical requisites in foodstuffs and labelling.

Symposium

S19 International Food Safety Policies

JAMES BALL, Delhaize Group, Belgium
MOHAMMED RAWASHDEH, Jordan Food and Drug Administration, Amman, Jordan
FLORIAN HEUPEL, Eurofins, Nantes, France
KEES AELBERS, PepsiCo, Amsterdam, The Netherlands
WILLIAM PAPPAS, Alliance One International, Morrisville, NC, USA
TERRY STILLMAN, Alliance One International, Morrisville, NC, USA

This symposium will provide a venue for an open discussion on the comparative differences of regulations and policies around the globe, updates on international legislation, and more importantly the effectiveness of an overall global food safety topology. Real-world examples of the challenges and accomplishments of multi-national organizations working in this environment will be sited to facilitate a "lessons learned" dialog. Speakers from Europe, Asia and the Americas will jointly explore current efforts to harmonize international standards, and gauge how near we are to a standardized approach and what's to come in the future. Global standardization has important ramifications for food safety and the efficiency of multi-national organizations and governments. Can a safer and more secure food system be established without a negative economic impact? In a world of increasing global trade, the importance of this discussion spans from public health and safety to the bottom line of every business. Key points of differentiation from previous similar presentations: 1. This session will provide an update on international food safety legislation 2. This session will gauge the effectiveness of the international food safety topology 3. This session will feature lessons learned by multi-national organizations 4. This session will measure how close we are to global standardization? 5. This session will weigh food safety cost/benefit considerations. Can we deliver on those expectations?

S20 Food Packaging Technology: Opportunities and Challenges That Enhance Food Safety

JOSEPH HOTCHKISS, Michigan State University, School of Packaging, East Lansing, MI, USA
FORREST BAYER, The Coca-Cola Company, Atlanta, GA, USA
MICHELLE TWAROSKI, U.S. Food and Drug Administration, Food Packaging Division, College Park, MD, USA
ROBERT BRACKETT, Grocery Manufacturers Association, Washington, D.C., USA
CARL WINTER, University of California-Davis, Dept. of Food Science and Technology, Davis, CA, USA

Food safety continues to be a priority for food manufacturers, regulatory bodies and government agencies in the United States and abroad. Over the years, food packaging technology has evolved to keep pace with our global food supply by enhancing the safety and quality of edible products. To that end, packaging offers the value-added benefits of keeping food affordable for consumers, extending shelf life, and in many instances, minimizing the risk of possible microbial contamination. However, with the advancement of food packaging technology we are becoming more aware of chemicals migrating from food packaging surfaces to food, which has implications for public health. This symposium will put past, present and emerging packaging technologies into context relative to food safety. Speakers will delve into the specifics of packaging's public health implications; the regulatory approval process associated with packaging and the benefits that food packing offers to enhance food safety.

S21 The Emergence of Non-culture Diagnostics and Their Impact on Global Foodborne Disease Surveillance

MARGUERITE A. NEILL, Brown Medical School and Memorial Hospital of Rhode Island, Divison of Infection Diseases, Pawtucket, RI, USA
ANDREA G. ELLIS, World Health Organization, Dept. of Food Safety, Zoonoses and Foodborne Diseases, Geneva, Switzerland
TIMOTHY F. JONES, Tennessee Dept. of Health, Nashville, TN, USA
JOHN BESSER, Centers for Disease Control and Prevention, Enteric Diseases Laboratory Branch, Atlanta, GA, USA
G. BALAKRISH NAIR, National Institute of Cholera and Enteric Diseases, Beliaghata, India

The symposium will aim to describe the evolving landscape of surveillance for foodborne illness, ranging from new surveillance challenges at the national and global levels to harnessing the power of rapid molecular subtyping methods being utilized by international laboratory networks. Examples will be provided along the way, along with provocative questions they raise for the clinical and public health communities. Emphasis will be placed not just on questions raised, but on looking toward the future and suggesting solutions to get us where we want to go.

S22 Food and Food Environment Test Considerations in View of Changing Regulations

DON ZINK, Food and Drug Administration, College Park, MD, USA
MARK MOORMAN, Kellogg's, Battle Creek, MI, USA
JOHN LEMKER, K and L Gates, Chicago, IL, USA
BOB BRACKETT, GMA, Washington, D.C., USA
TIM FRIER, Cargill, Minnetonka, MN, USA
MASI RAJIBI, ABC Research, Gainesville, FL, USA
JEFFREY L.KORNACKI, Kornacki Microbiology Solutions, Inc., McFarland, WI, USA

Responsible food processors have historically engaged in rigorous sampling of their production environments for pathogenic microorganisms and based remediation activities upon such data. This approach has resulted in great health risk reduction to consumers and business risk reduction to food producers. However, recent public events have created powerful incentives for regulatory agencies to seek this environmental and product pathogen test records previously considered confidential. These regulatory activities raise important concerns within the industry about how they should manage their pathogen control programs. The wrong approach (e.g., less environmental testing) will result in greater consumer and eventual business risk. This symposium will review the present situation, look at anticipated near-term regulatory changes, describe consequences of pathogen and indicator testing, and provide important suggestions to the industry that should result in a safer food supply and a less risky business environment.

S23 Way before the Fork: Impact of Pre-harvest Management Programs and Supply Chain Influences on the Control of Shiga Toxin-producing *Escherichia coli* Contamination in Beef

JAMES DICKSON, Iowa State University, Dept. of Animal Science and Interpartmental Program in Microbiology, Ames, IA, USA
GUY LONERAGAN, West Texas A&M University, Canyon, TX, USA
STUART REID, University of Glasgow, Veterinary Medicine, Glasgow, Ireland
DAVID SMITH, University of Nebraska-Lincoln, Lincoln, NE, USA
PATRICE ARBAULT, BioAdvantage Consulting, Orlienas, France
PATRICIA GRIFFIN, Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Atlanta, GA, USA

The negative effects and outbreaks of foodborne pathogens, including Shiga toxin-producing *Escherichia coli* (STEC) are significant and can be devastating. Beef post-harvest control methods have been successful in reducing the risk of contamination of STEC. The role of pre-harvest control methods and management programs is becoming increasingly critical for food safety and public health. Development and adoption of pre-harvest tools are the keys to farm-to-fork control of STEC. *E. coli* are a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others have the potential to cause human illness. Some kinds of *E. coli* cause disease by making a toxin called Shiga toxin. The bacteria that make these toxins are called Shiga toxin-producing *E. coli* (STEC). This term is interchangeable with the term verocytotoxic *E. coli* (VTEC). Those STEC that can cause human illness are sometimes called enterohemorrhagic *E. coli* (EHEC). The most commonly identified STEC in North America is *E. coli* O157:H7 (often shortened to *E. coli* O157 or even just O157). News reports about outbreaks of *E. coli* infections usually refer to *E. coli* O157. In addition to *E. coli* O157, many other serogroups of STEC cause disease and are sometimes called "non-O157 STEC." *E. coli* serogroups O26, O111, and O103 are the non-O157 serogroups that most often cause human illness in the United States. Most of what is known about STEC comes from outbreak investigations and studies of *E. coli* O157 infection, which was first identified as a pathogen in 1982. STEC live in the guts of ruminant animals, including cattle, goats, sheep, deer, and elk. The major source for human illnesses is cattle. The non-O157 STEC are not nearly as well understood as O157, partly because outbreaks due to them are rarely identified. As a whole, the non-O157 serogroups are less likely to cause severe illness than *E. coli* O157; however, some non-O157 STEC strains can cause the most severe manifestations of STEC illness. Addressing state-of-the-art pre-harvest efforts and tools to minimize the risks of STEC contamination of beef products, this symposium showcases input from global experts representing industry, academia and public health sectors.

S24 Advances in Detection Technologies to Address Food Safety and Food Defense Needs

ALEXANDER SIMONIAN, National Science Foundation, Biosensing and Bioengineering/CBET, Arlington, VA, USA
DANIEL V. LIM, University of South Florida, Advanced Biosensors Laboratory, Tampa, FL, USA
CHRIS R. TAITT, Naval Research Institute, Center for Bio/Molecular Science and Engineering, Washington, D.C., USA
ARUN K. BHUNIA, Purdue University, Dept. of Food Science, West Lafayette, IN, USA
BRUCE APPLEGATE, Purdue University, Dept. of Food Science, West Lafayette, IN, USA

The risk of unintentional contamination of food and ingredients with microbial and chemical agents continues to be an ongoing and relentless food safety challenge to all stakeholders. Moreover, the malicious contamination of food for political, financial and other purposes is a real and current threat, and deliberate contamination of food at one location could have global public health implications. Industries that comprise the U.S. food system from farm to table are potential targets for bioterrorism and terrorist hoax situations. Terrorists could create harm through: (1) final product contamination using either chemicals or biological agents with the intent to kill or cause illness among consumers, (2) disruption of food distribution systems, (3) damaging the agricultural economy (which makes up roughly 17% of the gross domestic product) by introducing devastating crop pathogens or exotic animal diseases or (4) hoaxes, which create anxiety and fear and which could severely impact an area of the food system. A variety of potential bioterrorism and chemical agents could contaminate the food supply, and the possible scenarios for deliberate contamination events are essentially limitless. One of several important detection methods for identifying credible threats, so that incidents may be appropriately triaged and responded to according to level of risk, includes enhanced laboratory capacity and technologies. Planning for possible crises should consider the ability of the surveillance and monitoring systems to rapidly detect food safety emergencies, including those caused deliberately. Investigation of a potential outbreak identified by surveillance should include identification of the food and the responsible agent in the food. Qualified laboratories are important requirements for food defense preparedness. The timely sampling, transport and analysis of suspected foods should be addressed as part of preparedness planning. Given these assertions, enhanced food testing capabilities are critical to successful food safety and defense programs. This symposium is designed to address and describe emerging technologies that can be considered state of the art laboratory tools for identifying food microbial and chemical biohazards of accidental and intentional origin.

S25 Human Noroviruses: Attribution, Transmission and Control

JUDY GREIG, Public Health Agency of Canada, Guelph, ON, Canada
NIGEL COOK, CSL, York, United Kingdom
CRISTOBAL CHAIDEZ, CIAD, Culiacán, Sinaloa, Mexico
ALVIN LEE, National Center for Food Safety and Technology, Chicago, IL, USA
MOSHE DREYFUSS, FSIS, Washington, D.C., USA
JAMES ARBOGAST, GOJO Industries, Akron, OH, USA

The transmission of the newly emerging norovirus genogroup II.4 that are highly virulent and capable of causing death in the elderly and immunocompromised needs to be better understood. Question revolve around foodborne related outbreaks of noroviruses in determining the types of foods that typically cause outbreaks, consumption trends, and changes in food commodities that are known to cause outbreaks over time and various geographical regions in comparison to the outbreaks of the recent years. Many questions involve new transmission routes or the ability to better detect these pathogens in foods that were not routinely tested for earlier. This symposium will increase our understanding of the risks associated with the transmission of enteric viruses from the farm to the table and what control measures can be put into place at the various levels. Questions will be answered related to what extent farm workers have in the spread of enteric viruses to produce. The importance of norovirus cross contamination in the commercial food service setting during preparation of fresh produce will be discussed. Information regarding the extent person-to-person transmission has on the spread of enteric viruses during outbreaks will be examined. Finally the impact of sanitizers for control of enteric viruses for produce and workers will be discussed.

Symposium

S26 Global Issues and Impact of Gluten Allergy and Celiac Disease

SAMUEL GODEFROY, Health Canada, Ottawa, ON, Canada
STEVE L. TAYLOR, University of Nebraska, FARRP, Lincoln, NE, USA
RHONDA KANE, FDA, Food Labeling and Standards, College Park, MD, USA
ROLAND E. POMS, International Association for Cereal Science and Technology, Cereal Science, Vienna, Austria
GLENN BLACK, GMA, Science Operations, Washington, D.C., USA

Wheat gluten is known to provoke a disorder known as celiac disease (CD) in a significant part of the worldwide population. In the United States alone, it is estimated that more than two million people suffer from CD. Moreover, wheat proteins are also responsible for allergies. This symposium elucidates the differences between CD and allergy, based on actual clinical and epidemiological data. There is currently no treatment for celiac disease or for wheat allergy, so people suffering from CD or allergy have to follow a strict gluten-free diet. As wheat, and more generally grains, represent a significant part of food ingredients, additives and final products, there is a tremendous need for food industry players to acquire an adequate understanding of the current needs and requirements of consumers with CD and wheat allergy and to fulfill their responsibilities in accordance with national and international regulations for gluten-free foods and labelling. This symposium will cover current regulations in the U.S. and Europe, consumer and patient needs, and current analytical and production technologies. The latest breakthroughs for gluten analysis in foodstuffs will be addressed, along with the benefits and limitations of the various technologies. To that end, some "real life" experiences during routine analysis will be highlighted. What's more, current progress by the food industry in the risk management of gluten in gluten-free food and manufacturing facilities will be presented. Such a topic has not been thoroughly approached for the last 2 years of the IAFP Annual Meeting.

S27 Food Safety in Developing Countries

BOBBY KRISHNA, Dubai Municipality, Food Safety Unit, UAB, United Arab Emirates
CAMERON SMOAK, Georgia Dept. of Agriculture, Madison, GA, USA
SHRI R. VIJAY, Food Safety and Standards Authority of India, Administration & Finance, New Delhi, India
FERNANDO G. QUEVEDO, San Marcos National University, Lima, Peru
PETER BEN EMBAREK, World Health Organization, Dept. of Food Safety, Zoonoses and Foodborne Illness, Beijing, China

Speakers will deliver the challenges that address different developing countries or regions and strategies taken to carry out the surveillance and control measures. Most of these speakers have not presented at IAFP meetings before.

S28 Foodborne Disease Outbreak Update

JEFFERY HIGA, Colorado Dept. of Public Health and Environment, Communicable Diseases, Denver, CO, USA
MAHA HAJMEER, California Dept. of Public Health, Food and Drug Branch, Sacramento, CA, USA
SHAUN COSGROVE, Colorado Dept. of Public Health and Environment, Communicable Diseases, Denver, CO, USA
SCOTT SEYS, USDA-FSIS, Office of Public Health Science, Minneapolis, MN, USA
MARK SOTIR, Centers for Disease Control and Prevention, Outbreak Response and Prevention Branch, Atlanta, GA, USA
TIMOTHY JACKSON, Nestle USA, Inc., Food Safety, Glendale, CA, USA

This symposium will describe the epidemiologic and environmental investigations of four foodborne disease outbreaks that have occurred in the past year.

S29 Maintaining Consumer Market Continuity during Animal Disease Outbreaks

TODD MCALOON, Cargill Animal Protein, Minneapolis, MN, USA
TIMOTHY CLOUSE, USDA-CEAH, Fort Collins, CO, USA
DARRELL TRAMPEL, Iowa State University, Ames, IA, USA
JON ZACK, USDA-APHIS, Riverdale, MD, USA
HOWARD MAGWIRE, United Egg Producers, Washington, D.C., USA
WILLIAM HUESTON, University of Minnesota, St Paul, MN, USA

For the last 5 years, a diverse team of egg industry, university and state/federal regulatory persons have proactively worked on a means to contain a Highly Pathogenic Avian Influenza disease outbreak while at the same time, maintaining market continuity. The process is highly collaborative and engaged. The team has garnered significant support by some respected animal health groups such as the United States Animal Health Association and National Poultry Improvement Plan as well published USDA APHIS Risk Assessments for various egg industry products, an MOU between Iowa and Minnesota with other states joining this coalition and the resulting work (Secure Egg Supply Plan) will be utilized by both egg industry and USDA APHIS as part of emergency preparedness and response plans. This group is changing the paradigm for animal disease outbreak control and is proof that collaboration between all stakeholders is the model going forward. Already a new group has been formed to address market issues related to a potential Foot and Mouth Disease (FMD) outbreaks in hogs, modeled after this team's efforts.

S30 A Practical Approach to Risk Communication: Engaging Stakeholders and the Public

ANDY BENSON, International Food Information Council, Washington, D.C., USA
JEAN KENNEDY, European Food Information Council, Brussels, Belgium
CARL WINTER, University of California-Davis, Dept. of Food Science and Technology, Davis, CA, USA
PETER BEN EMBAREK, World Health Organization, Dept. of Food Safety, Zoonoses and Foodborne Illness, Beijing, China
GENARO W. GARCIA, Centro Panamericano de Fiebre Aftosa, Health Surveillance and Disease Management, São Bento, Brazil
MARJORIE DAVIDSON, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA

Risk communication is vital to help prevent or reduce the risk of foodborne illness for the public and especially for target audiences. The fact that today's food supply is global and truly international in scope adds another set of food safety communication challenges. This session will help attendees build communication competence and capacity among IAFP members in the U.S. and abroad to be successful when communicating about food safety issues either in a local or global environment. The symposium is designed to facilitate a dialogue that enhances communication among food safety stakeholders (academic, government, industry and consumers) and will help develop best practices when communicating about food risks. In addition, the symposium will highlight examples/case studies from various food safety stakeholders in the U.S. and abroad including perspectives from the U.S., SE Asia, South America and Europe. These and other invited speakers will come together to share best practices for future risk communication initiatives.

Symposium

S31 Setting the Science-based Agenda for Co-management of Watershed Quality and Produce Safety

CHRISTINA FISHER, The Nature Conservancy, Arlington, VA, USA
DANIEL MOUNTJOY, USDA, Natural Resources Conservation Service, Salinas, CA, USA
MICHELLE JAY-RUSSELL, University of California-Davis, Western Institute for Food Safety and Security, Davis, CA, USA
JEFFERY LEJEUNE, The Ohio State University, Dept. of Veterinary Preventive Medicine, Wooster, OH, USA
MICHAEL BATZ, University of Florida, Emerging Pathogens Institute, Gainesville, FL, USA
TIM YORK, Markon Cooperative, Salinas, CA, USA

Providing fresh produce of high quality and safety, at all scales of production and distribution, is a vital national and global issue. In many areas, the seeming overriding focus on food safety issues and responses, in the absence of scientific certainty, has thrown fresh produce into a nexus of competing agendas between consumer health, environmental stewardship, and the economic sustainability of this agricultural sector. Efforts to ensure compliance with on-farm food safety audit requirements and microbiological standards may have resulted in significant local and regional negative impacts on watershed quality and wildlife in the central coast of California, a major cool-season fruit and vegetable producing region. A key feature in this dilemma is the limited specifically-applicable data on the role of non-crop vegetation as habitat for diverse vertebrate and invertebrate vectors on transference and persistence of enteric pathogens to fresh produce. In the absence of developing adequate risk-based data and analysis to support co-management strategies, the construction of sound, science-based policies that protect public health and balanced environmental systems will be unattainable. Adopting national mandatory standards for produce production and marketing based on conceptually limited or flawed options for co-management could have devastating economic impacts, especially to small farm and limited resource producers. This symposium is a novel and timely topic for IAFP and would bring together scientists and policy-drivers from a broad spectrum including the fresh produce industry, environmental advocacy, and regulatory communities. Attendees will attain a more in-depth understanding of the current conflicts and the efforts of a broad coalition of grower and environmental associations, agencies, regulators, and researchers to define the state-of-the science and a best path forward for developing effective co-management strategies. Symposia at IAFP in 2008 (S15 Harmonization of Irrigation Water Practices) and 2009 (S20 Environmental Reservoirs of Major and Emerging Foodborne Pathogens) have not recognized that the co-management of public health and the environment are critical, or considered the potential widespread impacts of inconsequential or ineffective food safety management practices.

S32 Bacterial Toxins: A Past or an Emerging Issue for Food and Beverage Safety?

PATRICE ARBAULT, BioAdvantage Consulting, Food Safety and Analytical Methods, Orliénas, France
SANDRA M. TALLENT, FDA, Division of Microbiology, College Park, MD, USA
JULIAN COX, University of New South Wales, Faculty of Science, Sydney, NSW, Australia
STEFANO MORABITO, Istituto Superiore di Sànita, Dipartimento di Sanita Pubblica Veterinaria e Sicurezza Alimentare, Rome, Italy
ERIC A. JOHNSON, University of Wisconsin-Madison, Dept. of Bacteriology – Food Research Institute, Madison, WI, USA
JULIAN COX, University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Bacterial toxins have never really disappeared from the radar screens of food safety and epidemiological data have underlined their perennial implication in foodborne intoxications worldwide. Recent outbreaks linked to consumption of foods and beverages (botulinum toxins in carrot juice and in "foie gras") have re-highlighted their significance, introducing some of them as 'a new threat', but are they really new or simply back from the oubliette? The symposium first gives an introduction to bacterial toxins and their relevance to food safety. Several of them (staphylococcal enterotoxins, *Bacillus* toxins, toxins excreted by some of the pathogenic *E. coli* groups, and *Clostridium botulinum* toxins) will then be discussed, including their characteristics, food matrices of concern, and current analytical technologies available for their detection. Such a global approach on bacterial toxins has not been presented for the last two IAFP Annual Meetings. This symposium wants to update on the impact of various bacterial toxins on food safety issues, including beverage, on the analytical technics for their detection in food, and to illustrate the lessons learned from some of the recent outbreaks.

S33 Tools for Predictive Microbiology and Microbial Risk Assessment

VIJAY JUNEJA, USDA, Wyndmoor, PA, USA
LEE-ANN JAYKUS, North Carolina State University, Dept. of Food, Bioprocessing and Nutrition Sciences, Raleigh, NC, USA
SARAH CAHILL, FAO, Nutrition and Consumer Protection Division, Rome, Italy
PANAGIOTIS SKANDAMIS, Agricultural University of Athens, Dept. of Food Science and Technology, Athens, Greece

Predictive models have been increasingly used by the food industry, regulatory agencies, and research institutes to predict the behavior of the pathogens in foods, for developing HACCP plans. The predictions serve as building blocks for microbial risk assessments. This symposium will give an update on the tools in predictive microbiology and risk assessment.

S34 WHO's Epidemiological Approach to Estimating Foodborne Diseases – WHO FERG

CLAUDIA STEIN, World Health Organization, Food Safety and Zoonoses, Geneva, Switzerland
ARIE HAVELAAR, RIVM, Bilthoven, The Netherlands
DANIEL ENGELJOHN, USDA OPPD FSIS, Washington, D.C., USA
VANESSA CRANFORD, Walt Disney Inc., Buena Vista, FL, USA

In 2007, WHO launched an initiative to estimate the global burden of foodborne diseases (FBDs), and established an international expert advisory group, the Foodborne Disease Burden Epidemiology Reference Group: FERG to assist with this effort. FBDs are caused by bacteria, viruses, parasites and chemicals and result in illnesses ranging from milder forms to life-threatening conditions, and from rare to abundant. Well-known foodborne pathogens are not always transmitted by food, but often by human-to-human transmission or by direct contact to reservoir animals, etc. In order to estimate the true burden (frequency, severity, and resulting life-years lost) of FBDs and provide useful inputs to policy makers and other stakeholders, we will learn how WHO FERG deals with the global quantification of foodborne diseases, understand the initiative's challenges and approaches, discuss what food safety professionals can expect from WHO FERG and find out how they can best support this work. This global epidemiological consideration is unique in IAFP compared to those discussed in the last few years.

S35 New Definitions in Imported Seafood Safety

BARBARA BLAKISTONE, National Fisheries Institute, McLean, VA, USA
PETER BEN EMBAREK, World Health Organization, Dept. of Food Safety, Zoonoses and Foodborne Illness, Beijing, China
ANA HOOPER, Darden Restaurants, Winter Spring, FL, USA
STEVE WILSON, NOAA, Washington, D.C., USA
DON KRAEMER, Food and Drug Administration, Washington, D.C., USA
BARBARA BLAKISTONE, National Fisheries Institute, McLean, VA, USA

Within this decade concern has increased about imported foods, especially seafood because over 80% is imported. Import Alerts from the Food and Drug Administration (FDA) have long existed but have increased in importance. In 2006 Import Alert 16–131 was issued by FDA because of unapproved antibiotic residues in seafood. The Alert targeted China's exports of shrimp, catfish, basa, eel, and dace. Seafood products from other nations too are under close scrutiny by the agency for unapproved antibiotic residues. Concerns are not strictly chemical. Other Alerts are cited on imports such as 16–18 on *Salmonella* spp. in shrimp. In response to public demand and the demand of Congress, FDA has now opened offices in other nations to more effectively address the real and consumer-perceived problems in imported seafood. And the agency continues to pursue third party certification programs to pre-empt export of seafood that does not meet FDA criteria. The United States is not alone in pursuit of improving the safety and quality of seafood. The EU and Canada have strict programs also and closely tabulate issues in seafood from outside their borders. The quest for safety and quality is not solely the responsibilities of the various governments. Private certifiers and are guardians too and offer restaurants, retailers, and industry oversight of wild and aquacultured product. In the mix too are the standards offered to countries by Codex Alimentarius and recent technical committee actions through ISO.

S36 Risk Benefit Analysis of Food Production and Consumption

CRISTINA TIRADO-VON DER PAHLEN, University of California-Los Angeles, School of Public Health, Los Angeles, CA, USA
KAZUKO FUKUSHIMA, WHO, Food Safety, Geneva, Switzerland
BERNARD BOTTEX, EFSA, Scientific Committee, Parma, Italy
STÉPHANE VIDRY, ILSI Europe, Brussels, Belgium

The Analysis of Risks and Benefits is necessary when these coexist; for example in foods (e.g., nutrients vs. food contaminants), or as a result of food technology processes, food production and farming systems etc. The assessment of risk to human health of food substances or nutrients is usually conducted independently of possible health benefits. Both health risks and benefits need to be balanced. At the same time, the risk and benefits of food technologies and food production systems in the environment and/or health should be also considered and integrated into the whole assessment. This new approach deserves special attention since the assessment of risks and benefits of foods, technologies, production systems, etc. will provide a powerful tool for risk managers for decision and policy making and for regulatory purposes. In particular, and as first step, there is a need for the development of a science based methodology for Risk Benefit Assessment. Objectives of the symposium on Risk Benefit Analysis include: Raise awareness on the need to address the risk and benefits of Food Consumption and Food Production Systems on Health, and/or the Environment in an integrated way. -Address the role of food scientist and producers in the provision of information and quality data for the assessment of the health risks and benefits of foods, processes, technologies, production systems, etc. for adequate risk management and risk communication. -Present the progress done at the international level - WHO/FAO, ILSI and the Scientific the European Food Safety Authority- (EFSA) on development of an harmonized methodology for health risk benefit assessment and -Offer a platform to discuss the need for an integrated science based methodology for risk benefit assessment for both health and the environment.

S37 Issues in the Production and Manufacture of Nuts and Nut-containing Products: Nuts to You

LINDA J. HARRIS, University of California-Davis, Dept. of Food Science and Technology, Davis, CA, USA
DOJIN RYU, Texas Woman's University, Dept. of Nutrition and Food Sciences, Denton, TX, USA
MATILDA FREUND, Kraft Foods Global, Global Microbiology and Food Safety, Tarrytown, NY, USA
TIM BIRMINGHAM, Almond Board of California, Quality Assurance/Industry Services, Modesto, CA, USA

This short symposium aims to address the production and processing of nuts, both as individual products and as ingredients. Speakers will cover all different types of nuts, such as almonds, pistachios, walnuts, pecans, hazel nuts, and the legume, peanuts, and will look at harvest, sanitation, storage, processing into other products and related problems, as well as contamination and outbreak issues. Current and new regulations related to nut cultivation, processing and use as ingredients will also be addressed.

Roundtable Abstracts

RT1 Research Needs A Roundtable: Retail and Foodservice Food Safety

JENNIFER QUILAN, Drexel University, Philadelphia, PA, USA
LARRY KOHL, Food Marketing Institute, Arlington, VA, USA
ANN MARIE MCNAMARA, Jack in the Box, San Diego, CA, USA
DONALD W. SCHAFFNER, Rutgers University, New Brunswick, NJ, USA
KEVIN SMITH, FDA, College Park, MD, USA

The Retail Food Safety Consortium is comprised of food safety professionals from five land-grant universities, professional societies (including IAFP), and government agencies. Its primary goal is to facilitate communication between food safety professionals at all levels. The Consortium was recently funded by the USDA by over half a million dollars to accomplish four specific objectives relative to retail and foodservice food safety. The last of these objectives (identify and prioritize retail food safety needs) will be accomplished with the help of this roundtable. The purpose of the roundtable is to allow various constituencies to raise the relevant issues and to begin group discussion to reach consensus on the important research issues to be addressed.

Technical Abstracts

T1-01 Aspects of Systems Theory in the Analysis of Molecular-biological Based Detection Methods

PETER ROSSMANITH and Martin Wagner
University of Veterinary Medicine Vienna, Christian Doppler Laboratory for Molecular Food Analytics, Vienna, Austria

Introduction: The implementation of molecular-biological based food-pathogen detection is a frequently and intensively discussed topic. Molecular-biological methods for food analysis comprise a detection chain consisting of sample preparation, target purification and a detection assay. Given this systemic character systems theory provides basis for discussion of principles and application of testing methods derived from various scientific areas for specification and validation of pathogen detection.

Purpose: This work describes the structure and strategy of a possible alternative approach for validation and specification of molecular-biological methods to accelerate their broad range implementation into food pathogen detection.

Methods: The hypothesis is established that systems theory provides the basis for implementation of test systems, derived from other scientific or technical areas, to specification and validation of molecular-biological food pathogen detection methods, or alternatively as supplemental to existing international standards. The categorization of black box and white box systems demonstrates a possible classification for pathogen detection methods. Furthermore, the transformation of models from systems theory to the analytical chain of food detection provides new insights for opportunities and restraints for such methods, especially when compared with conventional microbiological methods.

Results: The resulting applicability of Physical-Modeling-Synthesis and System-Identification as used in systems analysis provides two strong instruments for validation of the completely molecular-biological detection process. Equivalence-Class-Formation and Limit-Analysis, which is the underlying test principle for approvable application of System-Identification by means of Poisson-Analysis, both support specification of the enzymatic assay building the core of a molecular-biological detection chain. This alternative approach is based on validation of the method per se and supports conventional comparative validation according to ISO 16410.

Significance: The application of systems theory to problems of food detection by molecular-biological methods provides a strong tool and an alternative approach for the validation of new methods, specification of the enzymatic core method and evaluation of related and unanswered questions in this context.

T1-02 Utilization of Evolutionary Model, Bioinformatics and Heuristics for Development of a Multiplex *Escherichia coli* O157:H7 PCR Assay

FRANK R. BURNS and Jim Bono
DuPont, Wilmington, DE, USA

Introduction: E. coli O157:H7 is a devastating foodborne pathogen causing many foodborne outbreaks world wide with significant morbidity and mortality. The plasticity of the *E. coli* O157:H7 genome, the inconsistent expression of surface antigens, and the sharing of genetic elements with other non-pathogenic bacteria (many of whom have yet to be characterized) complicates the development of a reliable and specific PCR assays for this organism.

Purpose: The purpose was to develop a PCR based assay for *E. coli* O157:H7 that would be inclusive of all *E. coli* O157:H7 isolates, including all known atypical isolates (e.g., rough, sorbitol-fermenting, telluride-sensitive isolates), while maintaining sufficient specificity for use as a reliable screening tool.

Methods: The genetic evolutionary model for *E. coli* O157:H7 was used to determine, broadly that specific targets from both the O55:H7 parental lineage as well as the O157 encoding gene cluster (acquired as the last step in the evolution of this pathogen) should be employed. Bioinformatics and heuristic approaches were undertaken to identify multiple candidates from both these lineage elements. Targets were then tested against over 250 *E. coli* O157:H7 isolates from both the DuPont and USDA Meat Animal Research Center strain collections. In addition these targets were tested against 350 ground beef enrichments to evaluate potential for non-specific cross reaction with genetically uncharacterized bacteria.

Results: Of the eight targets evaluated, two failed at being completely inclusive; both missed isolates of the sorbitol-fermenting, telluride-sensitive lineage responsible for several European outbreaks. Of the remaining six targets, three had acceptably low rates of non specific cross reactivity, two of these from the O157 gene cluster. The first target chosen has greater specificity utilizing two single nucleotide polymorphisms and is non-reactive with most O157-bearing bacteria that are not O157:H7. The second target chosen was from the O55:H7 parental strain, and exploits de novo nucleotides flanking a truncated and disabled insertion sequence.

Significance: An understanding of *E. coli* O157:H7 evolution, coupled with bioinformatics and heuristic approaches to dealing with largely uncharacterized background flora has allowed the development of a highly specific and inclusive multiplex PCR assay without the complications and failure modes introduced by upfront antibody based selection.

T1-03 A Novel Colorimetric Screening Assay for *Escherichia coli* O157:H7 In Raw Ground Beef and Trim Utilizing Simultaneous Capture and *In Situ* Labeling during Automated Re-circulating IMS

Nicole Prentice, John Murray, Paul M. Benton, Katarzyna Brzegowa, Brooke V. Houston, Ian Sheldrake, Michael F. Scott, Christine Aleski and ADRIAN PARTON
MATRIX MicroScience Ltd. Cambridgeshire, United Kingdom

Introduction: E. coli O157:H7 is notorious because of its low infective dose and the severity of the disease in vulnerable individuals. Raw ground beef has been implicated as the source of *E. coli* O157:H7 in a significant number of food-borne disease outbreaks and food safety recalls during the past two decades. Detecting the presence of this STEC at low levels in raw beef presents significant challenges to both the food industry and regulatory agencies.

Purpose: This study describes the development and validation of a robust screening assay for *E. coli* O157:H7 in raw fresh beef samples based on *in situ* labeling of the target STEC captured during re-circulating IMS. The method is applicable to analyzing both ground beef and trim samples for the presence of *E. coli* O157:H7 where initial pathogen levels are in the 1—5 CFU per sample range.

Technical

Methods: Fresh ground beef patties or trim (25—375 g) were weighed into sterile stomacher bags; each sample received a low level inoculum (1—5 CFU) of cold stressed nutrient starved *E. coli* O157:H7 to mimic very low-level contamination. Samples were diluted 1:9 with pre-warmed Buffered Peptone Water and samples were briefly hand mixed prior to static pre-enrichment (7—16 h) at 42°C. Pathatrix re-circulating IMS was used to capture *E. coli* O157 from pre-enrichment aliquots and simultaneous specific labeling of target was achieved with an immunoconjugate having the same specificity as the capture ligand. Following automated wash and elution steps, Pathatrix beads were immersed in an enzyme substrate. Development of a blue color was indicative of the presence of *E. coli* O157:H7 in the sample. A proportion of the recovered Pathatrix beads were also streak plated onto selective agar plates for confirmation.

Results: Reliable detection of initial low level *E. coli* O157:H7 in raw ground beef and trim samples was achieved using the labeling of target cells in conjunction with the automated RIMS capture and washing procedure. No natural *E. coli* O157:H7 in uninoculated raw beef samples was encountered. Based on comparing the screening assay result with target isolation on selective agar plates no false positive or false negative results were obtained. Recovery of *E. coli* O157:H7 colonies on selective agar plates confirmed the colorimetric screening assay result in all cases.

Significance: The *E. coli* O157:H7 screening method described in this study offers a flexible and cost effective approach to identifying the presence of this STEC in beef samples. It offers next day results and reliability. User handling is minimized as target capture, labeling and washing steps are an integral part of the automated RIMS procedure.

T1-04 Sensitive and Rapid Detection of *Escherichia coli* O157:H7 in Food and Water

PEIXUAN ZHU, Shuhong Li, Platte T. Amstutz, Daniel R. Shelton and Cha-Mei Tang
Creatv MicroTech, Inc., Potomac, MD, USA

Introduction: FDA and USDA follow a zero tolerance policy for *Escherichia coli* O157:H7 contamination in food. Sensitive and rapid methods are needed to determine the presence of *E. coli* O157:H7 in food and water.

Purpose: An assay was developed to detect *E. coli* O157:H7 in food and water at low concentrations. The short enrichment time enabled rapid results.

Methods: The assay is based on Immunomagnetic Separation- Fluorescence Immunoassay (IMS-FIA). Antibody-coated magnetic beads were added to test samples to specifically capture *E. coli* O157:H7 cells. Bead-bound cells were recognized by an anti-O157:H7 Cy5-labeled polyclonal antibody to form an immuno-sandwich. Unbound dye was removed by washing. The fluorescence signal from the immuno-sandwich was compared to the fluorescence of a modified assay, where the dye-labeled antibody was dissociated from the magnetic beads. The fluorescence signals were measured using an ultra sensitive Signalyte™-II spectrofluorometer. Ten-fold serial dilutions of *E. coli* O157:H7 were tested.

Results: The results showed that the fluorescence intensity from the supernatant of the modified assay was highly correlated with the original *E. coli* O157:H7 cell concentrations. Limit of detection (LOD) threshold was established based on the average fluorescence intensity, plus three times the standard deviation of the negative controls. Fluorescence signals of the immuno-sandwich assay were more variable and less sensitive than the modified assay. LOD for *E. coli* O157:H7 of the modified assay was 10 CFU/ml in water, over two log better than the original assay. Comparable assays conducted for *E. coli* O157:H7 in ground beef were a factor of 10 less sensitive. Results were consistently reproduced monthly using the same reagents, which demonstrated a shelf life in excess of six months.

Significance: E. coli O157:H7 can be rapidly and consistently detected at low concentrations in food and water using the Signalyte™-II spectrofluorometer.

T1-05 Identification of Shiga Toxin-producing *Escherichia coli* on DNA Microarrays by Using a Novel Photoinduced Signal Amplification Method

BEATRIZ QUIÑONES, Ronald P. Haff, Amber W. Taylor and Erica D. Dawson
USDA-ARS, Western Regional Research Center, Produce Safety and Microbiology Research Unit, Albany, CA, USA

Introduction: Shiga toxin-producing *Escherichia coli*, with *E. coli* O157:H7 as a common serotype, is a leading cause of human gastrointestinal illnesses. The rise in foodborne-related outbreaks of pathogenic *E. coli* from food and waterborne sources has heightened the importance of developing improved methods to rapidly detect and characterize virulent strains. Therefore, the development of effective detection methods that identify the presence and distribution of *E. coli* O157 are needed with sufficient sensitivity, cost-effectiveness and suitability for routine testing.

Purpose: To evaluate better methods to rapidly detect and genotype *E. coli* O157 virulent strains, the present study explored the use of photopolymerization, a colorimetric and photoinduced signal amplification detection method, for pathogen identification on DNA microarrays.

Methods: A DNA oligonucleotide microarray was constructed with 30-mer oligonucleotide probes targeting intimin adherence protein (eae), perosamine synthetase (per), Shiga toxin 1 (*stx1*), and Shiga toxin 2 (*stx2*) in pathogenic *E. coli*. For detection on the microarray of these pathogen genetic markers, the microarray was hybridized with biotin-labeled PCR products and then labeled with a streptavidin-conjugated photoinitiator and a solution containing monomers. After irradiating at a specific wavelength, the formation of a colorless, cross-linked hydrogel polymer allowed the colorimetric detection of positive signals on the microarray.

Results: Analysis of the microarray data demonstrated polymer formation for only probes targeting virulence genes present in the tested *E. coli* O157 reference strains. Positive hybridization signals had average signal-to-noise ratio values above 10, and signal-to-noise ratio values below 1.5 were determined for the same virulence probes in a non-pathogenic *E. coli* strain. The quantification analysis of positive signals demonstrated that the detection sensitivity by using photopolymerization was approximately 100—1000 CFU/ml.

Significance: The use of DNA microarrays in combination with photopolymerization allowed the rapid and cost-effective identification of *E. coli* O157, compared to established methods that are more expensive and require several days for strain detection.

T1-06 Rapid Identification of *Listeria* Species: Comparison of a Real-time PCR Assay Versus Biochemical Galleries

Nicolas Desroche, Jean Guzzo and PATRICE ARBAULT
BioAdvantage Consulting, Food Safety and Analytical Methods, Orliénas, France

Introduction: Conventional methods for the detection and enumeration of *Listeria* sp. require biochemical tests to differentiate amongst the 6 species of *Listeria*. These tests are performed from colonies and rely on catalase activity, hemolytic characteristic and sugar fermentation. Rapid biochemical kits such as API® *Listeria* and Microbact™ *Listeria* are available for carrying out these biochemical test panels. Recently, a new automated real-time PCR method has been developed for the rapid identification of *Listeria* species (GeneDisc *Listeria* ID).

Purpose: The objective of this study was to evaluate the performances of this PCR assay and compare them to commercial biochemical galleries: API® *Listeria* and Microbact™ *Listeria*.

Methods: One hundred sixty-six (166) collection strains of the 6 main *Listeria* species were isolated onto agar plates. Colonies were picked up from the plates and then identified with the 2 biochemical galleries and the PCR-based assay, following the respective kit instructions. The *Listeria* identification results were compared. Any strain giving discordant results among the 3 assays was further analyzed through 16S DNA sequencing.

Results: The GeneDisc *Listeria* ID identified correctly all *Listeria* strains (166/166) while both biochemical galleries showed some erroneous results (5/166). The most important error was observed with the misidentification of 3 out of 11 *L. ivanovii* strains. No misidentification was observed for the species *monocytogenes* and *innocua*, using the biochemical galleries. Furthermore, the time to result was significantly lower with the GeneDisc ID *Listeria* (less than 1 hour) compared to the biochemical galleries (approximately 24 hours).

Significance: The automated PCR-based identification method seems a promising tool for the routine and rapid identification of *Listeria* isolates in food and clinical laboratories.

T1-07 Detection of *Listeria* spp. from Pooled Environmental Swab and Food Samples within 24 Hours Using Pathatrix Automated Re-circulating IMS Linked to Real-time PCR

John Murray, Nicole Prentice, Katarzyna Brzegowa, Paul M. Benton, Brooke V. Houston, Ian Sheldrake, Michael F. Scott, Marcie Van Wart and ADRIAN PARTON
MATRIX MicroScience Inc., Golden, CO, USA

Introduction: Listeriosis is an important public health problem that produces high mortality rates. Serious infections occur mainly in pregnant women, neonates, immuno-compromised and elderly individuals and result primarily from eating food contaminated with the bacterium *Listeria monocytogenes*. *L. monocytogenes* has a ubiquitous distribution and possesses properties that increase the risk of its persistence and dissemination in food processing facilities. Like other members of the genus, *L. monocytogenes* is psychrophilic and can grow at refrigeration temperatures of 1°C. The potential implications of *L. monocytogenes* being present in ready-to-eat foods which support growth has led to a zero tolerance approach from both the FDA and USDA-FSIS. RTE Food considered to be high risk for *Listeria monocytogenes* include those with pH > 4.4 and a_w > 0.92.

Purpose: The aim of this study was to assess the feasibility of using an enhanced enrichment protocol, recirculating IMS and real time PCR to develop a method which is capable of detecting the presence of *Listeria* contamination, including *L. monocytogenes*, at low level in pooled food and environmental swab samples within 24 hours.

Methods: A range of foods and environmental contact swabs were inoculated at low level (1-10 CFU per sample) with single *Listeria* species from a representative panel spanning the *Listeria* genus and including *L. monocytogenes*. All samples were diluted in pre-warmed demi Fraser and enriched statically at 37°C for 23 hours prior to pooling aliquots from inoculated samples with uninoculated samples in the ratio 1:4 or 1:9. Pooled samples were analyzed using Pathatrix Auto. Target capture employed IMS particles with proven inclusivity for all *Listeria* species. A 5-minute mechanical lysis step was used to release DNA from captured target cells prior to real time PCR detection. Selective agar plating was used to confirm the PCR results.

Results: The range of *Listeria* spp. were successfully isolated from pooled food samples down to 0.004 CFU/g and environmental contact swabs (1 CFU in 100 cm^2) using recirculating IMS. Detection was achieved using real time PCR and this was confirmed by isolation of target *Listeria* on selective agar plating.

Significance: The Pathatrix *Listeria* pooling method described allows food production facilities to increase sample throughput during routine *Listeria* monitoring of both food and environmental contact swabs. The method has the potential to enhance HACCP and pathogen testing regimes and can be employed to validate hygiene practices and sanitizing procedures aimed at reducing the incidence and spread of *Listeriae* in the food processing environment.

T1-08 Combined Thin Agar Layer and Centrifugation-plating Method for Enumeration of Injured *Salmonella*

Sangha Han and JULIAN M. COX
University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Introduction: Detection of sub-lethally injured *Salmonella* in foods is important because the organism may repair and ultimately cause illness. The thin agar layer (TAL) technique applies a non-selective agar to a traditional selective plating medium, which allows for the resuscitation and enumeration of injured cells. The combination of TAL with a centrifugation-plating technique (CP), potentially with a sample pre-treatment step, could allow for the enumeration of both vegetative and injured cells from food matrices.

Purpose: The aim of this study was to examine the efficacy of a combined TAL-CP method for the enumeration of acid and sodium chloride-injured *Salmonella*.

Methods: Strains of five *Salmonella* serotypes were injured by incubation at 5°C for 10 days in a nutrient broth containing NaCl (13.5%) and lactic acid (1%). Following injury, each strain was inoculated into chicken rinse, which was then centrifuged after treatment with protease A and Tween 80. The resuspended cells were enumerated onto tryptone soy agar (TSA), xylose lysine desoxycholate (XLD) and TAL (14 ml of TSA on 14ml of XLD). Counts on each medium were compared to determine recovery rates of injured *Salmonella*.

Results: Sublethally injured *Salmonella* cells were recovered well using CP. There was no statistically significant difference ($P > 0.05$) between TSA and TAL for enumeration of injured *Salmonella*. Both these media recovered higher numbers of injured *Salmonella* than XLD ($P < 0.05$). All three media showed no difference ($P < 0.05$) when inoculated with uninjured cells. Recovery of sub-lethally injured cells of four serotypes was about 90% after treatment with Tween 80 and protease, though recovery of *Salmonella* Infantis was only about 78%.

Significance: This study suggests that the TAL-CP method is effective for enumeration of *Salmonella*, including injured cells.

T1-09 Comparison of Vegetable and Animal Peptone-based Culture Media for Detection of *Salmonella* in Poultry

Rakesh Koorapati and JULIAN M. COX
University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Introduction: Peptones, polypeptides obtained from the hydrolysis of proteins, are primary protein sources in culture media; traditionally they are derived from animal sources. However, due to religious beliefs and concerns over transmissible spongiform encephalopathies (TSE), especially the bovine form (BSE), vegetable peptones have become an increasingly important alternative.

Purpose: The aim of this study was to compare the performance between vegetable peptone VP-based and conventional meat peptone (MP)-based culture media, using *Salmonella* as the analyte and poultry products as food matrices.

Methods: Growth of three strains of *Salmonella*, in fully vegetative or injured state, was determined in Buffered Peptone Water (BPW), Rappaport-Vassiliadis Soy (RVS) broth and Muller Kauffmann Tetrathionate novobiocin (MKTTn), as well as on a range of plating media,

Technical

from two commercial manufacturers, one supplying VP media and the other, a market-leader, supplying MP media. Naturally and artificially inoculated chicken samples (n = 80) were screened for the presence of *Salmonella* spp. using an ecometric streaking technique, in order to compare the performance between VP- and MP-based culture media. Plating media were formulated from individual ingredients with peptone as a solitary variable ingredient to assess specifically the performance of the peptones.

Results: No significant differences were observed in the growth of the three *Salmonella* serovars in broth or on solid culture media ($P < 0.05$). Absolute growth indices (AGIs) calculated from ecometric streaking on VP and MP plating media — XLD, Hektoen Enteric, Bismuth Sulphite and Brilliant Green agars — showed no significant difference in productivity rates for *Salmonella* and specificity, in terms of growth of the background microflora. A < 0.7 log CFU/mL difference in recovery rates was observed on media formulated with the same ingredients except VP or MP.

Significance: VP-based media have high specificity, productivity and perform similarly to MP media. Since vegetable peptone media are free from religious and BSE concerns, they serve as suitable replacements for animal peptone-based culture media.

T1-10 Development of Multi-parametric Tools for the Detection and Identification of Sporeforming Bacteria in the Food Chain

PATRICE CHABLAIN, Florence Postollec, Stephane Bonilla, Daniele Sohier and Sylvie Hallier-Soulier
Pall GeneSystems, R&D, Bruz, France

Introduction: Aerobic and anaerobic Gram positive sporeformers show a wide range of phenotypic and genotypic characteristics. These organisms are ubiquitous in the environment and have the ability to form endospores which enable them to survive treatments commonly used in food processes. User-friendly tools were developed for the rapid detection and identification of major sporeformers implicated in food spoilage and food poisoning outbreaks.

Purpose: A multi-parametric PCR-based method has been developed for the detection and identification of the most prevalent genera and species of sporeformers. The method performances were evaluated with both artificially and naturally contaminated samples.

Methods: A 28-h enrichment protocol has been defined to allow germination and growth of aerobic and anaerobic targeted strains. Chelex based extraction and developed GeneDiscs plates allow, at once, multiplex detection of several sporeformers commonly found in food industries. Inclusivity and exclusivity study has been tested on more than 220 collected strains, mostly composed of food isolates.

Results: Specificity study showed few discrepancy due to the taxonomic evolution. The developed biochip yields detection limit lower than 1 spore *B. cereus*/gram while standard detection method on agar yields more than 8 spore/g. PCR performances have been validated with both artificially (n = 60) and naturally contaminated food according to ISO 16140. Sporeformers' vegetative cells and spores present in naturally contaminated samples have been isolated without and with heat treatment, colonies have been 16SrRNA sequenced and identified according to the actual taxonomy in order to confirm the biochip molecular response. *Bacillus* genus was systematically confirmed while *Clostridium* detection could not always been confirmed on standard RCM medium due to difficult recovery on agar medium.

Significance: The lack of diagnosis tools is the major obstacle to control sporeformers contamination. The development of ready-to-use multiple parametric tools enable detection, identification and traceability of 5 genera and 9 species in less than 24 h rather than quantification of a few species. Sporeformers biodiversity and prevalence observed in raw materials or ingredients was not systematically correlated to the observed spoilage of the final product. Nevertheless, molecular detection offers the advantage to track mesophilic or thermophilic anaerobes which are extremely difficult to study with conventional methods.

T1-11 Differentiation and Speciation of Vibrios by PCR of 16S-23S rRNA Intergenic Spacer Region

MARIA HOFFMANN, Eric W. Brown, Peter C. Feng, Markus Fischer and Steven R. Monday
US Food and Drug Administration, CFSAN, College Park, MD, USA

Introduction: The genus *Vibrio* is comprised of 74 species. Among vibrios, *V. parahaemolyticus* and *V. vulnificus* have a greater potential to establish infection in humans, generally following consumption of raw or undercooked seafood. *Vibrio* infections are becoming more common worldwide. The United States Centers for Disease Control and Prevention (CDC) estimates that 8,028 *Vibrio* infections and 57 deaths occur annually in the United States. Of these infections, 5,218 are foodborne in origin. Consequently, it is important to have a method that can reliably identify *Vibrio* isolates.

Purpose: The purpose of this study was to design a PCR-based method using 16S-23S rRNA Intergenic Spacer Region (ISR) to reliably and efficiently differentiate numerous *Vibrio* species.

Methods: 16S rRNA gene sequencing was accomplished for all strains used in this study. *Vibrio*-specific PCR primers were designed to amplify 16S-23S rRNA ISR targets. ISR amplicons were resolved by capillary gel electrophoresis. The resulting patterns were analyzed using BioNumerics fingerprinting software.

Results: The data showed that this method easily discriminated the 69 *Vibrio* type strains at the species level. Furthermore, testing of 36 strains each of *V. parahaemolyticus* and *V. vulnificus*, isolated from numerous geographical locations demonstrated distinct intra-species ISR-typing patterns, making this technique equally useful for intraspecies differentiation, as well. Finally, vibrios isolated from sponges were characterized using16S rRNA gene sequencing and ISR-typing. The resulting data suggested both a panmictic population structure among geographically separate vibrios and evidence that sponge hosts may also be a source for speciation.

Significance: This fast, reliable and efficient ISR-typing system, which takes advantage of capillary electrophoresis technology, has been proven to be effective for identification of *Vibrio* species at both the species and subspecies level, suggesting it should also be useful for epidemiological investigations, as well.

T1-12 Detection of Low Numbers of Only Viable *Enterobacteriaceae* in Inoculated Pasteurized Milk Using Direct PCR after Ethidium Bromide Monoazide Treatment

FRANK SCHLITT-DITTRICH, Takashi Soejima, Tomoko Yaeshima and Keji Iwatsuki
Morinaga Milk Industry Co., Ltd., Biological Function Research Dept., Kanagawa-Pref., Zama-City, Japan

Introduction: The microbial analysis of milk by culturing methods is time consuming and laborious. Application of PCR in milk examination can significantly reduce the analysis time but various milk components inhibit the DNA-amplification. Furthermore, PCR does not distinguish between viable and dead bacterial cells.

Purpose: The purpose of this study was to develop a direct PCR (DPCR) assay that enabled the detection of only viable *Enterobacteriaceae* in milk after Ethidium Bromide Monoazide (EMA) treatment without DNA-isolation and purification.

Methods: Aliquots of 22 ml of pasteurized milk were single-strain inoculated with up to 7-log CFU of viable and heat-killed bacteria, respectively. Prior to DPCR (targeting an approximately 2490 bp DNA-fragment of the 16S to 23S rRNA gene) the sample preparation

Technical

and treatment were conducted including the following steps: pelletization, re-suspension and incubation in Brain Heart Infusion broth supplemented with proteinase for 3 h at 37°C, exposure to EMA and visible light, washing, and appropriate dilution in sterile water. The amplified DNA-fragment was identified by its melting point and visualized after gel electrophoresis and SYBRGold staining.

Results: Viable cells of 13 *Enterobacteriaceae* strains (members of 13 different genera) were detected in milk at inoculation levels of 14 ± 8 CFU (n = 26) in duplicates within 8 h of analysis time. Samples initially inoculated with up to 7 log CFU of dead *Cronobacter muytjensii* ATCC 51329 (formerly *Enterobacter sakazakii*) cells and viable Gram-positive bacteria cells (*Bacillus cereus* and *Staphylococcus aureus*) were tested negative with EMA-DPCR.

Significance: The analysis time was reduced to < 8 h which was significantly shorter compared with culturing methods which can take p to several days. We could detect < 10 viable *Enterobacteriaceae* cells in 10 ml of milk.

T2-01 Variation in Desiccation Tolerance among *Salmonella* Strains

REGINA C. WHITEMARSH, Aaron M. Gnas and Charles W. Kaspar
University of Wisconsin-Madison, Bacteriology, Madison, WI, USA

Introduction: *Salmonella* outbreaks associated with low-moisture foods, like peanut butter, powdered milk, and almonds have raised questions about the survival of this pathogen in ingredients and products that have traditionally been thought to inhibit growth and survival.

Purpose: A greater understanding of *Salmonella* persistence in and response to desiccation conditions is critical to developing and implementing intervention strategies.

Methods: Four strains of *Salmonella enterica* were used in this study; two strains (M-09-0001A, peanut butter outbreak isolate and LT2) of serovar Typhimurium, and one strain of serovars Tennessee and Agona. The ability of the strains to survive at low a_w (a_w=0.92) was examined using NaCl and sucrose to adjust the water activity. The influence of storage temperature (15 and 30°C) was also investigated. Survival was monitored by testing samples at time zero and weekly thereafter for the number of CFU/ml using trypticase soy agar.

Results: Survival of the *Salmonella* strains differed in low-a_w solutions prepared with sucrose and NaCl with greater survival in the NaCl-prepared solutions. In general, Typhimurium strain LT2 had the poorest survival. Incubation at 15°C significantly increased the survival of all six strains compared to survival at 30°C with less than a 1-log decrease in CFU/ml after 21 days of incubation at 15°C. *Salmonella* serovars and strains did not differ significantly in survival at 30°C with the exception of strain LT2 and the Enteritidis strain E40. Heat shock of *Salmonella* for an hour at 45°C prior to inoculation of low-a_w solutions enhanced survival.

Significance: These findings are significant because they demonstrate significant survival of *Salmonella* during desiccation. Low temperature or prior exposure to stress (heat shock) enhanced survival in low-a_w conditions. This information should be helpful in the development of effective intervention practices.

T2-02 Effect of Crust Freezing on the Survival of *Escherichia coli* and *Salmonella* Typhimurium in Raw Poultry Products

BYRON D. CHAVES-ELIZONDO, Inyee Y. Han and Paul L. Dawson
Clemson University, Food Science and Human Nutrition, Clemson, SC, USA

Introduction: *Escherichia coli* and *Salmonella* spp. are ubiquitous to the poultry production environment and hence their transmission to poultry products is a concern. Industry has widely used freezing as a strategy to halt pathogen growth and more recently, crust freezing has been claimed to improve operations, quality, and even safety of poultry products.

Purpose: To determine the effect of crust freezing and the presence of skin on the survival of *E. coli* and *S.* Typhimurium in raw poultry products.

Methods: Ampicillin-resistant *E. coli* JM 109 and nalidixic acid-resistant *S.* Typhimurium were used in the experiments. A set of cultures was subjected to cold-shock stress by storage at 4°C for 10 days. Commercial chicken breasts without skin and chicken thighs with skin were inoculated with each bacterium in separate experiments being either cold-shocked or non-cold-shocked prior to inoculation. Samples were crust frozen at -85°C for 20 min or completely frozen at -85°C for 60 min. *E. coli* and *S.* Typhimurium were recovered on appropriate selective and non selective media containing the corresponding antibiotic. Log reductions and injury extent were calculated and treatments were compared using ANOVA.

Results: No significant differences were observed in the reduction of cold-shocked or non-cold-shocked bacteria on products that were crust- or completely frozen, with or without skin. Reductions tended to be greater for *S.* Typhimurium than for *E. coli*, although none of the final reductions were greater than the desired target (1 log). Bacterial cell injury was not significantly different among any of the treatments.

Significance: The treatments did not show practical significance for initial reduction of these pathogens; thus freezing nor crust freezing should not be considered strategies for the reduction of these pathogens on poultry. However, additional studies are underway to compare crust freezing to refrigeration for inhibition of bacteria on raw poultry products.

T2-03 Characterization of the *Listeria monocytogenes* Transcriptional Response to Synergistic Growth Inhibition by Potassium Lactate and Sodium Diacetate

MATT STASIEWICZ, Teresa M. Bergholz and Martin Wiedmann
Cornell University, Food Science, Ithaca, NY, USA

Introduction: Combinations of organic acids are frequently used in the Ready-to-Eat meat industry to slow the growth of *Listeria monocytogenes*, a psychrotolerant foodborne pathogen, in refrigerated products. Although it has been demonstrated that the combination of diacetate and lactate causes greater than additive, i.e., synergistic, growth inhibition, the mechanisms of observed synergistic inhibition as well as how *Listeria* overcomes the inhibitory stress are not well understood.

Purpose: To use transcriptional profiling to investigate the mechanistic basis for diacetate and lactate synergy and identify genetic pathways *L. monocytogenes* uses to overcome their growth inhibitory effects.

Methods: *L. monocytogenes* h7858 and f6854 were exposed to the growth inhibitor treatments (0.14% water phase [w.p.] sodium diacetate, 2% w.p. potassium lactate, the combination of 0.14% diacetate and 2% lactate, or no inhibitors as control) in broth at 7°C for 8 h, and RNA was extracted and competitively hybridized to microarrays. ANOVA comparing the four treatments for each strain was used to identify differentially expressed genes ($P < 0.05$, fold-change > 1.5).

Results: The transcriptional response included 593 and 282 genes for h7858 and f6854, respectively, with a significant interaction between lactate and diacetate treatments; of these genes, 90 were significant in both strains. Cluster analysis showed the majority of the significant genes had either increased or decreased expression in both diacetate and lactate treatments compared to control and those

Technical

differences were magnified in the combination treatment. Gene Set Enrichment Analysis found some responses shared between strains, e.g., strong induction of fermentation genes particularly oxidoreductases and alcohol dehydrogenases, yet only 3 of 14 total role categories enriched were shared, suggesting the strains have very different transcriptional responses to these inhibitors.

Significance: These array data identify groups of genes synergistically affected by diacetate and lactate treatment and suggest physiological mechanisms used by *L. monocytogenes* to overcome the organic acid stress.

T2-04 High Pressure Inactivation of Noroviruses in Vegetables and Fruits

FANGFEI LOU, Hudaa Neetoo, Haiqiang Chen and Jianrong Li
The Ohio State University, Dept. of Food Science and Technology, Columbus, OH, USA

Introduction: Fresh produce is often a high-risk food because it can become contaminated at pre-harvest and post-harvest stages and it undergoes minimal or no processing. Disease surveillance shows that human norovirus ranks as the primary cause of fresh produce associated disease outbreaks (40%). Therefore, there is an urgent need to develop non-thermal processing technologies to inactivate foodborne enteric viruses in vegetables and fruits.

Purpose: The objective of this study was to determine the effectiveness of high pressure processing (HPP) to inactivate noroviruses in an aqueous medium, vegetables and fruits.

Methods: The cultivable murine norovirus was inoculated into Dulbecco's Modified Eagle Medium (DMEM), iceberg lettuce, fresh-cut strawberry and strawberry puree to a final concentration of approximately 10^7 PFU/ml or 10^7 PFU/g. The samples were treated at pressures ranging from 200 to 450 MPa for 2 min at either 4 or 20°C. The virus survivors were quantified by viral plaque assay and the inactivation kinetics of norovirus was determined.

Results: This study systematically investigated the inactivation of noroviruses in fresh produce by HPP. Murine norovirus was effectively inactivated by HPP in aqueous medium, lettuce, strawberry, and fruit puree. The pressure, pH and temperature all affected the inactivation of murine norovirus. Norovirus was more effectively inactivated at 4°C than at 20°C. Norovirus was also more sensitive to high pressure at pH 7.0 than at pH 4.0. Approximately 5 log PFU/g (or PFU/ml) reduction was achieved in all food items when pressurized at 400 MPa for 2 min at 4°C. High pressure affected the texture of lettuce and strawberries but not of strawberry puree, and did not affect the other sensory qualities such as color, aroma and freshness.

Significance: HPP effectively inactivates norovirus in fresh produce and can thus provide a novel intervention for processing fruits intended for frozen storage and fruit products such as puree, sauce, and juice.

T2-05 Phenotypic and Genotypic Characterization of Antimicrobial Resistance in *Salmonella* Serotypes Isolated from Retail Meats in Canada

MUEEN ASLAM, Valerie Bohaychuk, Gary Gensler, Richard Reid-Smith, Brent Avery and Patrick Boerlin
Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe, AB, Canada

Introduction: *Salmonella* spp. are one of the most important foodborne pathogens causing severe gastroenteritis in human. Meat is considered to be one of the main sources for the transmission of *Salmonella* spp. to humans, and the occurrence of antimicrobial resistance (AMR) in *Salmonella* poses a serious public health risk.

Purpose: The objective of this study was to perform phenotypic and genotypic analysis of AMR in *Salmonella* serotypes recovered from retail meats purchased in Alberta, Canada.

Methods: A total of 564 samples comprising chicken (206), beef (134), pork (133) and turkey (91) meats were collected. *Salmonella* isolates were recovered using standard cultural methods. Isolates were confirmed using biochemical tests and PCR employing *Salmonella* specific primer sets. Serotyping was performed with standard method. A total of 326 *Salmonella* isolates were analyzed. The AMR was determined using an automated microdilution method (Sensititre® system) using Gram Negative Plates. Results were interpreted according to the Clinical Laboratory Standard Institute guidelines. PCR was used to detect resistance genes including *tet*A,B,C, *sul1,2,3*, *blaCMY-2, blaSHV, blaTEM, blaPSE* and *aphA1, aphA2, aadB, strA/B.*

Results: All beef samples were negative for *Salmonella*. The most common *Salmonella* serotypes identified were Heidelberg (27%), Hadar (25%) and Kentucky (14%) in chicken and turkey. AMR differed among the serotypes; 100% isolates of serovar Anatum were resistant to seven antimicrobials, including category I antimicrobials (high importance to human health by Health Canada categorization). Multiple antimicrobial resistances were also common in serovars Heidelberg, Kentucky, Infantis, Typhimurium, Typhimurium var. Copenhagen, and Kiambu. Serovars Enteritidis, Johannesburg, Montevideo, Reading, Thompson and Tennessee were sensitive to tested antimicrobials. The presence of resistance genes generally correlated with resistance phenotypes and their prevalence differed among serotypes. The *tet*C and *sul3* genes were not found in any serotype. The *blaCMY-2* gene was found in serovars Anatum (100%), Kentucky (50%), Heidelberg (30%), Infantis (30%)and Kiambu (25%). The *blaPSE* and *blaTEM* genes were found in serovars Typhimurium var. Copenhagen (40%) and Heidelberg (40%), respectively. Serovar Rissen was positive for *sul1* and *tet*B genes without phenotypically resistant to these antimicrobials.

Significance: This study provides useful information for regulatory authorities and data may be used in risk assessment studies.

T2-06 Comparison of the rfb Cluster in 16 Rare *Salmonella* Serotypes

MATTHEW L. RANIERI, Andrea Moreno Switt, Henk C. den Bakker, Martin Wiedmann, Lavorka Degoricija, Craig A. Cummings, Elena Bolchacova, Greg Govoni and Manohar R. Furtado
Cornell University, Food Science, Ithaca, NY, USA

Introduction: *Salmonella* causes an estimated 1.4 million human cases per year by foodborne transmission in the United States, and is one of the leading causes of gastroenteritis worldwide. In support of epidemiological investigations, the Kauffman-White immunologic classification scheme, which is based on somatic (O) and flagellar (H) antigens, is commonly used. To eliminate the need for hundreds of antisera, molecular serotyping approaches offer an alternative to traditional methods, and have been investigated with respect to *Salmonella* serotypes commonly associated with foodborne illness.

Purpose: The purpose of this study was to analyze the rfb locus, which encodes for the nucleotide sugar biosynthesis pathways and transferases necessary for assembly of lipopolysaccharides that produce the O-antigen, for 16 less common human disease associated *S. enterica* subsp. enterica serotypes.

Methods: Sixteen *Salmonella* isolates representing serovars (serogroup) Adelaide (O), Alachua (O), Baildon (D2), Gaminara (I), Give (E1), Hvittingfoss (I), Inverness (P), Johannesburg (R), Minnesota (L), Mississippi (G), Montevideo (C1), Rubislaw (F), Senftenberg (E4), Uganda (E1), Urbana (N), and Wandsworth (Q) were sequenced by the SOLiDTM next generation sequencing system. By *de novo* assembly, the rfb locus for each strain was identified, and individual genes were putatively identified using the Rapid Annotation using Subsystem Technology (P-RAST).

Technical

Results: Within *Salmonella* serogroups or across serogroups sharing a common antigenic factor, there was a high degree of similarity, especially with genes related to sugar biosynthesis. However, comparisons among serogroups revealed considerably less homology in gene content. One common gene, identified as a flippase, was found in the majority of serotypes, and encodes for transmembrane proteins responsible for transferring O-units across the cytoplasmic membrane.

Significance: The identification of conserved genes across rare serotypes indicates potential for the application of molecular serotyping to the serogroups investigated in this study. Identification of O-antigens with molecular methods will provide an alternative to traditional serotyping, which is labor intensive, costly, and difficult to standardize.

T2-07 Sequences in the *comK* Prophage Junction Fragments Cluster *Listeria monocytogenes* Isolates of Epidemic Clones II, III and the 2008 Canadian Outbreak into Subclones That are Unique to Individual Meat and Poultry Processing Plants

MEI LOK, Yi Chen, Bindhu Verghese, Peter Evans, Sophia Kathariou and Stephen Knabel
Penn State University, Dept. of Food Science, University Park, PA, USA

Introduction: Listeria monocytogenes epidemic clones (ECs) II and III have been associated with large multistate outbreaks due to contaminated ready-to-eat (RTE) meat and poultry products in the U.S. The Food Safety and Inspection Service (FSIS) of the United States Dept. of Agriculture (USDA)'s RTE meat and poultry products monitoring program has generated a large number of *L. monocytogenes* isolates from meat and poultry processing facilities throughout the U.S. Based on pulsed-field gel electrophoresis profiles a small fraction of these isolates appeared to be ECII. Isolates with ECII profiles were also isolated from two turkey processing plants in the U.S. (Eifert et al., 2005). However, it is not known whether specific subclones of ECII and III, and the 2008 Canadian outbreak clone were associated with individual processing plants in the U.S.

Purpose: 1) To confirm that the isolates from FSIS and Eifert et al. (2005) were ECII, 2) To determine whether or not specific subclones of ECII and ECIII, and the 2008 Canadian outbreak clone were associated with individual plants and 3) To determine if *comK* prophages in ECII and ECIII can spontaneously induce, form phage and are infective or defective.

Methods: ECII PCR, multi-virulence-locus sequence typing (MVLST), comK prophage PCR, comK prophage junction fragment sequencing, attP and attB PCR, phage DNA PCR and plaque assay were performed.

Results: ECII PCR and MVLST confirmed that the isolates from FSIS and Eifert et al. (2005) were ECII. *comK* prophage PCR showed that most ECII isolates produced the same results as strains from the two known ECII outbreaks, except six FSIS isolates. *comK* prophage junction fragment sequencing identified subclones, most of which were unique to individual processing plants. *attP* and *attB* PCR revealed that the *comK* prophages in ECII and III could be spontaneously induced. Phage DNA PCR and plaque assays suggested that ECIII formed defective phages containing *comK* phage DNA.

Significance: Public health agencies can use *comK* prophage junction fragment sequencing, in conjunction with MVLST, to trace subclones of *L. monocytogenes* to identify reservoirs, sources and transmission pathways. This information will allow implementation of more effective intervention strategies for controlling *L. monocytogenes*. Further research is needed to determine whether the genes in the *comK* prophage junction fragments play a role in colonization and persistence of these unique subclones in individual plants, as well as in their transmission to foods and humans.

T2-08 An ABC Transporter Regulates Biofilm Formation by Controlling the Expression and Modification of Cell Surface Proteins in *Listeria monocytogenes*

Xinna Zhu, Weibing Liu, René Lametsch, Frank M. Aarestrup, Chunlei Shi, Qunxin She, XIANMING SHI and Susanne Knøchel
Dept. of Food Science, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China

Introduction: Listeria monocytogenes is a foodborne pathogen that can form biofilms in food processing environments. In a previous study, the lm.G_1771 gene encoding an ABC-transporter permease is involved in negative regulation of biofilm formation in *L. monocytogenes* 4b G by analyzing genetically a Tn917 insertion mutant LM-49 (phenotype: biofilm-forming enhanced).

Purpose: The aim of this study was to reveal the possible mechanism of this ABC-transporter permease controlling biofilm formation in *L. monocytogenes* by characterization of a Δ1771 mutant with lm.G_1771 gene deleted.

Methods: The ability of biofilm formation by the deletion mutant Δ1771 was performed using violet crystal staining assay. The characterization of the Δ1771 mutant was assessed by AI-2 bioluminescence assay, antibiotics assay and Triton X-100 induced autolysis. The differential proteome and gene expression between the wide-type strain and the mutant Δ1771 was compared using two-dimensional (2D) gel electrophoresis with combining mass spectrometry and complementary DNA (cDNA) Microarray, respectively.

Results: The Δ1771 mutant had shown the same enhanced ability of biofilm formation as LM-49. An AI-2 bioluminesence assay demonstrated that the Lm.G_1771 permease did not export the AI-2 signaling molecule, suggesting that the regulation must involve a novel signal transduction pathway. Functional proteomic/genomic analyses identified several differentially expressed proteins/genes in an lm.G_1771 deletion mutant, and these included 15 proteins revealed by 2D protein gel electrophoresis and 48 genes identified from transcriptomic analyses. For the Δ1771 mutant, the differential expression genes that increased in the Δ1771 mutant were related to cell envelope (six genes), stress response (two genes), protein secretion (three genes), transcriptional regulator (three genes), and unknown function (eight genes), and the repressed genes included the dlt operon and many genes with unknown function.

Significance: Our results suggest that the lm.G_1771-mediated signal transduction pathway regulates *L. monocytogenes* biofilm formation by controlling the expression of biofilm-mediating proteins and those involved in their modification.

T2-09 Maximizing Personnel Hygiene, Minimizing Washroom Contamination

John Holah and DEB L. SMITH
Campden BRI, Gloucestershire, United Kingdom

Introduction: Contamination of food by food workers has been identified as an important contributing factor during foodborne illness investigations. Hands must be washed prior to food handling, though the efficacy of such handwashing, and how to prevent the recontamination of the handwash user and the washroom environment during handwashing and drying have been little studied.

Purpose: This study assessed the microbiological efficacy of a novel automated handwash system as compared to a traditional handwash technique and assessed the impact of these techniques on the microbiological contamination of the user and the environment. Similar studies were undertaken for a range of hand drying techniques.

Methods: The efficacy of an automated hand wash system was assessed against a standard UK National Health Service (NHS) handwash protocol in a traditional sink using the methodology of BS EN 1499:1997 (hygienic biocidal handwash test) and 35 volunteers. The generation of large (ballistic) and small (aerosolized) water droplets and their associated microbiological components by these techniques, and their impact on the handwash user and the washroom environment, was measured using moisture indicator paper and microbiological air sampling. Similar droplet and microbial aerosol distribution studies were undertaken for a novel air knife hand drying system, a hot air hand dryer and paper towels using 30 volunteers.

Results: A 30 s standard NHS handwash achieved a 2.42 log reduction which was statistically different from the 2.67 log order reduction achieved by the automated handwash unit (30 s). Both techniques gave rise to microbiological contamination of the environment and user (including areas of the sleeves and body likely to come into contact with food), though the automated hand wash facility gave rise to much lower levels than the traditional sink. All hand drying techniques generated insignificant microbial aerosols though water droplet contamination of the environment was significantly greater for the air dryers than for paper towels.

Significance: The automated hand wash facility demonstrated a statistically significant improvement in hand wash efficacy over the UK approved NHS manual hand wash. Recontamination of the user and the washroom environment can be minimized by the hygienic design of automated handwash facilities and the correct siting of hand drying equipment.

T2-10 Developing a Fish Starter Culture Fermentation with a Local Nisin-producing Bacterium for Application in Small-scale Artisan Fishery Production in Senegal

MICHEL B. DIOP, Jacqueline Destain, Maïmouna Cisse, Ababacar S. Ndoye and Philippe Thonart
Ministère des Mines, de l'Industrie, de la Transformation Alimentaire des Produits Agricoles et des PME, Direction de la transformation alimentaire, Dakar, Senegal

Introduction: In Senegal, fish is the main animal protein source for populations. Traditional fish production, known to have some inherent food safety problems due to the high susceptibility of seafood to bacterial spoilage, is predominant because of the lack of financial resources that hamper the development of industrial preservation technologies.

Purpose: The safety problems are particularly acute in traditionally fermented fish products in which the catches, generally handled for many hours at ambient temperature, are preserved only by adding salt (NaCl) and sun drying.

Methods: Spontaneous fermentation assayed at 30°C for 10 h on fish (*Podamassys jubelini* and *Arius heudeloti*) purchased at a local market, led to the proliferation of enteric bacteria (that can include pathogenic bacteria) to over 10^8 CFU/g. Putrid odors developed after 5 to 6 h of incubation at 30°C and became very pronounced after 24 h. When these fish were supplemented with glucose (1%, wt/wt) and inoculated with a culture of a nisin-producing *Lactococcus lactis* (10^7 CFU/g), which was previously selected from a Senegalese fermented millet (*Pennisetum glaucum*) flour, the pH decreased to about 4.60 after 10 h at 30°C. Nisin activity was detected in juice from the two fish.

Results: In the new fermentation conditions, enteric bacteria contamination was reduced to 10^3 CFU/g. The putrid odors were not very perceptible in the products, indicating the inhibition of the enteric bacteria.

Significance: The results show the potentialities of this strain, justifying a more in-depth investigation into the use of millet flour as a carbohydrate source for the starter development in the perspective of its further application in small-scale fermented fish production in Senegal.

T2-11 Characterization of a Fish-specific Monoclonal Antibody

YI-TIEN CHEN and Yun-Hwa Peggy Hsieh
Florida State University, Nutrition, Food, and Exercise Sciences, Tallahassee, FL, USA

Introduction: Fish is one of the eight major allergen foods under the Food Allergen Labeling and Consumer Protection Act. Currently, there is no convenient method for fish detection to protect sensitized individuals and enforce the labeling law. Immunoassay is known as a simple and rapid method for detection of various food ingredients. In this study, we report a fish-specific monoclonal antibody (MAb), 8F5, which was raised against crude protein extract of cooked red snapper for the development of an immunoassay for fish protein detection.

Purpose: The specific objective of this study is to characterize the species specificity and antigenic protein of MAb 8F5.

Methods: Soluble proteins of all samples were extracted by 0.15 M NaCl. The species specificity of MAb 8F5 was screened against raw and cooked (100°C, 15 min) extracts from 55 common food fish species, 14 non-fish species and 4 food additives using an indirect enzyme-linked immunosorbent assay (ELISA). Protein extracts from raw and cooked (5, 10, 15 min at 100°C) extracts of four selected fish species were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then blotted with MAb 8F5 to reveal the antigenic component.

Results: MAb 8F5 belongs to IgG2a subclass. It strongly reacted with cooked extracts of all fish species tested without any cross-reactivity with land animals and food additives. The SDS-PAGE and immunoblot results showed that the antigenic component recognized by MAb 8F5 being a single protein with apparent molecular weight of 36 kDa. This antigenic protein maintained its molecular integrity up to 15 min of cooking suggesting the thermal-stability of this protein. In addition, the band intensity also increased with the increase of cooking time.

Significance: The fish-specific MAb 8F5, therefore, has great potential to be employed in an immunoassay for rapid and accurate detection of fish tissue in raw and cooked food products.

Technical

T2-12 Characterization of Food and Clinical *Listeria monocytogenes* Isolates Collected in Portugal

JOANA BARBOSA, Vânia Ferreira, Rui Magalhães, Isabel Santos, Gonçalo Almeida, Paul Gibbs and Paula Teixeira
Escola Superior de Biotecnologia-Universidade Católica Portuguesa, CBQF, Porto, Portugal

Introduction: The high incidence of *Listeria monocytogenes* in foods and the high fatality rate associated with listeriosis, makes this pathogen of high concern to the Food Industry. The collection and characterization of *L. monocytogenes* isolates from food are essential to a better understanding of the distribution of the pathogen through the food chain and the potential contribution of specific strains to human infection.

Purpose: We thus characterized both food and human clinical isolates of *L. monocytogenes* collected through 2003 to 2008 in Portugal. In this study 3698 *L. monocytogenes* isolates from different food products, namely vegetables (30), dairy (782), fish (19), pre-cooked meals (240), Ready-to-Eat (RTE, 217), and meat products (2410) and 75 *L. monocytogenes* isolates from human cases were investigated.

Methods: Isolates were tested for (i) the major serotype-specific genes: serotypes 1/2a and 3a (subtype A), serotypes 1/2b, 3b and 7 (subtype B), serotypes 1/2c and 3c (subtype C), serotypes 4b, 4d and 4e (subtype D) and serotypes 4a and 4c (subtype E); and (ii) resistance to arsenic (Ar), cadmium (Cd) and tetracycline (Tet).

Results: Subtype A isolates were most frequently detected on vegetables (46.7%), dairy (38.4%) and pre-cooked meals (92.9%) products; subtype B was most frequently detected on fish (57.9%) product isolates, and subtype D was most frequently detected on RTE (39.6%) and meat products (45.9%) isolates as well as on clinical isolates (68.0%). The most frequent profiles for Ar, Cd and Tet among isolates from different food products was as follows: ArSCdSTetS on vegetables (73.3%), dairy (54.9%) and clinical isolates; ArSCdRTetS on fish (47.4%), pre-cooked meals (80.0%), RTE (43.3%) and meat products (55.2%). Interestingly, profiles ArRCdSTetR and ArSCdRTetR were only detected on dairy and meat products isolates, respectively.

Significance: Overall, our data suggested that specific characteristics of isolates might be associated with their source.

T3-01 Prevalence and Distribution of *Salmonella* in Organic and Conventional Broiler Poultry Farms

WALID ALALI, Siddhartha Thakur, Roy D. Berghaus, Michael Martin and Wondwossen A. Gebreyes
University of Georgia, Center for Food Safety, Griffin, GA, USA

Introduction: Poultry is known to be an important vehicle of *Salmonella* transmission to humans, mainly via contaminated meat. Production of organic poultry in the U.S. has grown considerably to meet market demand in the past two decades. Very little is known, however, about the prevalence and dissemination of *Salmonella* in USDA-certified organic chicken farms.

Purpose: The objective of this cross-sectional study was to compare the prevalence of *Salmonella* and antimicrobial-resistant *Salmonella*, as well as investigate the distribution of this pathogen in organic and conventional broiler poultry farms.

Methods: Fecal, feed, and water samples were collected from birds at 3 weeks and 8 weeks of age for 2-flock cycles. One house was sampled per farm at 3 organic and 4 conventional broiler farms from the same company in North Carolina. All samples were analyzed for the presence of *Salmonella* using selective enrichment techniques. Further phenotypic (antimicrobial susceptibility) and genotypic (PFGE) testing were performed.

Results: The overall prevalence (across all farms and sample types) of *Salmonella* in organic farms was 4.3% (13/300) compared to 28.8% (115/400) in conventional farms. *Salmonella* prevalence in fecal samples were 5.6% (10/180) and 38.8% (93/240) from organic and conventional farms, respectively. From feed, 5% (3/60) and 27.5% (22/80) of the samples were positive for *Salmonella* from organic and conventional farms, respectively. None of the water samples were positive for *Salmonella*. Antimicrobial susceptibility testing was performed on 70 representative *Salmonella* isolates (12 from organic farms and 58 from conventional farms). In isolates from organic farms, 25% (3/12) were pansusceptible, 33.3% (4/12) had single antibiotic resistance (mostly to streptomycin), and 41% (5/12) were multi-drug resistant. In conventional farms, 1.7% (1/58) of the isolates were pansusceptible, 36.2% (21/58) were single-antibiotic resistant, and 62% (36/58) were multi-drug resistant; 43% (25/58) of the multi-drug resistant isolates were resistant to six drugs. Characterization of the 72 isolates using PFGE showed a high clonal nature of the isolates within and among the two types of farms.

Significance: The results of our study suggest that within this poultry company, prevalence of fecal *Salmonella* may be lower in certified-organic birds than in conventionally-raised birds. In addition, the prevalence of antimicrobial resistant *Salmonella* appears to be higher in conventionally-raised birds than in certified-organic birds.

T3-02 Molecular Surveillance of Multi-antibiotic Resistant *Staphylococcus aureus* and *Salmonella* Isolated from Co-op Rabbit and Poultry Processing Plants in Southeastern United States

LEONARD WILLIAMS and Olasunmbo Ajayi
North Carolina A&T State University, Center of Excellence for Post Harvest Technologies, Kannapolis, NC, USA

Introduction: The distribution practices and changes in food processing, by which potentially contaminated food could reach consumers across multiple geographic regions, presents a new and complicated challenge for public health and food safety experts in the United States.

Purpose: A study was conducted to determine the prevalence and genetic diversity of multi-antibiotic resistant strains of *Salmonella* and *Staphylococcus* spp. isolated from co-op rabbit and poultry processing plants in five states (Florida, Alabama, Tennessee, Mississippi and Georgia).

Methods: Phenotypic characteristics, antimicrobial and genetic relationship were analyzed in one-hundred and two *Salmonella* and *Staphylococcus* isolates collected from 360 broiler and rabbit samples from four sampling points (pre- and post-evisceration; pre- and post-chilling) from commercial broiler and rabbit processing facilities. Biochemical and antimicrobial analysis using 16 different antibiotics and API strips were used to determine the biochemical patterns among the isolates. Polymerase chain reaction and pulsed field gel electrophoresis analysis were performed to detect methicillin (mecA) and clumping factor (cflA) gene for *Staphylococus aureus* and fimbrin (fimA) gene for *Salmonella* and the genetic relatedness of the strains using SmaI restriction enzyme and analyzed using Bionumeric software program.

Results: Results indicated that ten different biochemical profiles were detected among the 53 (15%) *Staphylococcus aureus* and 60 (18%) of *Salmonella* isolates recovered from the broiler and rabbit samples. Isolates of distinct phenotypic profiles were detected within a sampling point. Fifty-three (100%) isolates were positive for clfA gene, 19 (36%) for mecA gene and 19 (30%) for fim A gene using PCR. Antibiotic testing revealed that of the 113 *Staphylococcus* and *Salmonella* strains tested, 67% of the strains were sensitive, while 63% were resistant to multiple antibiotics. Both *Salmonella* and *Staphylococcus* isolates were all typed by pulsed field gel electrophoresis, which identified 8 different types of *Salmonella* and 4 different types of *Staphylococcus aureus*. Type A and its subtypes comprised of 45 and 30% of all *Salmonella* and *Staphylococcus aureus* isolates recovered from post-evisceration sampling points, respectively.

Significance: Similar strains were prominent among several co-op processing facilities, suggesting possible cross-contamination and a combination of molecular or antimicrobial typing tools maybe an effective epidemiological tool for source tracking in meat processing facilities.

T3-03 Prevalence and Antimicrobial Resistance of *Campylobacter* Isolated from the National Antimicrobial Resistance Monitoring System Retail Meat: 2002—2007

SHAOHUA ZHAO, Linda English, Jason Abbott, Emily Tong, Niketta Womack, Sharon Friedman and Patrick McDermott
U.S. Food and Drug Administration, Laurel, MD, USA

Introduction: Campylobacter is a leading cause of foodborne diarrhea illness. Antimicrobial resistance in *Campylobacter* spp. from the food supply is a global public health concern.

Purpose: The objectives of this study were to determine the prevalence, antimicrobial susceptibility, and genetic relatedness of *Campylobacter* spp. recovered from the National Antimicrobial Resistance Monitoring System (NARMS) retail meat program.

Methods: We compared the prevalence of *Campylobacter* in a sampling of 24,566 meats including chicken breast (n = 6,138), ground turkey (n = 6,109), ground beef (n = 6,171) and pork chop (n = 6,148) from ten FoodNet sites collected during 2002—2007. Isolates were further speciated by PCR and analyzed for antimicrobial susceptibility by agar dilution or broth microdilution and compared genetic relatedness using pulsed-field gel electrophoresis (PFGE).

Technical

Results: A total of 2,258 of *C. jejuni*, 925 *C. coli* and 7 *C. lari* were identified. Chicken breast showed the highest contamination rate (49.5%), followed by ground turkey (1.6%), and both pork chops and ground beef had < 0.5% of contamination. Resistance was most frequent to doxycycline/tetracycline (46.6%), followed by nalidixic acid (18.5%), ciprofloxacin (17.4%), azithromycin and erythromycin (2.8%), telithromycin (2.4%), and clindamycin (2.2%) and gentamicin (< 0.1%). All isolates were susceptible to meropenem and florfenicol. With exception of doxycycline/tetracycline, *C. coli* showed higher resistance to all other antimicrobials than *C. jejuni*. PFGE fingerprinting profiles showed that *Campylobacter* were genetically diverse with 1,226 PFGE profiles generated from the 2318 isolates. Several clones were found to be geographically dispersed and to persist throughout the six-year sampling.

Significance: Results demonstrated a high prevalence of antimicrobial-resistant *Campylobacter* in chicken meat, emphasizing the importance of sustained monitoring of the food supply.

T3-04 The Effect of Heat on the Antimicrobial Efficacy of Cinnamic Aldehyde, Carvacrol and Eugenol

GRISHMA KOTWAL and Faith J. Critzer
University of Georgia, Food Science and Tech., Griffin, GA, USA

Introduction: Several natural compounds have been evaluated to determine their antimicrobial capacity; however, research is needed to study the different parameters that affect the activity of these compounds in order to determine the conditions that may alter efficacy and help establish appropriate use in foods.

Purpose: The objective of the study was to quantify the antimicrobial activity of three phytophenolic essential oil components (cinnamic aldehyde, carvacrol and eugenol) after exposure to various temperature conditions, against *Escherichia coli* O157:H7 and *Salmonella enterica* serovars.

Methods: Pure aromatic volatiles (≥ 98% purity) in black micro centrifuge tubes were subjected to temperature exposure for 0 h, 0.5 h, 1h, 4h, at 60°C and 70°C, and for 0 h, 12 h, 24 h, 48 h, 72 h, 7 d, 14 d and 21 d at 4°C and 25°C. The in vitro determination of antimicrobial activity was carried out through the microbroth dilution assay. Concentrations of 1, 2, 5, and 10 mM of each antimicrobial were evaluated in order to determine the Minimum Inhibitory Concentration (MIC) against five strains of each foodborne pathogen independently.

Results: The MIC results for the untreated antimicrobial components showed carvacrol to be the most effective with an MIC of 1mM, followed by cinnamic aldehyde MIC = 2 mM and eugenol MIC = 5 mM. The MIC increased to 2, 5 and 10 mM for carvacrol, cinnamic aldehyde, and eugenol, respectively, when samples were held at 70°C for 4 h. A slight reduction in activity was observed in carvacrol with exposure ≥14 days (MIC = 2 mM) and cinnamic aldehyde ≥ 21 days (MIC = 5 mM) when held at 4°C and 25°C, while eugenol retained activity at these temperatures.

Significance: It is important to establish efficacy of antimicrobial compounds in a wide array of processing conditions. The retention of antimicrobial activity by these naturally occurring essential oil components will allow flexibility when processors seek to incorporate them into food. Further work is being conducted to determine the activity of these compounds in a model milk system.

T3-05 Cinnamaldehyde Induces Cell Elongation in *Escherichia coli* O157:H7

Richard A. Holley and VISVALINGAM JEYACHCHANDRAN
University of Manitoba, Food Science, Winnipeg, MB, Canada

Introduction: Plant essential oils and their components have been widely studied for their ability to inhibit pathogenic and spoilage organisms in foods. One such compound, cinnamaldehyde, is active against many foodborne pathogens including *Escherichia coli* O157:H7; however its mechanism of antimicrobial action is not fully understood. It has been suggested that cinnamaldehyde may damage the cell membrane and inhibit polymerization of the cell septum protein ftsZ.

Purpose: To examine changes in growth and cell morphology of *E. coli* O157:H7 during exposure to sub-lethal concentrations of cinnamaldehyde.

Methods: Log phase *E. coli* O157:H7 (02/0627) cells were treated with 100, 200 or 300 mg/l cinnamaldehyde (< minimal inhibitory concentration, 400 mg/l) at 37°C for 4 h. Growth was monitored by hourly plate count and absorbance (A_{600}) while filament formation and cell viability were monitored by photomicroscopy after 5- cyano-2, 3- ditolyl tetrazolium chloride (CTC) and Live/Dead BacLight vitality staining. Mean cell length values, the number of viable cells and differences in growth response were compared by ANOVA.

Results: Cinnamaldehyde at ≤ 200 mg/l significantly ($P < 0.05$) delayed *E. coli* O157:H7 growth, causing a ≤ 2 h lag while 300 mg/l prevented growth for 4 h. The greatest extent of filamentation (94.7%) and greatest mean cell length (6.2 µm) occurred at 2 h exposure to 200 mg/l cinnamaldehyde. After 2 h at 200 mg/l, changes to normal cell length and growth rate were noted. Cell viability by vital staining was unaffected by ≤ 200 mg/l cinnamaldehyde, but after 1 h at 300 mg/l, only 75 % of cells were viable, 30% of cells were still filamentous, and little change was noted for the next 3 h.

Significance: At sub-lethal concentrations, cinnamaldehyde caused a delay in *E. coli* O157:H7 replication because of filament induction which was reversible at ≤ 200 mg/l.

T3-06 Can Hand Hygiene Regimens Offer Reduced Risk in Food Service Environments?

SARAH EDMONDS, Christopher Fricker and David Macinga
GOJO Industries, Research and Development, Akron, OH, USA

Introduction: The FDA Food Code specifies that food handlers must maintain clean hands by washing with soap and water. Alcohol-based hand sanitizers (ABHS) may be used in conjunction with hand washing, but are not allowed as a substitute for hand washing. There is currently limited data to demonstrate the added antimicrobial benefit of a wash-sanitize regimen in comparison to hand washing with soap and water only.

Purpose: To compare the antimicrobial activity of a wash-sanitize regimen as compared to the standard hand wash with soap and water. To evaluate the impact of product formulation (handwash or ABHS) on antimicrobial efficacy.

Methods: A modification of ASTM E1174 was used. Hands were contaminated with approximately 1×10^9 *Escherichia coli* suspended in chicken broth to replicate a moderate food soil. Eighteen subjects participated for each of the six test configurations described below. $\log_{10}$ reductions from baseline were calculated for each configuration. Statistical analyses were performed using an ANOVA ($P < 0.05$).

Results: The $\log_{10}$ reductions for each configuration are as follows: non-antimicrobial handwash (3.10 ± 0.61), antimicrobial handwash (3.56 ± 0.74), non-antimicrobial handwash followed by ABHS foam (3.81 ± 0.89), antimicrobial handwash followed by ABHS foam (4.16 ± 0.91), non-antimicrobial handwash followed by advanced formula ABHS gel (5.13 ± 0.71), and antimicrobial handwash followed by advanced formula ABHS gel (5.22 ± 0.60). When used individually, the non-antimicrobial handwash and antimicrobial handwash were statistically equivalent. All wash-sanitize regimens were statistically superior to any type of hand washing regime. Wash-sanitize regimens incorporating the advanced formula ABHS gave statistically significantly higher efficacy than those incorporating the ABHS foam.

Significance: Hand washing is an appropriate hand hygiene intervention in low risk environments (i.e., clearing tables), however a wash-sanitize regimen can provide an additional antimicrobial benefit and should be considered for high risk situations such as handling raw meat.

T3-07 SaniTwice™: A Hand Hygiene Solution for Reducing Contamination on Heavily Soiled Hands When Water is Unavailable

SARAH EDMONDS, Robert McCormack, Christopher Fricker and David Macinga
GOJO Industries, Research and Development, Akron, OH, USA

Introduction: The FDA Food Code specifies that food handlers must clean their hands by washing with soap and water. However, food is frequently prepared and served in areas where clean water or sinks are unavailable. SaniTwice, a two-step hand hygiene procedure using an alcohol-based hand sanitizer (ABHS), has been shown to be an effective option for reducing bacteria on lightly soiled hands in low to no water situations. However, the effectiveness of SaniTwice against bacterial contamination on heavily soiled hands is currently unknown.

Purpose: Evaluate the antibacterial efficacy of the SaniTwice method on hands in the presence of heavy food soils.

Methods: A modification of ASTM E1174 (Health Care Personnel Handwash Test Method) was used. Hands were contaminated by either handling raw hamburger or cooked chicken chunks seeded with *Escherichia coli* to represent heavy soils found in food service settings. Two test product configurations were evaluated with each soil: a non-antimicrobial handwash and SaniTwice using a 62% ABHS foam. The SaniTwice procedure involved "washing" with an excess of ABHS and paper towel drying; followed by reapplication of ABHS according to label instructions. Five participants evaluated each of the four configurations and $\log_{10}$ reductions from baseline were calculated for each configuration.

Results: When hands were contaminated by handling chicken chunks, the non-antimicrobial handwash and SaniTwice achieved $\log_{10}$ reductions of 2.96 ± 0.48 and 3.32 ± 0.43, respectively. When hands were contaminated by handling raw hamburger, the non-antimicrobial handwash and SaniTwice achieved $\log_{10}$ reductions of 2.58 ± 0.41 and 2.69 ± 0.34, respectively.

Significance: The SaniTwice method is an effective option for hand hygiene when handwashing resources are unavailable, as it achieved bacterial reductions on heavily soiled hands that are equivalent to traditional hand washing.

T3-08 Analysis of Plasmids and Mobile Elements Carrying Antimicrobial Resistance Genes in *Salmonella* Isolates by Whole Genome Sequencing

ANDREA I. MORENO SWITT, Matthew Ranieri, Henk den Bakker, Lovorka Degoricija, Craig A. Cummings, Gregory Govoni, Elena Bolchakova, Manohar Furtado and Martin Wiedmann
Cornell University, Dept. of Food Science, Ithaca, NY, USA

Introduction: Foodborne salmonellosis causes an estimated of 1.4 million human cases annually in the United States. Plasmids carrying antimicrobial resistance genes have been described among diverse *Salmonella* serovars. Furthermore, dissemination through the food chain of plasmids encoding resistance genes is a global public health concern.

Purpose: The purpose of this study was to identify the repertoire of antimicrobial resistance genes in mobile elements among diverse human disease related *Salmonella* serovars by full genome sequencing.

Methods: Sixteen *Salmonella* isolates representing serovars Senftenberg, Rubislaw, Gaminara, Hvittingfoss, Minnesota, Urbana, Alachua, Adelaide, Wandsworth, Johannesburg, Baildon, Mississippi, Inverness, Montevideo, Uganda and Give were sequenced by the SOLiD next generation sequencing system.

Results: By *de novo* assembly, putative large plasmids were identified among the genomes. In the serovar Montevideo isolate an IncW plasmid was detected carrying sulfonamide and aminoglycoside resistance genes in an integron class 1. This element includes a cluster of genes homologous to a previously sequenced plasmid of a multidrug resistant *Salmonella* Newport isolate. No other isolates harboring plasmid associated resistance genes were identified. However, in serovar Inverness, we identified one large IncI1 plasmid of approximately 120 kb, and one 64 kb putative integrated conjugative element. In addition, we found putative IncI1 plasmids lacking resistance genes, but with characteristics of conjugative plasmids in serovar Mississippi and Urbana isolates.

Significance: Plasmids carrying antimicrobial resistance genes and mobile elements with self transmissible features were identified using full genome sequencing of *Salmonella*. Identification of a number of plasmids without resistance genes suggests a larger repertoire of transmissible genetic elements in *Salmonella* than previously recognized; these elements may allow for emergence of new plasmids carrying antibiotic resistance genes.

T3-09 Considering the Design and Analysis of Efficacy Trials for Antimicrobial Treatments of Raw Meat and Poultry

MARK POWELL
USDA, Washington, D.C., USA

Introduction: Recent policy developments have spurred interest in the design and interpretation of experiments to evaluate the efficacy of antimicrobial treatments for raw meat and poultry products. Current domestic and international guidelines for approval of antimicrobial treatments differ markedly and provide limited guidance on statistical design and analysis of efficacy trials. Determining whether a treatment effect exists for the entire target population is complicated when results vary among replicate trials (e.g., days or plants).

Purpose: The study compares the statistical power of an illustrative antimicrobial efficacy experiment under the standard analysis of variance (ANOVA) model with fixed effects to that under a mixed model with fixed treatment and random replicate effects.

Methods: Statistical power is analyzed for an experimental design with three treatment groups: (1) no treatment, (2) treatment without antimicrobial additive (e.g., water-only wash), and (3) treatment with antimicrobial additive; and three replicate trials. To optimize power, the design plans for two independent contrasts: (1) no treatment versus treatment (with or without additive) and (2) treatment without additive versus treatment with additive. Compared to no treatment, it is anticipated that treatment without additive averages a 0.5 $\log_{10}$ reduction in microbial contamination, and the antimicrobial treatment averages a 1 $\log_{10}$ reduction, with antimicrobial efficacy varying among replicates. Because standard procedures do not consider such variation, power for the mixed model is estimated using bootstrap sampling.

Results: The power of the test of the incremental antimicrobial effect is lower under the mixed model, and the disparity increases with variance among replicates; however, inferences from the standard ANOVA cannot be generalized due to heterogeneous treatment efficacy among replicates.

Significance: The reduced power under the mixed model illustrates the inherently greater challenge of generalizing from limited experimental conditions to the entire population. For a fixed total sample size, mixed model power increases with the number of replicates (fewer samples per trial), but this comes at the expense of inferential power within replicates that may help explain the observed heterogeneity.

T3-10 Evaluation of a Predictive Model for Total Viable and Lactic Acid Bacteria on Refrigerated Vacuum-packed Beef Primals

MARK TAMPLIN, Alison Small, Sheila Peddell, Olivia McQuestin, Bianca Porteus, Danielle O'Callaghan, John Sumner and Ian Jenson
University of Tasmania, Food Safety Centre, Sandy Bay, TAS, Australia

Introduction: The shelf-life of fresh beef is affected by intrinsic and extrinsic factors including temperature, pH, packaging atmosphere and microbial species. The storage life of meat can be controlled, in part, by understanding the kinetics of microbial growth that contributed to spoilage. Such information can be converted into predictive models that aid in managing cold chains.

Purpose: The purpose of this study was to produce and validate a predictive model for the viability of total viable count (TVC) and lactic acid bacteria (LAB) on vacuum-packed beef primals.

Methods: TVC and LAB models were produced by studies of microbial growth on commercially-packed primal cuts, and separately for cuts irradiated and then inoculated with a 6-strain spoilage cocktail. Microbial counts and surface pH were measured at selected time intervals on samples stored at -1.5, 0, 2, 4 and 7°C for up to 30 weeks. Growth parameters were estimated for lag time, growth rate and maximum population density, and then transformed into secondary models. Model estimates were compared to independent data of growth rates in the published literature, ComBase and industry data.

Results: The model based on laboratory-inoculated meat over-estimated TVC and LAB levels when compared to data in ComBase and the published literature. For example, the bias and accuracy factors were 2.8 and 3.1 for TVC, respectively. Producing the model from commercially-packed primal cuts improved bias and accuracy factors to 1.6 and 2.8 for TVC, respectively. However, growth rate predictions for TVC and LAB were still 3 to 4 times higher than the rates observed for whole beef primals produced by six Australian abattoirs and stored at -0.5°C.

Significance: These findings demonstrate that microbial communities, and their interactions, are likely complex in fresh beef, and that these differences may affect the validity of model applications for commercial products.

T3-11 Inactivation of *Bacillus coagulans* Spores in Tomato Juice by Pressure-assisted Thermal Processing

HOSSEIN DARYAEI, Chelsea Johnson and V.M. (Bala) Balasubramaniam
The Ohio State University, Food Science and Technology, Columbus, OH, USA

Introduction: Bacillus coagulans is a heat-resistant spore-forming bacterium, spoiling thermally-sterilized acidic foods. Pressure-assisted thermal processing (PATP) is a promising technology capable of inactivating bacterial spores and producing shelf-stable food products. Lesser thermal effects due to uniform compression heating, as well as rapid cooling, allows better quality preservation in PATP comparing to conventional processing.

Purpose: This study was aimed to evaluate the efficacy of PATP for inactivation of *B. coagulans* spores in tomato juice.

Methods: A spore crop of *B. coagulans* 185A (selected as the most pressure-heat resistant strain amongst three strains) was prepared on nutrient agar plus 500 mg/L dextrose and 3 mg/L $MnSO_4$. H_2O, incubated at 50°C for 7 d. Spores were suspended (4.2×10^8 CFU/mL) in a commercial tomato juice (pH 4.2) and treated at 600 MPa and process temperatures of 95, 100 and 105°C for ≤ 2.5 min. The inactivation data was fitted to estimate kinetic inactivation parameters using selected linear and non-linear models. The models were evaluated using the mean square error, regression coefficient and accuracy factor.

Results: Depending upon the process temperature, a 600 MPa PATP treatment reduced the spore population by 3.0—3.6 logs during the 30 s pressure come-up time. Treatment times of 2.5, 2 and 0.5 min were sufficient to inactivate the spores to below the detection limit (< 10 CFU/mL) at 95, 100 and 105°C, respectively. Pre-process temperature history also significantly influenced ($P < 0.05$) PATP-induced spore inactivation. Kinetic model analysis indicated that the non-linear models produced better fits than the linear model to all survivor curves.

Significance: This study suggests that combined pressure-heat treatment may be used as a viable alternative to inactivate *B. coagulans* spores in acidic food products, like tomato juice.

T3-12 Non-thermal Pasteurization of Almonds and Pistachios with Organic Citrus Bioflavonoid Extracts

PEYMAN FATEMI, Cliff Coles and Yves Methof
Aurora Food Safety Solutions, Pleasanton, CA, USA

Introduction: Thermal treatments of nuts have been widely used because of lack of effective alternative pasteurization technologies. There is a need for non-thermal treatments that will achieve the minimum 4-log reduction of *Salmonella* without impacting the sensory attributes of nuts. Antimicrobial properties of citrus bioflavonoids have been used in the meat and other industries. To be effective, treatments must overcome the natural surface barriers of nuts.

Purpose: Objective of this work was to evaluate effectiveness of organic citrus bioflavonoid extract (BioSecur 428D) in improving the safety of nuts by combining this technology with an electrostatic spray method that created negatively charged 30—40 µm droplets for better nut surface coverage.

Methods: Whole almonds or pistachios were inoculated with either *Enterococcus faecium* or *Salmonella* Enteritidis Phage Type 30 per protocols of the Almond Board of California. Twenty-five g samples were added to sterile bags and rinsed or electrostatically sprayed with equal amounts of treatment at various time and concentration combinations. Controls included un-rinsed nuts as well as those rinsed/sprayed with water alone. Controls indicated that rinsing/spraying with water alone for up to 5 minutes did not remove *E. faecium* from the nut surfaces. Therefore, any reduction of bacteria levels was due to the action of treatment.

Results: Rinsing/spraying the almonds and pistachios with BioSecur 428D was effective in achieving greater than 5-log reduction of *E. faecium* and *Salmonella* SEPT30. Lower treatment concentrations required longer exposure times of up to 5 minutes while higher concentrations achieved greater than 6-log reduction after 1 minute of exposure. Overall, these results show that it is possible to achieve the target microbial reduction on nuts using citrus extracts at concentration and exposure times applicable to industrial settings.

Significance: Improve the safety of nuts. Introduce a novel and cost effective approach in antimicrobial treatments. Application to other commodities.

Technical

T4-01 Field Assessment of Surface Contamination and Systemic Transference of an Attenuated *Salmonella* Typhimurium to Melon Fruit from Controlled Contamination of Irrigation Water

TREVOR SUSLOW, Adrian Sbodio, Alejandro Tomás-Callejas, Francisco López-Gálvez and Sharyn Maeda
University of California-Davis, Plant Sciences, Davis, CA, USA

Introduction: Establishing microbiological quality standards for irrigation water, within food safety management programs for fresh produce is a contentious topic. The California melon industry requested the initiation of a study on the potential for systemic transfer of *Salmonella* to mature fruit in a field environment.

Purpose: Evaluate the survival and dispersal of applied *Salmonella* Typhimurium following controlled irrigation-source contamination events in an open field environment.

Methods: Cantaloupe ('Oro Rico') and Honeydew ('Summer Dew') in replicated plots in Davis, CA (Reiff Coarse Loam) were irrigated, by furrow or sub-surface emitter tape, containing a rifampicin resistant (100 mg/L) isolate of virulence-attenuated *Salmonella*. "Contamination" levels were targeted at log 4 and 6 CFU/ml of water applied. At several periods, samples of water, furrow and seed-bed soil, rhizosphere soil, soil surrounding the emitter tape, and environmental samples were processed by standard protocols including selective enrichment and molecular confirmation. Mature fruit in the central bed (crown) as well as fruit developing in contact with furrow slopes were processed, with and without disinfection with mercuric chloride (10 min + 1 min sterile water rinse), as above.

Results: Applied *Salmonella* Typhimurium was readily recovered from irrigation inputs, soil, and environmental samples throughout the trial. The applied strain was regularly recovered from cantaloupe but not honeydew developing in "contaminated" furrows. No *Salmonella* was recovered from the rind or in the sub-rind tissue taken below the abscission zone in over 200 crown fruit. The applied *Salmonella* survived in the rhizosphere of melons irrigated by sub-surface drip injection but no evidence of transfer to fruit was detected.

Significance: These results support greenhouse outcomes in our lab that strongly suggest a very high threshold of contamination from irrigation water would be required to make the risk of root uptake and transfer to fruit a significant concern. External contamination of fruit remains a plausible direct or indirect route from irrigation water.

T4-02 Tracking an *Escherichia coli* O157:H7 Contaminated Batch of Leafy Greens through a Commercial Processing Line

ANNEMARIE L. BUCHHOLZ, Gordon R. Davidson, Rudolph E. Sloup, Bradley P. Marks, Ewen C. Todd and Elliot T. Ryser
Michigan State University, Food Science and Human Nutrition, East Lansing, MI, USA

Introduction: Cross-contamination of uncontaminated leafy greens with small amounts of *Escherichia coli* O157:H7-contaminated product during commercial processing has been suggested as one cause for recent outbreaks.

Purpose: The goal of this study was to quantify the spread of *E. coli* O157:H7 during small-scale commercial processing of iceberg lettuce, occurring via transfer within the system or by transport of contaminated particles.

Methods: Radicchio (9.1 kg) was dip-inoculated to contain 10^6 CFU/g of a 4-strain avirulent, GFP-labeled, ampicillin-resistant *E. coli* O157:H7 cocktail. The processing line consisted of a commercial shredder, step conveyor, flume tank, shaker table and centrifugal dryer. After priming this line with 45 kg of iceberg lettuce, the inoculated Radicchio was processed and immediately followed by 907 kg of iceberg lettuce (triplicate experiments). Forty bags of lettuce/Radicchio (~22.7 kg/bag), 40 water samples (500 ml) and 50 equipment swabs (100 cm^2) were targeted for collection. All visible shreds of Radicchio were retrieved from these 40 bags, the equipment, and the floor and were weighed and counted. Twenty-five grams of only iceberg lettuce was collected from each bag and from 10 of the 40 bags after centrifugal drying. All samples were examined for *E. coli* O157:H7 by direct plating or membrane filtration with trypticase soy agar containing 0.6% yeast extract and 100 ppm ampicillin used as the growth medium.

Results: After processing, an average of 614.92 g (93.64%), 6.93 g (1.25%), 5.00 g (0.78%) and 2.85 g (0.47%) of inoculated Radicchio appeared in the shredded lettuce from bags 1 to 10, 11 to 20, 21 to 30 and 31 to 40. These same bag groupings contained average *E. coli* O157:H7 counts of 1.69, 1.22, 1.10 and 1.11 log CFU/g, respectively, with most of this contamination coming from the water and equipment surfaces. No significant decrease in *E. coli* O157:H7 was seen in the iceberg lettuce after centrifugal drying. Hundreds of Radicchio pieces were recovered from the equipment surfaces after processing with the conveyor harboring the most in terms of weight (9.79 g) followed by the shredder (8.32 g), flume tank (3.53 g) and shaker table (0.08 g).

Significance: Based on these findings, *E. coli* O157:H7-contaminated leafy greens are able to perpetuate in a processing line long after a contamination event, and thereby contaminate large quantities of previously uncontaminated product.

T4-03 Recovery of *Escherichia coli* O157:H7 from Inoculated Spinach Fields as Affected by Inoculum Dose, Plant Material and Environmental Conditions

EDUARDO GUTIÉRREZ-RODRÍGUEZ, Steven Koike, Michael Cahn and Trevor Suslow
University of California-Davis, Plant Science, Davis, CA, USA

Introduction: Leaf colonization by epiphytic bacteria and human pathogens has been described as irregular and dependent on phyllosphere characteristics and environmental conditions. Bacterial attachment is influenced by physicochemical properties of bacteria and the leaf being colonized. Extreme variability in bacteria populations within leaves reduces the efficacy of sampling and the ability of detection methods to minimize distribution of contaminated product.

Purpose: To determine the heterogeneity of persistence on spinach leaves over time by *E. coli* O157:H7 under field conditions following foliar inoculation.

Methods: Spinach was cultivated in the Salinas Valley, California from June to November 2009. Spinach was sprayed with log 0.3 and 0.56 CFU/m^2 of a mixture of two attenuated strains. Individual leaves and composites up to 150 g were used in the recovery of *E. coli* O157:H7 with and without disinfection with 1% $AgNO_3$. Quantitative recovery was by selective and differential culture. Assurance GDS-O157™ was used for qualitative detection of *E. coli* O157:H7 below the limit of plating recovery.

Results: Composite 150 g sample units consisted of 165 to 220 leaves. Average populations after 12 to 36-h post-inoculation were 1.4 and 3.1 CFU/g, respectively. Fifty percent of plants inoculated with log 0.56 CFU/m^2 and 25% of those from low dose were positive for *E. coli* O157:H7 after 6 days post-inoculation. On average, populations recovered from both treatments were of 1.35 CFU/g and remained constant in all trials over 28 days. Recovery of *E. coli* O157:H7 from 30 individual leaves was 35% and 57% (lower dose and higher dose, respectively) without disinfection, while 8% and 17% after disinfection.

Significance: Our findings indicate that even homogeneous contamination events result in heterogeneous distribution over time. Pathogen sampling protocols for leafy greens should be standardized for sample mass units greater than the standard 25 g from raw or processed samples due to the significant variability observed in the surviving populations.

T4-04 Molecular Ecology of *Listeria* spp., *Salmonella*, *Escherichia coli* O157:H7, and Non-O157 Shiga Toxin-producing *E. coli* in Northern Colorado Wilderness Areas

CHRISTINA AHLSTROM, Clyde Manuel, Kelly Horgan, Martin Wiedmann and Kendra Nightingale
Colorado State University, Animal Science, Fort Collins, CO, USA

Introduction: *Listeria monocytogenes, Salmonella, Escherichia coli* O157:H7, and non-O157 shiga toxin producing *E. coli* (STEC) represent clinically important foodborne pathogens in the U.S. A knowledge gap exists regarding the presence, transmission and molecular ecology of these foodborne pathogens in pristine environments

Purpose: The objective of this study was to collect a set of foodborne pathogens from pristine environments to aid in interpretation of a shared subtype between clinical and food isolates during an outbreak.

Methods: Five wilderness locations in Colorado were selected to represent pristine environments and three areas approximately 100 m^2 within each location were designated; where each area was sampled during the spring, summer, and fall of 2009. A total of 225 soil, 225 water, 45 drag swab and 117 fecal samples were collected. Up to five soil samples and five water samples from each area were pooled. The average pH of soil samples was 5.2 ± 0.6, which may be explained by sampling coniferous forest soil overlaid by decomposed acidic needles. Furthermore, all samples were microbiologically analyzed to detect *Listeria* spp., *Salmonella, E. coli* O157:H7, and non-O157 STEC. After non-selective pre-enrichment, samples were divided and analyzed to detect each target organism using a modified version of the Food and Drug Administration Bacteriological Analytical Manual. Up to four presumptive colonies for each target organism from each sample were confirmed by PCR to detect gene fragments specific to those organisms.

Results: Overall, two samples tested positive for *L. monocytogenes,* including one soil and one fecal sample. Eight samples were positive for *Listeria* spp. other than *L. monocytogenes*, including four fecal, one water and three soil samples, which were all determined to be *L. welshimeri* via *sigB* sequencing. No *E. coli* O157:H7 or *Salmonella* were detected.

Significance: Our results demonstrate a rare presence of foodborne pathogens in pristine environments and further characterization of these isolates will provide insight into epidemiological associations between isolates in nature and clinical isolates.

T4-05 Development of a Simple Method to Detect Coliphages in Fresh Produce as Evidence of Fecal Contamination

CHOI-IOK R. WONG and Fu-Chih Hsu
Scientific Methods Inc., Granger, IN, USA

Introduction: The consumption of ready-to-eat produce has become a major source of foodborne outbreaks in the USA because it is especially prone to contamination that may go unchecked and result in severe illness. Due to the difficulty of identifying potential pathogens that may be present in contaminated fresh produce, reliance on indirect measures of contamination, such as the presence of indicator microorganisms, becomes critical to protect public health. Monitoring traditional bacterial indicators such as *E. coli*, along with other alternative indicators like coliphages as viral indicators, may provide more food safety measures.

Purpose: In this study, a simple method was developed to detect male-specific (F+), somatic coliphages, and *E. coli* from ready-to-eat produce.

Methods: The method included a pectin-based medium containing chromogenic substrates, bacterial stain and an elution buffer for the recovery of coliphages from vegetables and fruits. A modified membrane filtration method was used to detect *E. coli* from washes.

Results: In spiking experiments, the recoveries of F+ coliphages from washes using the pectin medium were 72% (lettuce), 44% (strawberries), 48% (baby carrots), 29% (cherry tomatoes), and 58% (green onions). In a field study, 181 samples were collected from retail stores or farmers markets in the USA. Of the 181 field samples tested, 81.2% were positive for *E. coli*, 21.3% were positive for F+ coliphages, and 35.7% were positive for somatic coliphages with ranges of 0 to 3×10^6 CFU/100 g (*E. coli*), 0 to 1.3×10^4 PFU/100 g (F+ coliphages), and 0 to tntc (somatic coliphages). Among vegetables analyzed, alfalfa sprouts, ready-to-eat baby spinach, and cilantro tested positive for all fecal indicators with higher concentrations and more frequent than other vegetables tested.

Significance: The results from this study show that it is feasible to use the pectin-based medium to detect coliphages in vegetable washes collected from markets. The coliphage results may provide additional safety measures for ready-to-eat produce along with other bacterial indicators such as *E. coli*.

T4-06 Internalization of Murine Norovirus-1 to Romaine Lettuce

JIE WEI, Yan Jin, Tom Sims and Kalmia E. Kniel
University of Delaware, Animal and Food Sciences, Newark, DE, USA

Introduction: Norovirus is a leading cause of foodborne illness associated with consumption of fresh produce. Irrigation with surface water such as river or canal water has resulted in increasing interests concerning virus internalization into plants through root tissue. Little is known on the uptake of enteric viruses into leafy greens.

Purpose: To study the likelihood of virus internalization into Romaine lettuce through root tissue, lettuce sprouts were measured over time for virus viability and infectivity.

Methods: Five-day old Romaine lettuce sprouts were fed 1×10^7 or 1×10^4 PFU murine norovirus-1 (MNV) daily for up to three days. MNV was pipetted at the roots carefully without contact to shoots. After one or three days, shoots were collected and blended with a tissue homogenizer. The samples were then applied to cell culture to determine the concentration of infectious virus, or viral RNA was extracted and applied to reverse transcription and quantitative PCR (RT-qPCR). MNV antiserum was produced by specific pathogen-free birds and IgY was purified with aluminum sulfate. Shoots were stained with chicken anti-MNV IgY and observed under confocal microscopy to evaluate the presence of MNV. Control uninoculated sprouts in the presence of Hanks' Balanced Salt Solution were grown or assessed identically in each experiment.

Results: For both day one and day three incubation, in two out of three trials, shoots samples incubated with 1×10^7 PFU MNV contained ~ 5 log RT-qPCR units MNV; however, the cell culture assay showed that only 1 to 1.5 log PFU MNV was infectious. While the sprouts incubated with 1×10^4 PFU MNV, no virus was detected in either day one or day three samples. MNV was observed in both stem and leaf of the sprout incubated with 1×10^7 PFU MNV for one day under confocal microscopy. No virus was visible with the lower inoculation or control samples.

Significance: This is the first study to show that MNV could internalize into lettuce sprouts through the root. Current data suggests that this could occur when high numbers of virus are present, i.e., a flood or sewage leak.

T4-07 Hydroponic Internalization of Enteric Viruses into Green Onions and Spinach

KIRSTEN A. HIRNEISEN and Kalmia E. Kniel
University of Delaware, Animal and Food Science, Newark, DE, USA

Introduction: Enteric viruses including both hepatitis A virus (HAV) and human noroviruses have been the cause of outbreaks involving both green onions and leafy greens. During the washing of fruits and vegetables, internalized pathogens evade removal and inactivation by sanitizers. Internalization of non-viral pathogens through intact roots has received much debate. Previous studies on viruses indicated limited internalization into plants grown in soil.

Purpose: This study addresses the internalization of enteric viruses into spinach and green onion plants when grown in hydroponic solution contaminated with HAV and a common norovirus surrogate, murine norovirus (MNV).

Methods: Spinach and green onion sprouts were grown in Hoagland's solution containing 0.2% bacto agar, inoculated with HAV (7 log TCID50) or MNV (5.5 log PFU). After seven days, plants were washed with Virkon® to inactivate viruses present on outer surfaces prior to detection by qPCR infectivity studies. Survival of viruses in soil and homogenized spinach and green onions was determined by qPCR or by TCID50 and plaque assay in mammalian cell culture.

Results: Virus persistence and internalization were evaluated. HAV persisted in soil for 90 days; qPCR data indicated a 2.35 ± 0.7 log genomic copies/g reduction in titer. Both HAV and MNV were able to persist in homogenized green onion and spinach tissue for 10 days with no significant reduction in titer as shown by TCID50 and plaque assay ($P < 0.05$). When grown in hydroponic solution, 6.05 ± 0.12 log genomic copies of HAV and 3.25 ± 0.23 log PFU of MNV were internalized into spinach. Similarly, 4.08 ± 0.5 PFU of MNV was internalized into green onions.

Significance: Many commercial crops are grown hydroponically. HAV and MNV internalize and persist within spinach and green onion tissues increasing the potential for contamination. This study increases the knowledge of virus-plant interactions and provides information useful for risk assessment.

T4-08 Controlled Environment Assessment of Preharvest Internalization and Transference of *Salmonella* into Melon Vines from Irrigation Water Using a Tube Nucleation Assay

CAROL B. D'LIMA, Kin H. Tan and Trevor V. Suslow
University of California-Davis, Davis, CA, USA

Introduction: Melon vines harbor endophytes, including enteric bacteria that may enter fruit at some point in maturation. The risk of systemic transfer of pathogens from roots to fruit needs clarification.

Purpose: Evaluate potential for dose-dependent root uptake of *Salmonella* in controlled environments by tube nucleation assay (TNA).

Methods: Cantaloupe ("Oro Rico" F1) vines were irrigated at 4—5 leaf stage with a single inundative treatment, at various doses, of *S. Poona*, transformed with the *ice*C gene. Inoculum was applied to the rhizosphere by sub-irrigation. "Contamination" levels were uninoculated, log 2, 4, 6 and 8 CFU/ml of water applied. Successive internodal sections of log 8 CFU/ml irrigated plants were also analyzed to determine the extent of acropetal systemic movement. Following surface sterilization, detection of up to 80 vines/treatment was conducted by TNA, confirmed by culture and molecular techniques.

Results: Recovery of internalized *Salmonella* after one day was detectable within 0, 7, 57 and 96 percent at log 2, 4, 6 and 8, respectively. After one week 14% of the vines inoculated at the highest concentration were positive for internalization. *Salmonella* was detected among 90 percent of the basipetal internodal segments among vines analyzed on Day 1. Detection was reduced to 10 percent after 2 weeks. Analysis of successive internodal sections on Day 1 showed a decrease to 30% recovery in the adjoining section, dropping to 10% in the top most section. Basipetal sections analyzed after 2 weeks were consistent with earlier outcomes (< 10% positive).

Significance: These model system experiments indicate that, upon a contamination event of a high dose of *Salmonella* into the soil via irrigation water, there exists the high potential for immediate, but apparently transient, transfer of enteric pathogens into melon vines. Systemic transference to fruit must be considered. However, results indicate that there is a very high threshold of contamination from irrigation water to make this plausible.

T4-09 The Effect of Total Organic Carbon Content and Repeated Irrigation on the Persistence of *Escherichia coli* O157:H7 on Baby Spinach

DAVID T. INGRAM, Cheryl Mudd, Sean Ferguson, Dallas Hoover, Kalmia E. Kniel and Manan Sharma
USDA-ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA

Introduction: In response to U.S. foodborne illnesses caused by contaminated spinach, growers have adopted regulations stated in the California Leafy Greens Marketing Agreement (LGMA). The LGMA permits a maximum population mean of 126 Most Probable Number (MPN) generic *E. coli* per 100 ml irrigation water. These metrics, however, do not include other measurements of water quality that might enhance the epiphytic survival of bacterial pathogens.

Purpose: We investigated the effect of repeated irrigation of baby spinach plants with water containing differing levels of total organic carbon (TOC) on the epiphytic survival of enterohemorrhagic *E. coli* O157:H7 (EHEC).

Methods: Sterile, diluted bovine manure was prepared to contain TOC concentrations of 0ppm, 12 — 15 ppm and 120 — 150 ppm. An inoculum of three nalidixic acid-resistant EHEC strains was introduced into each irrigation solution at either a high (5—6 log CFU/100 ml) or low (0—1 log CFU/100 ml) population, and sprayed twice a week onto baby spinach plants using a fine mist air-brush. A 3-tube MPN was used to determine the persistence of EHEC on the aerial tissues, which were harvested daily.

Results: Plants irrigated with high EHEC populations, regardless of TOC levels, showed a 3-log reduction within the first 24 h. EHEC then exhibited low levels of persistence for up to 16 days on all treatments, ranging from 76.4 MPN per plant (day 1) to 0.40 MPN per plant (day 16). For the irrigation events containing low EHEC populations, no viable EHEC were detected after 24 h. The TOC content of irrigation water did not affect the persistence of EHEC on baby spinach tissues.

Significance: This study suggests that EHEC populations in irrigation water which comply with LGMA guidelines will not survive for more than 24 h on foliar surfaces of spinach plants. The TOC content of irrigation water did not influence EHEC survival.

T4-10 Effect of Modified Atmosphere on Persistence and Virulence Expression of *Escherichia coli* O157:H7 on Shredded Lettuce

Sudesna Lakshman, Sean Ferguson, David T. Ingram, Jitendra R. Patel, Yaguang Luo and MANAN SHARMA
USDA-ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA

Introduction: Fresh-cut lettuce contaminated with *Escherichia coli* O157:H7 has caused multiple foodborne outbreaks. Packaging and storage temperature may affect the persistence and ability of the pathogen to cause illness.

Purpose: To evaluate packaging atmospheres and storage temperatures on the behavior of *E. coli* O157:H7 on shredded lettuce.

Methods: Shredded lettuce was inoculated with 4.07 CFU/g of nalidixic-acid resistant *E. coli* O157:H7, and packaged into: gas permeable films with an initial oxygen level set at 2 Pka (treatment A), micro-perforated films (treatment B) or non-gas permeable films (treatment C). Inoculated and uninoculated samples were stored at 4 or 15°C for up to 10 days. Populations of *E. coli* O157:H7 and aerobic heterotrophs were enumerated on MacConkey and tryptic soy agar, respectively. *E. coli* O157:H7-RNA from inoculated lettuce at 15°C was analyzed by reverse transcriptase real-time PCR for expression of 5 virulence factors (VF) on each day - *stxII*, *eae*, *iha*, *rfbE*, and *espA*. Two replicate experiments were performed.

Results: No significant ($P < 0.05$) differences in *E. coli* O157:H7 populations were observed among the three packaging atmospheres at 4°C. At 15°C, *E. coli* O157:H7 populations under treatment B were significantly lower than other treatments on days 7 and 10. Heterotrophic populations from uninoculated lettuce under treatment B were significantly greater than populations from other treatments on day 3 at 4°C and day 1 at 15°C. Expression of *stxII*, *eae*, *iha*, and *rfbE* under treatment B was significantly greater than under other treatments on day 7, but significantly lower than under treatment C on day 10.

Significance: Different packaging atmospheres affected the persistence and virulence factor expression of *E. coli* O157:H7 on lettuce at 15°C, as well as heterotrophic populations at 4 and 15°C. Packaging atmospheres of fresh-cut lettuce may affect the ability of *E. coli* O157:H7 to cause illness.

T4-11 Inactivation of *Escherichia coli* O157:H7 on Spinach and Parsley Using Low-energy X-ray Irradiation

SCOTT R. MOOSEKIAN, Sanghyup Jeong, Bradley P. Marks and Elliot T. Ryser
Michigan State University, Food Science and Human Nutrition, East Lansing, MI, USA

Introduction: Several multistate *Escherichia coli* O157:H7 outbreaks in the United States have been linked to consumption of baby spinach and parsley. These outbreaks highlight the inability of standard commercial washing practices to reduce bacterial pathogens to safe levels on fresh produce, re-emphasizing the need for improved microbial inactivation strategies.

Purpose: In this study, low-energy X-ray irradiation was investigated to inactivate *E. coli* O157:H7 on the surface of baby spinach and flat-leaf parsley.

Methods: Baby spinach leaves obtained at retail were round-cut (5.07 cm^2) using a sterile cork borer, whereas single flat-leaf parsley leaves were removed from the stem and digitally imaged with these pixilated images factored into a computer algorithm to determine the leaf surface area. Both products were dip-inoculated in a 3-strain cocktail of *E. coli* O157:H7 (K3995 - 2006 spinach outbreak, K4830 - 2006 lettuce outbreak A, K4492 - 2006 lettuce outbreak B) (9.43 log CFU/ml) to obtain ~5.8 log CFU/cm^2 on the leaves. After 24 h of storage at 4°C, triplicate samples were processed in a prototype low-energy X-ray irradiator (Rayfresh Foods, Ann Arbor, MI). Each side of the sample was irradiated to achieve five combined surface doses of up to 0.176 kGy for spinach and 0.205 kGy for flat-leaf parsley, with these dose levels confirmed using radiochromic film dosimeters. *E. coli* O157:H7 survivors were quantified in sample homogenates by plating appropriate dilutions on Sorbitol MacConkey Agar containing cefixime and tellurite (no evidence of sublethal injury seen in previous work), followed by incubation at 37°C for 24 h.

Results: The analyses yielded D_{10}-values of 0.035 and 0.052 kGy for *E. coli* O157:H7 inoculated spinach and parsley, respectively. These are similar to the value reported for iceberg lettuce (0.039 kGy using low-energy X-ray). Given the enhanced efficacy of X-ray irradiation, the dose required to achieve a 5 log reduction (0.175 and 0.260 kGy) would be significantly lower than the FDA maximum allowable dose of 4 kGy for spinach and lettuce.

Significance: Based on these findings, low-energy X-ray irradiation appears to be an effective means for inactivating *E. coli* O157:H7 on spinach and parsley, and is also likely applicable to other leafy green vegetables.

T4-12 Inactivation of *Salmonella* on Tomato Surfaces Using Gaseous Chlorine Dioxide Treatment

BASSAM A. ANNOUS, Angela Burke and Joseph E. Sites
U.S. Dept. of Agriculture, ARS-ERRC, Food Safety Intervention Technologies, Wyndmoor, PA, USA

Introduction: Tomatoes have been implicated in fourteen outbreaks of salmonellosis in the U.S. since 1996. Previous research in our laboratory has documented the inadequacy of washing processes to inactivate and/or remove microorganisms on tomatoes, including human pathogens, due to biofilm formation and inaccessibility of microbial attachment sites to washing systems.

Purpose: The objective was to develop gaseous chlorine dioxide (ClO_2) treatment capable of inactivating human pathogens attached to inaccessible sites within biofilm on the tomato surfaces.

Methods: Tomatoes were inoculated with *Salmonella* Poona RM 2350 to an approximate final concentration of 5 log CFU/g, and stored at 4°C for 24 h prior to treatment. Tomatoes were fumigated with ClO_2 for up to 6 h in a closed chamber that was developed at ERRC, using two different technologies for generating ClO_2. Following treatment, residual (non-injured and injured) populations of *Salmonella* Poona on tomatoes were enumerated using XLT-4 selective agar and TSA overlaid with XLT-4 agar media, respectively.

Results: There was in excess of 4.5 log CFU/gm reduction in *Salmonella* Poona populations following ClO_2 treatment for 6 h. Population reductions following ClO_2 treatment were similar irrespective of the technology used to generate ClO_2. The treatment helped increase the shelf life of the tomatoes by reducing the spoilage microorganism populations on the surface, and did not seem to have adverse effects on the quality of this commodity.

Significance: The work presented here showed that gaseous $CClO_2$ treatment of tomatoes was able to inactivate *Salmonella* Poona cells attached to inacessible sites such as the stem scar area. Also, this treatment was shown to extend the shelf life at 4°C and had no adverse effects on the quality of the tomatoes.

Technical

T5-01 Time-temperature Dependent Growth Patterns of *Salmonella* spp. in a Model Food System with Natural Microflora

AMIT MOREY, Shelly R. McKee and Manpreet Singh
Auburn University, Dept. of Poultry Science, Auburn, AL, USA

Introduction: The USDA-FSIS *Salmonella* Initiative Program requires post-chill temperatures to be below 40°F as a critical control point to reduce *Salmonella* prevalence in raw poultry products. Alternative parameters to regulations on time and temperature during chilling of poultry have potential to achieve the food safety objective that the FSIS is pursuing. Models predicting *Salmonella* growth at abused temperatures are developed using sterile medium without competitive microflora, while studies on growth dynamics of the pathogen at temperatures ranging from 4 to 10°C (40 to 50°F) are of particular interest to poultry processors, yet lacking.

Purpose: Experiments were conducted to study the growth of *Salmonella* Typhimurium and Heidelberg at various temperatures in brain heart infusion broth (BHI) and non-sterile chicken slurry (CS).

Methods: Nalidixic acid (60 ppm) resistant *Salmonella* Typhimurium and Heidelberg were inoculated (3 log CFU/mL) separately in non-sterile CS and BHI. The inoculated medium (5 mL/well) was dispensed in a 12-well plate (5 wells each of BHI and CS; and 2 wells as negative control) and maintained at 4, 7 and 10°C. Samples were taken every 24 h for 6 days, spread plated on XLT4 agar and Naladixic acid and incubated at 37°C for 24 h. Simultaneously, inoculated CS was incubated at 37°C and sampled every h for 18 h. Bacterial enumerations were reported as log CFU/mL. Analysis of variance was conducted to determine significant differences in growth patterns at different temperatures ($P < 0.05$).

Results: Populations of *S.* Typhimurium and Heidelberg in CS and BHI were not different ($P > 0.05$) over a 6-day period at all temperatures. Significant differences ($P < 0.05$) in the populations of *S.* Typhimurium and Heidelberg were observed at 4°C as compared to 7 and 10°C, irrespective of the medium. At 37°C, populations of *S.* Typhimurium and Heidelberg increased from 2 to 9 log CFU/mL in CS with natural flora over 18 h period.

Significance: Growth patterns of *Salmonella* at near refrigeration temperatures during carcass chilling can be useful to develop mathematical models and help processors determine that they are maintaining process control.

T5-02 Analysis of ALLRTE and RTE001 Sampling Results for *Salmonella* Species, Calendar Years 2005—2008

Kristina E. Barlow, Stephen W. Mamber, TIM B. MOHR and Evelyne Mbandi
USDA-FSIS, Washington, D.C., USA

Introduction: Salmonella may survive in underprocessed meat and poultry products (e.g., beef jerky) or contaminate Ready-to-Eat (RTE) meat and poultry products from the production environment (e.g., raw materials, food handlers, or animal vectors). In addition to *Listeria monocytogenes*, RTE meat and poultry products samples from the Food Safety and Inspection Service's (FSIS) ALLRTE and RTE001 sampling programs are tested for *Salmonella* species. ALLRTE is a random sampling program for all types of RTE products, while RTE001 is a risk-based sampling program that targets post-lethality exposed RTE products.

Purpose: To obtain, tabulate and analyze ALLRTE and RTE001 sampling results for *Salmonella* collected over a 4-year period.

Methods: The FSIS analyzed results of *Salmonella* testing of meat and poultry product samples collected under the ALLRTE and RTE001 sampling programs for calendar years 2005 through 2008. Samples were tested using methods in the FSIS Microbiology Laboratory Guidebook.

Results: The analyses included 11,823 ALLRTE samples collected from 1,989 establishments and 33,277 RTE001 samples from 2,556 establishments in calendar years 2005 through 2008. Results showed low incidences of *Salmonella*-positive samples from the ALLRTE and RTE001 sampling program, with 8 and 14 positives, respectively over the study time period. Positive product results averaged 0.07% for ALLRTE samples (range, 0 to 0.13%) and 0.04% for RTE001 samples (range, 0.01% to 0.08%). Similarly, percentages of establishments with *Salmonella*-positive samples ranged from 0 to 0.27% for ALLRTE and from 0.07 to 0.54% for RTE001. Three types of products (i.e., head cheese, pork barbecue and sausage products) accounted for about half of all *Salmonella*-positive samples.

Significance: This detailed examination of *Salmonella* species data from the ALLRTE and RTE001 sampling programs will help guide changes in policies, regulations, inspection procedures and enforcement actions relevant to the prevention of *Salmonella* contamination in RTE products.

T5-03 Molecular Characterization and Serotyping of *Salmonella* Isolated from the Shell Egg Processing Environment

MICHAEL MUSGROVE, Karen L. Liljebjelke, Kimberly D. Ingram and Arthur Hinton
USDA-ARS, Egg Safety and Quality Research Unit, Athens, GA, USA

Introduction: Salmonellosis may be contracted by the consumption of raw or undercooked eggs. In order to develop effective sanitation practices it is helpful to understand the location of *Salmonella* reservoirs in processing environments. Shell egg processing reservoirs for *Salmonella* have not been well characterized, previously.

Purpose: A study was conducted to determine which equipment and facility surfaces in the shell egg processing environment were contaminated with Salmonella. Isolates were further characterized to determine serotype and relative molecular similarity.

Methods: On 7 visits, 35 surfaces were swabbed individually using sterile gauze pads soaked in 10 mL of phosphate buffered saline. Each sample was subjected to a cultural procedure to recover *Salmonella*: pre-enrichment in buffered peptone overnight at 37°C, selective enrichment in TT and RV broths at 42°C for 18—24 h, selective plating (BGS and XLT-4) incubated at 37°C for 18—24 h, biochemical reactions (LIA and TSI slants) incubated at 37°C for 18—24 h,and confirmed using poly-clonal anti-sera. Isolates were re-streaked three times and saved prior to subsequent analyses. Isolates were sero-typed by the Kauffman-White scheme. DNA from each isolate was harvested and purified using a commercial kit. DNA was analyzed by repetitive *Enterobacteriaceae* palindrome polymerase chain reaction using an automated system. Scatter plots and dendrograms were created to visualize DNA similarity.

Results: Salmonella serotypes recovered were Braenderup (1), Kentucky (2), Heidelberg (12), Typhimurium Copenhagen (Tyco-1), and Typhimurium (7). *Salmonella*-positive surface locations were wash tanks (Kentucky, Typhimurium, Tyco), drains (Braenderup, Heidelberg), and post-wash equipment surfaces (Heidelberg, Kentucky). All Typhimurium were recovered from the wash tanks at a single rep and were similar (95%). Drain isolates were similar regardless of rep (90%). Almost 70% of the isolates were recovered from wash tank equipment surfaces from 2 reps. However, drains were a *Salmonella* reservoir in 4/7 reps.

Significance: Wash tanks and drains were important *Salmonella* reservoirs in the shell egg processing environment. These results indicate that more effective sanitation is needed for washing equipment surfaces and drains.

T5-04 Persistent and Sporadic *Listeria monocytogenes* Strains in Fermented Meat Sausage Processors

VâNIA FERREIRA, Joana Barbosa, Kitiya Vongkamjan, Andrea Moreno Switt, Tim Hogg, Paul Gibbs, Paula Teixeira and Martin Wiedmann
Escola Superior de Biotecnologia, CBQF, Porto, Portugal

Introduction: Persistence of *Listeria monocytogenes* in food-associated environments represents a key factor for contamination of foods with this pathogen. While several authors attempted to identify specific characteristics that confer a better survival and resistance capability of *L. monocytogenes* strains in the food-processing environment, including biofilm formation and resistance to disinfectants etc., little attention is being given to the potential contributions to persistence of prophages and phage resistance.

Purpose: To identify persistent as well as transient strains associated with production of fermented meat sausages in Northern Portugal, samples of raw material and finished product from 7 processors were collected at multiple dates either at retail establishments or at processing plants. We also investigated whether presence of lysogenic prophages in *L. monocytogenes* and phage susceptibility may be associated with strain persistence in the environment.

Methods: A total of 202 *L. monocytogenes* isolates were characterized by pulsed-field gel electrophoresis (PFGE) typing. A subset of 41 isolates, including (i) 19 representing strains that persisted in a given processor and (ii) 22 representing strains that did not show evidence for persistence were tested for lysogen induction. Twenty-six prophages where induced and their lytic spectrum against the 41 strains of *L. monocytogenes* was investigated.

Results: For all seven processors PFGE provided evidence for persistence of *L. monocytogenes* for 10 to 32 months. Results obtained revealed that (i) there was no significant association between lysogeny and persistence (ii) molecular serogroup D (4b, 4d, and 4e) isolates were more susceptible to phages as compared to serogroup A (1/2a and 3a) or B (1/2b, 3b, and 7) isolates, (iii) there was no evidence for differences in phage susceptibility between persistent and sporadic strains.

Significance: Our data support that while *L. monocytogenes* serotypes differ in phage resistance, phage resistance does not seem to be associated with strain persistence.

T5-05 Impact of Chlorine and Temperature on *Listeria monocytogenes* Survival Growth Behavior on Ready-to-Eat Meats

SHIOWSHUH SHEEN, Cheng-An Hwang and Vijay Juneja
USDA-ARS-ERRC, Wyndmoor, PA, USA

Introduction: Listeria monocytogenes (Lm) continues to pose a food safety hazard in ready-to-eat (RTE) meat due to potential cross-contamination. Chlorine is commonly used to sanitize processing equipment. However, Lm may survive on processing equipment surfaces, which then contaminate food products.

Purpose: The objective of this study was to characterize the behavior of chlorine-exposed Lm on RTE meat products stored at 4, 8, or 16°C.

Methods: A 2-strain cocktail of Lm serotype 4b was pre-treated with chlorine (0, 25, and 50 ppm) for one hour, and then inoculated onto RTE meat surfaces to obtain about 3.0 log CFU/g. Samples were stored at three temperatures (4, 8, and 16°C) and Lm was enumerated at frequent intervals. The lag phase and growth rate of Lm were estimated using DMFit (Combase website, Baranyi's model).

Results: Our results indicated that Lm growth was repressed by chlorine treatment. The lag phase of Lm after exposure to 0 ppm of chlorine (4.2 days) was shorter than that of Lm shocked with 25 ppm (5.4 days) and 50 ppm (6.8 days) at 4°C. The lag phase decreased with an increase in temperature. For example, at 25 ppm, lag times were 5.2, 3.8 and 2.6 days for 4, 8 and 16°C, respectively, and increased with an increase in chlorine concentration. At 16°C, lag times were 1.2, 2.6, and 4.0 days for 0, 25, and 50 ppm, respectively. However, the growth rate increased with an increase in temperature and decreased with an increase in chlorine. The growth rate and lag phase as a function of temperature and chlorine concentration can be described using a modified Ratkowsky model and a modified Zwietering model, respectively.

Significance: The results showed that the use of chlorine can suppress the growth of Lm. The predictive models developed will contribute to microbial risk assessment of RTE meats.

T5-06 Comparison of Data from FSIS Routine and Intensified Sampling Programs for *Listeria monocytogenes* from Ready-to-Eat Establishments

KRISTINA E. BARLOW, Stephen W. Mamber, Evelyne Mbandi and Tim B. Mohr
USDA-FSIS, Washington, D.C., USA

Introduction: The U.S. Dept. of Agriculture (USDA) Food Safety and Inspection Service (FSIS) samples Ready-to-Eat (RTE) meat and poultry products and processing environments for *Listeria monocytogenes* (Lm) through routine sampling (RLm) and intensified verification testing (IVT) programs. RLm sampling is performed at each RTE establishment at least once every four years, while IVT is performed in response to positive results from all Lm testing programs.

Purpose: To compare data from FSIS routine and intensified sampling programs for Lm from RTE establishments.

Methods: Samples were collected from FSIS establishments producing post-lethality exposed RTE meat and poultry products. Product samples were collected in the final packaged form. Food contact surface (FCS) and environmental samples were collected using sterile spongesicles hydrated with Dey-Engley (DE) broth. Samples were analyzed using methods in the FSIS Microbiology Laboratory Guidebook. Samples were collected proportional to establishment size (RLm) or based on investigative needs (IVT).

Results: From 2005 to 2008 a total of 135/16,284 (0.83%) of RLm samples and 486/13,241 (3.67%) of IVT samples were positive. For RLm, 7/2,633 (0.27%) of product, 38/8,945 (0.42%) of FCS and 90/4,706 (1.91%) of non-FCS environmental samples tested positive. For IVT, 68/2148 (3.17%) of product, 125/5,915 (2.11%) of FCS and 293/5,178 (5.66%) of non-FCS tested positive. From 2005 to 2008, RLm positives decreased slightly from 9/795 (1.13%) to 59/6,005 (0.98%), while IVT positives decreased from 164/3,060 (5.36%) to 135/3,681 (3.67%).

Significance: Overall, percentages of positives were highest for the IVT program, likely because of the investigative nature of IVT. For the RLm program, positives were highest for the non-FCS, followed by the FCS, and product samples. For IVT, positives were highest for non-FCS, followed by product, and FCS. FSIS uses the results from both sampling programs to identify cross contamination and harborage and to help protect public health.

T5-07 Comparison of a Novel Sample Collection Device and Cellulose Sponge for the Collection of *Escherichia coli* from Beef Carcasses

KEVIN CHURCH, Jared G. Maughan, Kelly M. Tesar and Amanda S. Vos
Microbial-Vac Systems, Inc., Jerome, ID, USA

Introduction: Current collection methods for the sampling of beef carcasses routinely employ cellulose sponges. The M-Vac sampling system was designed as an alternative to conventional methods for microbial sampling in diverse applications, including surface sampling of beef tissue. The device utilizes directed spray of sterile diluent coupled with vacuum suction to collect a sample from the surface of interest. Comparative studies of the M-Vac against the cellulose sponge are required to determine performance in collection of samples from beef carcasses.

Purpose: To evaluate the performance of the M-Vac sampling system in comparison to the cellulose sponge for collection of *E. coli* O157:H7 on artificially inoculated flank cuts from beef carcasses. A secondary objective was to determine if the sampling devices could potentially spread contamination to uncontaminated sites.

Methods: Flank cuts were obtained from hanging beef carcasses from a local slaughter facility. Duplicate 100 cm^2 sites were outlined on external surface and artificially inoculated with *E. coli* O157:H7 ATCC 700728 at either low (5.0×10^{-2} CFU/cm^2) or high (1.5 CFU/cm^2) inoculum levels. Following inoculation, flank sections were cooled (4°C) for 18 h prior to sampling. M-Vac sampling was conducted with 40 ml of Butterfield's Buffer per site. Cellulose sponges were hydrated with Butterfield's Buffer prior to sampling. Following sampling of an inoculated site, sampling devices were used to sample a secondary, non-inoculated site before processing. The secondary sites were excised using a sterile scalpel. Samples were enriched in mEHEC broth and incubated at 42°C for 18 h prior to analysis by PCR for presence/absence.

Results: The rate of positive samples for *E. coli* O157:H7 from inoculated sites was higher for the M-Vac than the sponge. Mantel-Haenszel Chi-square analysis of the data revealed a significant difference in the rate of positive samples from the artificially inoculated sites between the M-Vac and sponge at low inoculum level ($X^2 = 7.84$), but no significant difference at the high inoculum level. Excision results from secondary sampling sites showed that the sponge had a significantly higher rate of positive samples at high inoculum ($P < 0.05$), but no significant difference was observed at the low inoculum.

Significance: These data suggest that that the M-Vac may be a suitable alternative to sponge sampling of beef carcasses. The M-Vac may provide a more representative sample of surface bacteria when present in low numbers, and potentially reduce the risk of spreading contamination to different areas on the carcass.

T5-08 Three Sampling Methods to Recover Bacterial Populations on Beef Trimmings in Commercial Settings

MICHAEL DE LA ZERDA, Mansour Samadpour and Mohammad Koohmaraie
IEH Laboratories and Consulting Group, Meat Division, Lake Forest Park, WA, USA

Introduction: Escherichia coli O157:H7 is a potential pathogen in beef trimmings. Implementing good dressing procedures prevents pathogens from entering the meat supply, and along with a robust sampling and testing program can verify the efficacy the food safety system. Identification of beef trimmings contaminated with *E. coli* O157:H7 is reliant upon the execution of a robust sampling program.

Purpose: Identify a sampling method to recover a larger quantity of organisms from beef trimmings to provide sufficient opportunity to accurately detect organisms within the volume of beef trimmings available for sampling.

Methods: Forty-two combo bins of beef trimmings (85% lean) in a commercial processing facility were sampled using Excision 60 pieces (E), surface shaving (SS), and core drilling (CD). Samples were tested for Aerobic Plate Count (APC), Total Coliform Count (TCC), and Biotype I *E. coli* (ECC). Results were compared on the basis of log CFU/sample, log CFU/g, and log CFU/375 g.

Results: T-tests determined log CFU results for all comparisons were equivalent for E and CD ($P > 0.05$), while SS recovery was higher across all comparisons for all tests ($P < 0.05$). SS recovery was higher by 1.08, 0.85, and 0.58 log CFU/sample for APC, TCC, and ECC compared to the next numerically higher value recovered by CD. SS recovery was higher by 1.27, 1.04, and 0.77 log CFU/g for APC, TCC, and ECC compared to CD. SS recovery was higher by 1.27, 1.03, and 0.77 log CFU/375 g for APC, TCC, and ECC compared to CD.

Significance: Sampling method may be a limiting factor in the ability of commercial beef operations to recover a large amount of organisms in a sample. Surface shaving collected more organisms overall compared to Excision 60 pieces or core drilling, which were equivalent.

T5-09 Prevalence of *Clostridium difficile* in Various Types of Ground Meat and Poultry Products

Jeff Bussey, Clarissa Sugeng, Jeffrey M. Farber and JOHN AUSTIN
Health Canada, Bureau of Microbial Hazards, Ottawa, ON, Canada

Introduction: The increasing incidence of community-acquired *Clostridium difficile* associated disease has raised concerns over the possibility of foodborne transmission of *C. difficile*. Recent surveys of ground beef for the presence of *C. difficile* indicate its presence in up to 20% of Canadian ground beef samples and up to 50% of U.S. ground meat samples.

Purpose: The current study was undertaken to confirm the presence of *C. difficile* in Canadian ground beef and determine whether other types of ground meats may contain this organism.

Methods: Seventy-five samples of retail ground meat and poultry products were tested for the presence of *C. difficile* using selective enrichment, followed by ethanol shock and plating on selective agar. Presumptive positive colonies were confirmed as *C. difficile* by detection of L-proline aminopeptidase activity and PCR detection of the triose phosphate isomerase gene.

Results: Clostridium difficile was found in 13 of the 75 samples tested (17.3% positive). *Clostridium difficile* was detected in every type of ground meat including 4 of 22 (18%) samples of ground beef, 1 of 9 samples of veal (11%), 4 of 12 (33%) samples of ground turkey, 1 of 15 samples of ground chicken (7%), 1 of 9 samples of ground pork (11%) and 2 of 16 (13%) samples of ground lamb.

Significance: The results confirm the presence of *C. difficile* in Canadian ground beef, and are the first demonstration of *C. difficile* in Canadian ground turkey and pork. This is the first report of *C. difficile* in ground chicken and lamb.

T5-10 The Control of *Salmonella* Typhimurium in Poultry: From Vaccination to Specific Immunotherapy

Anthony Pavic, Peter Groves and JULIAN M. COX
University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Introduction: Salmonella Typhimurium has been associated with poultry as a vehicle and vector for zoonotic infection in humans. As this serovar is prevalent in both humans and poultry in Australia, poultry vaccines (killed and attenuated) have been developed against it.

Purpose: The objectives of this study were to measure the serological and bacteriological response in vaccinated hens, the yolks of their eggs, and their live progeny, to challenge with 10^4 CFU *S.* Typhimurium, and to determine whether prophylactic immunotherapy (anti-Typhimurium IgY), administered through feed, would reduce carriage of the bacterium in chicks.

Methods: Commercially available killed vaccine was injected intramuscularly at 12 (primer) and 18 (booster) weeks of age (woa) into Cobb hens, which were serologically tested every two weeks post-primer to challenge with *S.* Typhimurium at 20 woa. Post-euthanasia (at 24 woa) the blood and eggs (serological) and caeca (bacteriological) were tested for *S.* Typhimurium. The progeny of vaccinated and non-vaccinated hens were challenged (10^4 CFU/ml) at 3 days of age (doa) and, at 20 doa, blood and caeca were tested. The egg yolks from vaccinated hens were collected and tested (ELISA) for the presence of IgY. From those with high titre (>1000), crude IgY was prepared and used to resuspend dried egg yolk powder. This suspension was fed to day-old chicks (at 3% of daily nutrient intake) prior to challenge (104 CFU) at 3 doa. At 10 doa, the chicks were euthanized and caeca cultured.

Results: The serum ELISA results post-primer showed slow seroconversion (8% positive at 14 and 16 woa, 26% at 18 woa and 39% at 20 woa) which still afforded significant (X^2 $P = 0.001$) protection to vaccinated hens, based on culture of caeca. Both serum (33% positive) and egg yolk (16% positive) ELISA titres means (n = 60) were significantly different (Students *t*-Test $P < 0.05$) between vaccinated and non-vaccinated hens. While negative for circulating anti-Typhimurium IgG, the progeny from vaccinated hens showed significantly (X^2 $P = 0.047$) lower colonization rates. The IgY titres in egg yolk, crude IgY extract and supplemented feed ranged from 181 to 1890/ml, 20 to 1000/ml and 40—400/g, respectively, throughout the trial. The populations of salmonellae in caeca were no different in treated (4.5 $\log_{10}$ CFU/g) and control (4.2 $\log_{10}$ CFU/g) chicks.

Significance: The vaccine afforded protection against *S.* Typhimurium in hens, and their progeny (day-old chicks). However, the prophylactic use of crude anti-Typhimurium IgY, supplemented into poultry starter feed, did not prevent or reduce colonization in chicks.

T5-11 Enhanced Recovery of *Campylobacter jejuni* from Chick Paper under Hatchery Incubation Conditions

Jeremy W. Chenu, Anthony Pavic and JULIAN M. COX
University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Introduction: *Campylobacter jejuni* is a foodborne human pathogen often associated with broiler (meat) chickens. The route of transmission in the broiler flock is unclear and although horizontal transmission is likely, vertical transmission may occur. Isolation from low-moisture samples using standard cultural techniques is difficult. As incubation in the hatchery may provide the ideal environment for persistence of *C. jejuni* because of its high temperature, relative humidity and CO_2 levels, such conditions may also improve recovery during analysis of environmental sources.

Purpose: To determine the recovery rates of *C. jejuni* from artificially inoculated chick paper during incubation under aerobic and enriched CO_2 (ca. 5% O_2, 10% CO_2, 85% N_2), high humidity and elevated temperature conditions.

Methods: Eighty chick paper samples (5 cm^2) were inoculated with ca. 10^2 CFU of a field isolate of *C. jejuni*. Half the samples were incubated aerobically at 38°C. The other forty samples were incubated in jars with a microaerobic atmosphere and high humidity at 38°C. Samples were removed and placed in 25 mL of 3M Tecra Campylobacter Enrichment Broth at 15 (10 samples), 30 (10 samples) and 60 (20 samples) mins. Following delayed selective enrichment, samples were streaked onto standard selective agars and screened using an ELISA.

Results: All papers were positive after 15 min. After 30 min, 9 of 10 papers were positive under modified storage whereas none were positive after aerobic storage. After 60 min, the results were 4 of 20 and 0 of 20, respectively. The ELISA and streak plating showed equivalence for the detection of positive samples.

Significance: As the hatchery pad paper dried during storage, *C. jejuni* died or became unculturable. However, under specialized storage conditions the cells were able to survive longer than under aerobic conditions. In the humid environment of the hatchery incubators, *C. jejuni* may be able to survive for a longer period than expected. Holding environmental samples under conditions emulating the hatchery may enhance recovery of *C. jejuni*.

T5-12 Validation of a High Throughput DNA Extraction and Real-time PCR Detection of *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes* and *Listeria* spp.

WENDY LAUER and Jean-Philippe Tourniaire
Bio-Rad Laboratories, Hercules, CA, USA

Introduction: To meet the demands of labs with large sample numbers, a high throughput DNA extraction procedure was developed using a 96-well microplate format. This procedure was evaluated followed by detection of target organism by real-time PCR.

Purpose: The objective of this study was to compare iQ-Check high throughput DNA extraction protocol and real-time PCR detection to the appropriate US reference method for detection of *E. coli* O157:H7 in raw ground beef, *Salmonella* spp. in raw chicken breast, raw ground beef, raw pork and fresh spinach, *Listeria monocytogenes* in deli turkey and *Listeria* spp. on stainless steel.

Methods: Matrices were inoculated with a low level of target organism and an MPN was determined on the day of analysis. Samples were processed with the high throughput DNA extraction procedure and according to reference method specifications. Chi-square analysis was calculated according to McNemar for paired samples and according to Mantel-Haenszel for unpaired samples.

Results: At a 95% confidence level, there was no significant difference between the PCR test method and the reference method. All samples that were positive by the test method were confirmed according to reference method protocol. The PCR test was able to identify more confirmed *E. coli* O157:H7 samples than the reference method.

Significance: Sample processing can lead to a large bottleneck in a microbiology lab. A high throughput DNA extraction followed by real-time PCR can meet the demands of high volume labs.

T6-01 Pathogen Presence and Indicator Organism Levels during Turned Pile Composting of Broiler Litter and Aerated, Static Pile Composting of Mixed Feedstocks

ACHYUT ADHIKARI, Andy Bary, Craig Cogger and Karen Killinger
Washington State University, Food Science, Pullman, WA, USA

Introduction: Assessing pathogen risk associated with composting is important to understand potential pathogen introduction in produce farming.

Purpose: In an on-farm, two-year study of composting (aerated static pile-ASP composting with mixed feedstocks, including dairy manure and broiler litter, and turned pile-TP composting of broiler litter), pathogen presence and indicator organism levels were examined.

Methods: In the process to further reduce pathogens (PFRP), ASP must reach 55°C for 3 days and TP for 15 days with 5 turns. Piles were built each year; five locations were sampled at three depths (surface, 30 cm, 60 cm); and homogeneous samples were collected after turning or moving. TP was sampled at initiation, turnings, moving and field application. ASP was sampled twice after meeting PFRP, moving and field application. Samples were quantified for fecal coliforms and generic *Escherichia coli* (*E. coli*), and analyzed for pathogen presence (*E. coli* O157 and *Salmonella*).

Results: For TP, at initiation through turn 3, fecal coliforms and generic *E. coli* in samples collected after turning were higher than samples collected at the surface, 30 and 60 cm before turning. Generic *E. coli* at all depths were < 0.5 log MPN/g dw. In Year 1, pathogens were detected at initiation, and turns 2, 3, and 5. In Year 2, *E. coli* O157 was detected at turns 2 and 5, and after meeting PFRP. For ASP, pathogens and generic *E. coli* were not detected in Year 1. Fecal coliforms remained below the detectable limit until moving (2.7 log MPN/g dw) and field application (4.7 log MPN/g dw). In Year 2, fecal coliforms at the surface remained above 3.0 log MPN/g dw. At field application, fecal coliforms were 3.5 log MPN/g dw and generic *E. coli* were 0.47 log MPN/g dw. Pathogens were detected in the initial feedstocks and at two sampling periods after meeting PFRP.

Significance: Pathogens were detected in samples with low generic *E. coli levels*. In Year 2, pathogens were detected in composted broiler litter and mixed feedstock compost after meeting PFRP using two composting methods. Pathogens were not detected at field application in this study.

T6-02 Produce Microbial Quality is Associated with Surface Microbial Contamination in Packing Sheds: An Assessment of Risk Factors for Produce Contamination

JUAN LEON, Elizabeth C. Ailes, Kizee A. Etienne, Lynette M. Johnston, Lee-Ann Jaykus and Christine L. Moe
Emory University, Hubert Dept. of Global Health, Atlanta, GA, USA

Introduction: Over the past decade, several high profile produce-associated outbreaks were thought to have originated with a contamination event(s) at the farm or packing shed. There is a need to prospectively identify and quantify risk factors for produce contamination at the farm and packing shed.

Purpose: The goal of this study was to determine whether microbial levels on produce were associated with microbial levels on equipment surfaces in the packing shed, and if so, to quantify the degree of association.

Methods: A total of 292 produce samples (cabbage, cilantro, cantaloupe, celery, parsley, turnip greens) and 292 matched equipment surface swab samples from 6 locations (bin, wash-tank, turn-table, conveyor belt, rinse-cycle, box) were collected from eight packing sheds in the southwestern United States between November 2000 to December 2003. To identify the potential for microbial pathogen contamination, these samples were assayed by enumerative tests for proxy indicators including coliforms, *Enterococcus*, and *Escherichia coli*. We used the Spearman's rank correlation test and multivariate regression analyses to examine the association between indicator organisms on produce and equipment surfaces.

Results: We found that specific produce samples (e.g., cilantro 42%) and equipment surfaces (e.g., conveyor belt 38%) had high prevalence of *E. coli*, a marker of fecal contamination. In both univariate and multivariate analyses, specific microbial indicators on produce samples were significantly associated with the same microbial indicators on equipment surfaces (e.g., *Enterococcus* on produce and *Enterococcus* on equipment surfaces). These data suggest that equipment surfaces may contaminate produce or produce may contaminate equipment surfaces but unfortunately our data do not allow for determination of causality.

Significance: Our evidence suggests that targeted interventions to reduce equipment surface contamination would decrease produce microbial contamination and may provide some protection for consumer health.

T6-03 Attachment, Persistence and Infectivity of *Cryptosporidium parvum* Oocysts in Fresh Produce

DUMITRU MACARISIN, Monica Santin, Gary Bauchan and Ronald Fayer
USDA-ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA

Introduction: Cryptosporidium parvum is an environmentally resistant, abundant, and ubiquitous protozoan parasite that causes severe diarrheal disease in humans and livestock. Consumer dietary preference towards organically grown fresh produce, which are largely fertilized with composted or fresh manure, might result in a higher occurrence of foodborne outbreaks of *Cryptosporidium*. Irrigation waters were also suggested among major routes of *Cryptosporidium* contamination of fresh produce.

Purpose: The objective of this study was to elucidate the ability of *C. parvum* to attach and persist in fruits and vegetables exposed to parasite oocysts *via* contaminated water. Also, of importance was to evaluate if *C. parvum* oocysts persisting in vegetal matrices remain infectious.

Methods: Fruits and leafy greens were experimentally exposed to water containing *C. parvum* oocysts. Low temperature scanning electron microscopy and laser scanning confocal microscopy were used to examine vegetal matrices for the presence of the parasite. After several washing methods fruits and vegetables were tested for persistence of parasite oocysts by targeting and amplifying *C. parvum* DNA. Infectivity of *C. parvum* oocysts in produce was assessed by mouse infectivity assays.

Results: Under experimental conditions, oocysts of *C. parvum* strongly adhered to apples and leafy vegetables and resisted removal by washing techniques recommended for the recovery of the parasite oocysts from fresh produce. Oocysts were localized on the surface and within pores of apple peel, spinach leaves and scallions. An adjacent amorphous extracellular matrix observed on and around oocysts appeared to be involved in the adherence to plant surfaces. The parasite remained infectious on apples for up to one month under conditions of postharvest storage.

Significance: Under experimental conditions waterborne *C. parvum* can adhere, persist and remain infectious on produce meant to be eaten raw. Extension of these findings to the possibility and likelihood that this occurs under natural conditions raise concerns regarding food safety.

T6-04 Efficacy of Commercial Produce Sanitizers against *Escherichia coli* O157:H7 in a Pilot-scale Leafy Green Processing Line

GORDON R. DAVIDSON, Annemarie L. Buchholz, Paul J. Sirmeyer, Ewen C. Todd and Elliot T. Ryser
Michigan State University, Food Science and Human Nutrition, East Lansing, MI, USA

Introduction: During processing of leafy greens, chemical sanitizers are routinely added to the recirculating washwater to reduce the persistence and spread of bacterial pathogens. Although the effectiveness of these sanitizers against various foodborne pathogens is well documented from numerous bench-top studies, the microbial reductions seen in such small-scale experiments are not necessarily directly transferable to commercial-sized processing lines.

Technical

Purpose: The goal of this study was to assess the ability of three commonly used commercial sanitizers to reduce *Escherichia coli* O157:H7 populations on inoculated shredded lettuce, in circulating wash water, on equipment surfaces, and in centrifugal waste-water during simulated commercial processing.

Methods: Heads of iceberg lettuce (5.4 kg) were cored, dip-inoculated with a 4-strain avirulent, GFP-labeled *E. coli* O157:H7 cocktail at 6 log CFU/g, shredded, and conveyed to the top of a flume tank containing 890 L of recirculating water to which ~0.01% (w/v) lettuce solids and the sanitizer were added. Fourteen mesh produce bags were then filled with shredded lettuce (25 g/bag) from the conveyor, exposed to water with/without 30 ppm free chlorine (XY-12, Ecolab, St. Paul, MN), 30 ppm peroxyacetic acid (Tsunami 100, Ecolab) or 30 ppm mixed peracid (Tsunami 200, Ecolab) in the flume tank for 90 s, dried on a shaker table and then spin-dried. Water (50 ml) and the previously bagged 25-g lettuce samples were collected every 10 s and added to neutralizing buffer. Additional samples included lettuce after shaker and centrifugal drying, equipment surfaces (100 cm^2) and the centrifugation water. *E. coli* O157:H7 was quantified on trypticase soy agar containing 0.6% (w/v) yeast extract and 100 ppm ampicillin after 24 h of incubation at 37°C.

Results: After 90 s of lettuce washing, XY-12, Tsunami 100 and Tsunami 200 respectively decreased *E. coli* O157:H7 populations 3.70, 0.74 and 2.06 log CFU/ml in the recirculating wash water compared to the water control. Regardless of the sanitizer used, *E. coli* O157:H7 populations on the lettuce generally decreased ≤ 1 log by the end of processing. Using these sanitizers, the pathogen was detected in the centrifugation water and on the equipment surfaces at levels up to 5.48 log CFU/ml and 5.08 log CFU/100 cm^2.

Significance: These widely used produce sanitizers proved to be far less effective than reported in previous bench-top studies and can only be relied upon for maintaining a low microbial load in the wash water.

T6-05 Enhanced Removal of Noroviruses from Fresh Fruits and Vegetables by Combination of Surfactants and Sanitizer

ASHLEY PREDMORE, Claire Herbert and Jianrong Li
The Ohio State University, Dept. of Food Science and Technology, Columbus, OH, USA

Introduction: Fruits and vegetables are a major vehicle for the transmission of foodborne enteric viruses. One major foodborne virus, human norovirus, accounts for more than 40% of fresh produce-associated disease outbreaks. However, the majority of food safety research studies focus on bacterial contamination, with little attention to viral contamination in fresh produce. It has been reported that commonly used sanitizers are ineffective at removing foodborne viruses from fresh produce. Therefore, development of new interventions to remove noroviruses from fresh fruits and vegetables is desperately needed.

Purpose: The objective of this study is to determine whether surfactants can enhance the sanitization of noroviruses from fresh fruits and vegetables.

Methods: Cultivable murine norovirus (MNV-1) was inoculated onto 50 g samples of either whole fresh strawberries or romaine lettuce at a concentration of 3.0×10^7 PFU/g. The samples were shaken for 1 hour to allow attachment of viruses to the fresh produce. To enhance the removal of the viruses, four surfactants, sodium dodecyl sulfate (SDS), Nonidet P-40 (NP-40), Triton X-100, and Tween 20, were added to a chlorine solution. The fresh produce was washed with these sanitizers, and the amount of surviving virus in the fresh produce was quantified by plaque assay.

Results: Our results showed that all four surfactants significantly enhanced the removal of viruses from fresh fruits and vegetables. As expected, tap water alone and traditional chlorine solution (200 ppm) had a 1.5 log reduction or less of the virus in the fresh produce. However, sanitization efficiency was significantly improved when 50—500 ppm of each detergent was added to the chlorine solution. A 2-4 log virus reduction was achieved by combination of the surfactants with chlorine solution. As expected, the removal efficiency was further enhanced by increasing the concentration of the surfactants in chlorine solution.

Significance: Combination of a proper surfactant with commonly used sanitizer (chlorine) significantly enhanced the sanitization of foodborne viruses. SDS is an FDA approved food additive. Triton X-100 and NP-40 both exist in dish soaps, liquid detergents, and many other detergents. FDA recognizes Tween 20 as a GRAS (Generally Recognized as Safe) substance. Thus, our intervention could be implemented by not only the food industry, but also the average consumer who has been searching for a way to sanitize fruits and vegetables before consumption in his or her own home.

T6-06 Columbus Public Health: 2009 Samuel J. Crumbine Consumer Protection Award Recipient

CHRISTINA R. WILSON
Columbus Public Health, Columbus, OH, USA

Introduction: Columbus Public Health (CPH) was the 2009 Samuel J. Crumbine Consumer Protection Award recipient.

Purpose: Columbus Public Health's Food Protection Program utilizes a multi-faceted approach to keeping and bringing licensed retail food facilities into compliance the Ohio Uniform Food Safety Code via educational and enforcement activities.

Methods: Columbus Public Health developed a comprehensive strategic plan to affect fundamental change to the Food Protection Program. This plan addressed the following issues: (1) Food safety education and training; (2) Knowledge and understanding of the changes in the new risk-based code and the science of food safety; (3) Bridging language and cultural barriers; 4) Providing easy access to public information; (5) An increase in the number of inspections to meet state-mandated frequency of inspection criterion; (6) Addressing the concerns of food security and defense; and, (7) The need to develop an improved enforcement model.

Results: CPH has been communicating these risk factors associated with foodborne illness to the operators and the public by performing risk-based inspections, providing extensive food safety information on the CPH website, and developing the SIGNS Public Information Initiative to promote transparency and better disclose compliance and enforcement information to the public. As part of the SIGNS initiative, consumers can search our website for details of critical violations observed during previous inspections, which empowers consumers to make informed choices regarding patronage. A durable plastic sign which uses a color-coded system to inform the public of the facility's compliance and enforcement status is posted at the entrance of licensed facilities. CPH has taken an active role in providing risk-focused food safety education by presenting regularly scheduled Person-in-Charge (PIC) and ServSafe Managers' Certification training classes in English, Spanish, Mandarin, and Somali.

Significance: All the CPH staff worked together as a team to contribute to the successful application for the 2009 Samuel J. Crumbine Consumer Protection Award. The staff persevered after an unsuccessful attempt in 2008, and incorporated the comments of the Crumbine Award Jury to improve both the program and application.

T6-07 Modifying the Behavior of Food Employees Using Educational Materials and Methods Designed for Oral Culture Learners

Alan M. Tart and JOANN PITTMAN
FDA, Office of Regulatory Affairs, Atlanta, GA, USA

Introduction: Many factors affect food workers' ability and motivation to prepare food safely, including, but not limited to, receiving proper training (Green and Selman, 2005). Research suggests that food workers are predominantly oral culture learners and that training materials and methods commonly used today are contrary to oral culture learner characteristics (Beegle, 2004).

Purpose: The purpose of this project is to improve the effectiveness of training related to the control of foodborne illness risk factors by developing educational materials and methods more appropriate for oral culture learners.

Methods: Funded through grants and contracts from the FDA's Center for Food Safety and Applied Nutrition (CFSAN) and Office of Regulatory Affairs/ORA U, a project team consisting of FDA National Retail Food Team members and Public Affairs Specialists, behavior and communication experts, and over 50 representatives from the state and local regulatory community, industry, academia, and trade and professional organizations, have developed/collected and field tested educational materials (posters, storyboards, videos, audio testimonials, demonstrations, and exercises) designed specifically for oral culture learners.

Results: Preliminary results suggest that using stories, sayings, and pictures with vivid examples that allow food workers to "feel" the impact of a behavior, as well as using interactive demonstrations and two-way communication provided in a focus group format, may assist with motivating food workers to change their food safety practices by helping them to understand "why" proper behaviors and practices are important in preventing foodborne illness. In 2010 and beyond, the Agency plans to test the long term effectiveness of the materials in changing behavior, develop strategies for market penetration and implementation, and design additional materials/methods for other foodborne illness risk factors.

Significance: This project will provide a unique foundation for improving the effectiveness and efficiency of food employee behavior modification efforts in the U.S. The materials and methods developed in this project will enhance existing training materials and methods to better improve employee behavior and practices related to the five foodborne illness risk factors. The scope of this project impacts the safety of the U.S.'s ~300 million residents and improves the training infrastructure and approach of ~3,000 state, local, tribal, and territorial regulatory jurisdictions and the ~1.3 million retail and foodservice establishments they regulate.

T6-08 Applying GFSI Recognized Management Systems to the Peanut Industry — A Case Study

John G. Surak, Patrick Archer, TATIANA A. LORCA, Rena Pierami and Rhonda Starling
EcoSure (A Division of Ecolab Inc.), St. Paul, MN, USA

Introduction: Ensuring food safety is a major concern in the global food supply. As a result, the Food Business Forum (CIES) launched the Global Food Safety Initiative (GFSI) in May 2000. GFSI has three business objectives: (1) Achieve a harmonization of food safety management system standards through a benchmarking process; (2) Improve cost efficiency throughout the food supply chain through a common acceptance of recognized standards; and (3) Provide a unique international stakeholder platform for networking, knowledge exchange and sharing of best food safety practices and information. As a result, GFSI has approved five management system schemes which include BRS, Dutch HACCP, FSSC 22000, IFS, and SQF 2000 code.

Purpose: The GFSI initiative is impacting food safety management systems in the United States with retailers announcing that private label suppliers must have their processing plants certified to a GFSI standard. Trade associations are working to help their members comply.

Methods: This symposium will discuss the GFSI initiative, compare the benchmarked standards and detail the certification process in order to provide critical information to make the proper management decision with regard to the certification process. In addition, it will present the peanut industry association's perspective on the need for their membership to implement GFSI recognized schemes as well as a case study on the process a peanut processor used to obtain certification of their GFSI recognized food safety management system.

Results: Certification against a GFSI recognized program has assisted the processor to provide additional assurance over the safety, quality and legality of their product and process.

Significance: This symposium will provide practical experience of a food processor with the BRC Standard for Food Safety.

T6-09 The Economic Cost of Foodborne Illness from Contaminated Produce in the United States

ROBERT SCHARFF
The Ohio State University, Consumer Sciences, Columbus, OH, USA

Introduction: Contaminated produce has increasingly been identified as a source of foodborne illness in the United States. Produce items such as tomatoes, leafy greens, and peppers have been implicated in outbreaks involving a wide range of bacterial, parasitic, and viral pathogens. In this study I examine the health-related economic costs associated with these illnesses.

Purpose: This study seeks to estimate the economic cost of foodborne illnesses associated with tainted produce.

Methods: The cost of produce-related illness is a function of food attribution data, the burden of illness, and pathogen-specific economic cost estimates. I use 2003—2007 food attribution data from the CDC's Foodborne Disease Outbreak Surveillance System to derive the proportion of foodborne illnesses that originate in contaminated produce items. For bacterial pathogens, the proportion of illnesses due to contaminated produce range from 0% for *Vibrio* infections to 54% of *E. coli* illnesses. Using burden of illness estimates based on Mead et al. (1999), these figures suggest that almost 20 million foodborne illnesses are annually contracted after consumption of tainted produce. I use the enhanced cost of illness method (Scharff 2009) to apply economic values to identified illnesses. This method provides a comprehensive health loss value, including costs from medical care, lost quality of life, and death.

Results: The annual economic cost of illness from produce contaminated with foodborne pathogens is $38.6 billion (90% CI $9.9 — $67.4 billion). The cost per case of illness is $1,961 (90% CI $506—$3,418), as compared to $1,814 for other foodborne illnesses. State differences in costs range from $1,756 per case in Kentucky to $2,184 per case in Hawaii.

Significance: Economic analysis is increasingly being relied upon by both the federal government and state governments as a guide to policymakers who must allocate increasingly scarce resources to programs that aim to improve the health and well being of their respective communities. By assessing the economic cost associated specifically with produce, I provide a measure that can be used to inform debate on the efficacy of new initiatives designed to make produce safer.

T6-10 Assessing Vegetable Producers' Beliefs Regarding Food Safety Issues

MELANIE L. LEWIS IVEY, Jeffrey T. LeJeune and Sally A. Miller
The Ohio State University, Plant Pathology, Wooster, OH, USA

Introduction: Foodborne disease outbreaks caused by fresh vegetables contaminated with *Salmonella* sp. and *E. coli* O157:H7 continue to be a concern in the United States despite efforts by industry, academia and the government to reduce their incidence. Given the lack of progress toward a reduction in foodborne human infections and the increase in identified foodborne disease outbreaks associated with produce there is an interest in identifying and promoting the adoption of good agricultural practices (GAPs) that will result in a safer food supply.

Purpose: The purpose of this study was to identify gaps, misconceptions or emerging perceptions among vegetable growers with regard to their decision-making process concerning prevention of and response to pre- and post-harvest contamination.

Methods: Gaps and misconceptions of vegetable grower's beliefs were identified using a five-stage mental model approach. A comprehensive questionnaire was designed and mailed to 621 vegetable producers in Ohio, Michigan, Kentucky and Indiana. Returned questionnaires were manually coded and these data were analyzed using non-parametric statistical tests.

Results: The survey response rate was 33.8%. Fifty percent of the respondents grew vegetables in Michigan, 29.3% in Ohio and 17.7 and 6.7% in Indiana and Kentucky, respectively. Growers very familiar with GAPs practiced them more often than those who were familiar with or unfamiliar with GAPs ($P = 0.00$). However, the level of familiarity with GAPs was poorly associated with the extent to which they were practiced ($P = 0.437$). The extent to which farmers stated they practiced GAPs was poorly correlated with actual practice of water and equipment sanitation ($P \leq 0.379$). The median response to whether or not transplants and pre- and post-harvest plant diseases were a source of contamination was "neither agree or disagree". However the largest proportion of growers either "strongly or somewhat agreed" that transplants and plant diseases were sources of contamination. Fifty percent of the growers disagreed that seed were a source of contamination.

Significance: These data indicate that there is a gap in knowledge between familiarity with GAPs and their implementation. They also highlight that growers believe plant diseases are sources of contamination thereby warranting further studies in plant-human pathogen interactions on vegetables. These findings will allow for the development of target-specific methods of communication and response.

T6-11 The Knowledge and Behavior of Parents of Young Children Concerning Domestic Food Safety

LOUISE FIELDING and Aoife O'Gorman
UWIC, Cardiff School of Health Sciences, Cardiff, United Kingdom

Introduction: The public is increasingly concerned about food-related risks but the rise in foodborne illness suggests that people still make decisions on food consumption, storage and preparation that are less than ideal from a food safety perspective. Many reasons have been cited for this. People make risk-benefit decisions and might tolerate or ignore risks in order to obtain the benefits related to the consumption of certain foods. Young children are among the most vulnerable to foodborne illness as they have not yet developed a strong immune or digestive system. *Listeria monocytogenes* is of particular concern as it is able to grow at refrigeration temperatures.

Purpose: The aim of this study was to determine the knowledge and behavior of parents of children less than five years of age in terms of domestic refrigerated food storage.

Methods: Qualitative and quantitative data collection methods were used. The study consisted of a self-completed questionnaire and an examination of the food storage practices and temperature of domestic refrigerators of 20 participants.

Results: Eighty five percent of respondents did not know the temperature of their refrigerator and only 5% checked their refrigerator to ensure that it was within the correct temperature range. There was no correlation between knowledge of the correct temperature range and the measured temperature of the participants' refrigerators. A significant difference ($P \leq 0.05$) was shown between the ages of participants in relation to their food safety knowledge.

Significance: The study shows an acceptable level of food safety awareness among the participants but also identified gaps in food safety knowledge and practices. Food risk communication should concentrate not just on the best practice 'education' but also on understanding the reasons why consumers fail to adopt safe practices. This presentation will also outline new work being carried out regarding the behavior of elderly consumers with respect to *Listeria monocytogenes*.

T6-12 Examining Consumers' Perceptions of Nanotechnology for Food Safety: A Baseline Study

MARY NUCCI and William K. Hallman
Rutgers, the State University of NJ, Food Policy Institute, New Brunswick, NJ, USA

Introduction: Nanotechnological applications for food safety—nanosensors, antimicrobial packaging, and thin films—offer the promise to significantly enhance food protection and quality. While these technologies are progressing to market readiness, little is known about consumer knowledge about these applications. These perceptions are important, because the promise of nanotechnology depends upon consumers' openness to and acceptance of nanotech. This first study to examines consumers' understanding of food nanotechnology details perceptions of food nanotechnology and the potential for acceptance of the technology.

Purpose: Rather than investing in the creation of nanotech-based products and then trying to convince consumers that they should accept those, many have called for a more thoughtful approach to investing in the development of food nanotechnology. This study aims to provide this information about nanotechnology applications for food prior to market readiness.

Methods: Approximately thirty participants participated in individual semi-structured interviews designed to elucidate knowledge and perceptions about the food nanotechnology. Participants were asked similar questions pre and post reading a backgrounder on nanotechnology. After the interview, the participants were offered the opportunity to taste foods described as either containing edible nanosensors or stored in nanocomposite films, and asked structured questions about the taste and general likability of the product. Participants were then presented with a series of possible food nanotech applications and asked about their potential acceptance of these products.

Results: In line with previous research on nanotechnology, initial awareness and understanding of food nanotechnology was low in all participants. However, concerns regarding safety, risks and benefits, environmental impacts, and links to other food technologies indicated that consumers acceptance of food nanotechnology is still not a given.

Significance: Given the potential of nanotechnology to address specific issues in food safety, understanding consumers' awareness and acceptance will ease the use of this technology in promoting food protection and quality.

T7-01 Application of Kinetic Models to Describe Heat Inactivation of Selected New Zealand Isolates of *Campylobacter jejuni*

ALI M. AL-SAKKAF, Goeff Jones and John Mawson
Massey University, Institute of Food, Nutrition and Human Health, Palmerston North, New Zealand

Introduction: New Zealand has a high rate of reported campylobacteriosis compared to other developed countries. One possible reason is that local strains have greater heat tolerance and thus are better able to survive undercooking. The first-order kinetic model has been used extensively in the calculation of the thermal inactivation parameters, D and z. However, non-linear survival curves have been reported and a number of models have been proposed to describe the patterns observed.

Purpose: The objective of this study was to investigate the thermal inactivation parameters of NZ isolates and to compare the conventional first-order model with some selected non-linear models.

Methods: Survival data for seven *Campylobacter* MLST strains (those most commonly implicated in human cases) were obtained after heating inoculated Brain Heart Infusion Broth using a submerged coil apparatus. The survival data were fitted by the log-linear and non-linear primary models, which were selected to cover most observed shapes of survival curves for vegetative bacterial cells. Standard measures of goodness-of-fit were used for comparison of the applied models.

Results: The survival kinetics did not support the hypothesis that NZ strains of *C. jejuni* have a higher thermal resistance than overseas strains. In general the non-linear models fitted the individual inactivation data sets better than the log-linear model. However, only the linear model and Weibull model could be successfully fitted to all data sets. When the mean shape factor was used (η = 1.1) for all the data sets in the Weibull model, the goodness-of-fit was poorer than when fitting η and δ individually. The Weibull model was now no better than the log-linear model, providing a better fit for only about 50% of the data sets.

Significance: The high rate of campylobacteriosis in New Zealand is not due to the heat-tolerant strains and the likelihood of a systematic error from using the log-linear model in thermal processing calculations, leading to a safety risk or over processing of the products, appears to be low.

T7-02 Modeling the Survival and Growth of *Salmonella* on Chicken Skin Stored at 4 to 12°C

THOMAS P. OSCAR
USDA-ARS, Princess Anne, MD, USA

Introduction: The minimum growth temperature for *Salmonella* is in the range of 6 to 8°C, which is within temperatures encountered during cold storage of poultry.

Purpose: The objective of this study was to investigate and model survival and growth of *Salmonella* on chicken skin during cold storage.

Methods: Chicken skin was inoculated with a low initial dose (0.9 ± 0.06 log) of a single strain of *Salmonella* Typhimurium DT104 (ATCC 700408) followed by storage at 4 to 12°C for 0 to 10 days. A general regression neural network (GRNN) model that predicted the log change of *S.* Typhimurium DT104 as a function of time and temperature was developed. Percentage of residuals in an acceptable prediction zone from -1 ('fail-safe') to 0.5 ('fail-dangerous') log was used to validate the GRNN model using a criterion of 70% acceptable predictions.

Results: Performance of the model for predicting dependent data (n = 163) was 85.3% acceptable predictions. The model was also evaluated for interpolation and for extrapolation to another serotype of *Salmonella* (i.e., Kentucky). Performance of the model for predicting independent data for interpolation (n = 77) was 84.4% acceptable predictions, whereas performance of the model for predicting independent data for extrapolation (n = 70) to serotype Kentucky was 87.1% acceptable predictions. Thus, the model was found to provide acceptable predictions for survival and growth of *Salmonella* Typhimurium and Kentucky on chicken skin during cold storage.

Significance: Mathematical models that predict the behavior of microbial pathogens on food are valuable tools for assessing and managing food safety risks because they can provide valid predictions of pathogen behavior in food under storage and handling conditions that were not investigated but that are within the conditions investigated and modeled and thus, save time and money associated with performing microbiological tests on food.

Technical

T7-03 FSIS *Escherichia coli* O157:H7 Beef Establishment Risk-assessment Project

James L. Withee, Eric D. Ebel, W. T. Disney, Dare Akingbade, Michael S. Williams, Wayne D. Schlosser, Nathan J. Bauer, Janell R. Kause, John M. Hicks, William K. Shaw, Jennifer Webb and DENISE R. EBLEN
USDA-FSIS, Washington, D.C., USA

Introduction: The USDA Food Safety and Inspection Service has worked since 1994 to reduce *E. coli* O157:H7 illness from beef consumption. The Agency is developing new policy options to further reduce the risk of illness from beef.

Purpose: The Beef Establishment Risk Assessment Project was initiated to (1) identify new options for managing the risk of *E. coli* O157:H7 illness from beef consumption; (2) evaluate the public health benefits of these options; and (3) identify mechanisms for measuring improvements in public health when options are implemented.

Methods: E. coli O157:H7 data on live cattle, ground beef and human illnesses were fit to multiple models including a trend model that accounts for annual variation, seasonal models, and a description of the residual variability.

Results: Based on FoodNet data and CDC outbreak evidence, there are approximately 11,000 *E. coli* O157:H7 illnesses from beef consumption per year. The outbreak evidence also suggests that ground beef is responsible for a substantial proportion of these illnesses. Since 2003, beef-associated *E. coli* O157:H7 outbreaks have increased while FoodNet reported cases have remained steady. In the past two years, there has been an increase in *E. coli* O157:H7-contaminated beef in the FSIS sampling program. Our analysis highlighted risks associated with the seasonal increase of *E. coli* O157:H7. These models inform us that the seasonal increase of *E. coli* O157:H7 in live cattle likely accounts for the observed seasonal increase in human illnesses each year. Our model estimates that eliminating the summer spike of *E. coli* O157:H7 in ground beef will prevent approximately 4,000 illnesses each year.

Significance: The results are being used to facilitate development of policy options to reduce the risk of foodborne illness from *E. coli* O157:H7 in beef.

T7-04 Predictive Modeling for *Listeria monocytogenes* Transfer during Slicing of Delicatessen Meats

KEITH VORST, Lindsey Keskinen, Gary Burgess, Jeff Danes and Elliot Ryser
Cal Poly State University, Industrial Technology and Packaging, San Luis Obispo, CA, USA

Introduction: Listeria monocytogenes can persist for weeks or months on difficult-to-clean stainless steel surfaces including delicatessen slicer blades. The proposed model predicts an exponential decay in the number of cells versus slice number. The delicatessen product sliced was found to have the greatest impact on the fit of the model and thus a predictor of transfer.

Purpose: The proposed predictive models can be used to help a processor determine whether an overall greater reduction in *L. monocytogenes* prevalence in a production facility could be achieved by requiring better raw material quality or by improved sanitation efforts.

Methods: A model based on the following three assumptions was developed to predict the previously calculated transfer coefficients by: a) the number of Listeria cells transferred from the blade to the meat during slicing is a fraction (f1) of the number of *Listeria* cells on the blade just before each sequential slice, b) the number of *Listeria* cells transferred to surrounding areas is a different fraction (f2) of the number of cells on the blade just before each sequential slice, and c) the CFU on the blade before any slicing begins is N0. Fitting the equation to experimental data (finding "k" "a").

Results: The cumulative *Listeria* transfer amounts were based on the experimental data for each mechanical slicer scenario. A similar trend was seen for all scenarios, with 99% of the total *Listeria* transfer occurring within the first 10 slices. Significantly more transfer to turkey than to salami had been observed previously. Transfer of weak biofilm-forming *L. monocytogenes* strains to turkey and salami resulted in the lowest variance ($R^2 = 0.94$) for observed vs. predicted values for all models tested. Transfer of cold-injured *L. monocytogenes* to turkey and salami showed the greatest deviation from the predicted values. In all possible combinations of variables, the fraction transferred to the surroundings (f2) always exceeded the fraction transferred to each slice of delicatessen meat (f1).

Significance: This work confirms the previous findings of the authors suggesting the greatest number of *Listeria* (> 90%) will be found in the first 15 slices of delicatessen meats after mechanical or knife slicing. Despite the researchers previously developed model being an empirical model, it appears to be accurate for certain underlying microbiological mechanisms that may affect survival (cold-injury and desiccation over time) and may affect attachment and persistence on surfaces (biofilm forming ability).

T7-05 Evaluating the Factors Important in Norovirus Transmission in Foodservice Systems

DI LI and Donald Schaffner

Rutgers University, Food Science, New Brunswick, NJ, USA

Introduction: Human norovirus (HuNoV) can spread easily and rapidly via multiple routes unless effective interventional actions are taken. The U.S. Centers for Disease Control and Prevention estimates that approximately 23 million acute gastroenteritis cases are caused by HuNoV annually. Almost 40% of all norovirus outbreaks occurred in restaurant settings, most often due to poor hygiene practices and cross contamination.

Purpose: The purpose of this research is to develop a simulation model that mimics the complex interactions involved in norovirus transmission that may take place in a foodservice system and to use the model to study interventions that reduce risk.

Methods: Data from the peer-reviewed literature were collected and used to build the model. Our model focused primarily on quantifying the effects of (1) virus transfer between food, hands and food contact surfaces, (2) virus survival on different surfaces, (3) the effect of handwashing and related cleaning treatments on virus reduction and (4) probability that hand washing would occur. The model was built with the discrete-event simulation package, Arena® (Rockwell International), and then used to simulate foodservice worker movement, as well as virus transfer and survival.

Results: The results show that in the model food preparation system, if 10^6 norovirus particles are brought into the food processing area either by an infected food handler or a batch of contaminated produce early in the day, about 30% of the prepared food can put diners at risk by the end of the day, even if handwashing is performed as required.

Significance: The model shows key points in the virus transmission process that lead either to further spread or to reduction in the spread of the virus. It has the potential to reduce the morbidity and economic loss currently associated with HuNoV outbreaks, and to identify key foodservice worker behaviors that affect virus transmission.

T7-06 Network Science Methods to Analyze Food Import-Export Networks

JóZSEF BARANYI, Mária M. Ercsey-Ravasz, Zoltán Toroczkai and Zoltán Lakner

Institute of Food Research, Computational Microbiology, Norwich, United Kingdom

Introduction: The science of Complex Networks was heralded as one of the potential hits of the new disciplines in the 21st century (Science, 2009; 325. 405-432). One of its main findings is that some organizational principles of complex networks show fundamental laws regardless of the area where it is applied to, and this makes Network Science probably the most multidisciplinary modern scientific tool. Food science is especially suitable for network science applications, since food-related problems (microbiology, risk assessment, transport, epidemiology, food security and food trade, etc.) involve MANY players in various social contexts, entangled in a COMPLEX NETWORK OF INTERACTIONS where emergent properties of the system cannot be described and predicted by studying only its parts.

Purpose: The purpose of this study is to evaluate the world's food import-export network by methods stemming from the science of Complex Networks.

Methods: A FAO database is used to set up a directed, weighted network where the nodes are countries which reported on their food import and export. Information on the category, the value and the quantity of the food items, as well as on the (agri-food) population of the node-countries is used to analyze the basic properties of the network.

Results: Expected and intuitive, as well as surprising conclusions can be drawn from the topology and the dynamics of the network. It is possible to identify significant middle-men countries which despite their small size can play crucial roles in the network. It is also possible to identify and visualize that group of countries which are vulnerable to food crises even though their economy is not among the poorest.

Significance: Network Science methods can play a crucial role in modern food science; especially in food security, food trade, risk assessment and epidemiology. Network-analysis can also be applied to optimize agro-food trade, with special attention on decreasing the environmental burden of transport systems.

T7-07 Sources and Settings: Contaminated Food Vehicles and the Settings of Foodborne Disease Outbreaks

DANA COLE, Karen Herman and Barbara Mahon

CDC, Enteric Diseases Epidemiology Branch, Atlanta, GA, USA

Introduction: Local, state and territorial public health departments voluntarily report foodborne disease outbreaks to the Centers for Disease Control and Prevention (CDC). Analysis of the foods and settings associated with reported foodborne outbreaks is important both to inform and to evaluate the impact of food safety policies.

Purpose: This study examined the common food vehicles and food preparation settings associated with reported foodborne disease outbreaks from 1998 to 2008.

Methods: Data from foodborne disease outbreak investigations reported from 1/1/1998 through 12/31/2008 were obtained from the electronic Foodborne Outbreak Reporting System (eFORS). Analyzed fields included the outbreak year, contaminated food, and food preparation settings. Contaminated foods were categorized as either simple or complex, based upon whether ingredients could be grouped into one of 17 commodities (e.g., beef, eggs) or included multiple commodities, respectively. Time trends and the distribution of contaminated commodities in different food preparation settings were examined.

Results: Data from 13,517 outbreaks were analyzed. The most common food preparation settings were restaurants/delis (59%), private homes (12%) and caterers (6%). On average, the annual number of outbreaks associated with food preparation by restaurants/delis and caterers declined over the study period (0.3%/year and 2.2%/year, respectively), but the annual number associated with preparation in private homes increased (2.3%/year). Most outbreaks with reported and classifiable contaminated foods (n = 6,968) involved complex food vehicles (54%). Poultry was the most common simple food commodity reported in outbreaks associated with restaurants/delis, followed by fish; whereas in outbreaks associated with private homes, fish was most common, followed by beef.

Significance: Since these data do not identify the source of contamination of implicated food vehicles, the findings cannot be used to identify changes in contaminated food sources or contamination settings. Rather, these differences likely represent differences in food storage, preparation, and consumption patterns, and may also reflect an effect of recent efforts to improve food safety in retail food service settings.

T7-08 The Potential for Cross-contamination of Foods through Improper Storage in Home Refrigerators

SANDRIA GODWIN, Fur-chi Chen, Richard Stone, Edgar Chambers and Delores Chambers
Tennessee State University, Family and Consumer Sciences, Nashville, TN, USA

Introduction: Cross-contamination occurs when bacteria from uncooked foods are transferred to foods that will be eaten without further heating. Improper storage of refrigerated foods in the home increases the potential for cross-contamination. Consumers are told to separate fresh meats, seafood, poultry and eggs from other foods in the refrigerator, to place them on the bottom shelf of the refrigerator, and to store opened packages of foods or foods that may leak in properly sealed containers or bags on the bottom shelf.

Purpose: This study was designed to assess whether consumers followed these recommendations when storing foods in home refrigerators.

Methods: An observational study examined the contents of the refrigerators in 200 homes in Florida, Kansas, and Tennessee. Trained researchers completed a checklist documenting the location in refrigerator, storage containers or packaging used, and expiration dates of specific refrigerated items (milk and other dairy products, raw meat and poultry, Ready-to-Eat (RTE) meats, other RTE foods, raw eggs, and fresh fruits and vegetables). The checklist also included a section for researchers to comment specifically on areas pertaining to cross-contamination.

Results: Circumstances that could allow for cross-contamination were noted in many refrigerators. Storage locations of raw meat products in relation to RTE products or fresh fruits and vegetables demonstrated the greatest risk for cross-contamination. Raw meat was found stored next to RTE meats in 45 homes. Open packages or uncovered storage containers of RTE foods were noted in 26 refrigerators. Raw meat was stored in its original package on upper shelves in refrigerators, with nothing to catch raw meat drippings under the package in 7 homes.

Significance: This study demonstrated consumers lack of knowledge or inability to implement proper storage practices in household refrigerators, thus increasing the risk for cross-contamination. A targeted educational program that reaches a large number of consumers is needed.

T7-09 A Mathematical Survival Model for *Escherichia coli* O157:H7 and *Staphylococcus aureus* on Stainless Steel Surfaces

FERNANDO PEREZ RODRIGUEZ, Antonio Valero, Elena Carrasco, Ewen Todd, Guiomar Denisse Posada Izquierdo, Andrés Morales Rueda, Rosa Maria Garcia-Gimeno and Gonzalo Zurera
University of Cordoba, Dpto Bromatologia y Tecnologia Alimentos, Cordoba, Spain

Introduction: Bacterial survival on surfaces is recognized as an important factor contributing to pathogen and spoilage flora contamination of foods through transfer on contact. In Quantitative Microbiological Risk Assessment (QMRA), combining survival and cross contamination models is necessary to obtain more accurate estimations on cross-contamination impact on the final risk.

Purpose: To study survival of *Escherichia coli* O157:H7 and *Staphylococcus aureus* on stainless steel under different substratum conditions simulating soiled and clean surfaces; and based on results, to develop mathematical models describing survival of pathogens as a function of time.

Methods: Cocktails of *S. aureus* and *E. coli* O157:H7 were deposited on different sterile 10 cm^2 # 304 stainless steel coupons at 5 log CFU/cm^2 using PBS and meat juice extract to mimic clean and dirty surfaces, respectively. Surface samples were taken at different times by swabbing inoculated area and analyzed for bacterial enumeration. Log-linear and the Weibull models to describe survival data were statistically assessed.

Results: *S. aureus* could be detected up to 16 days after inoculation with PBS (0.5 log CFU/cm^2). On meat juice extract, *S. aureus* was recovered after 34 days a relatively high levels 2.5 log CFU/cm^2. In contrast, *E. coli* O157:H7 was recovered up to 24 days after inoculation in PBS. On meat juice extract, *E. coli* O157:H7 showed a great increase in its survival ability, remaining viable on surfaces after 9 days (1.5 log CFU/cm^2). Survival curves for *S. aureus* could be well described using a log-linear model ($R > 0.93$). In turn, *E. coli* O157:H7 presented an evident tail zone (for all conditions assayed) which made the Weibull model more appropriate ($R > 0.91$).

Significance: Results for heavily soiled conditions stressed the importance of performing effective cleaning and disinfection procedures to decrease persistent pathogenic microorganisms in food environment. These are used as inputs to forthcoming mathematical models incorporating bacterial survival on surfaces to estimate the risk of food contamination over different time periods. QMRA studies not including survival models could overestimate the risk associated to bacterial transfer.

T7-10 The Value and Challenges of Providing Sound, Effective and Timely Risk-based Scientific Advice for International Food Safety Standard Setting

Sarah M. Cahill and MARIA DE LOURDES COSTARRICA
Food and Agriculture Organization of the United Nations, Nutrition and Consumer Protection Division, Rome, Italy

Introduction: The WTO SPS Agreement requires that all food safety standards be based on an appropriate risk assessment. At the international level, the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) provides risk-based scientific advice to support standard setting on microbiological food safety issues by the Codex Alimentarius Commission (CAC) as well as by member countries of FAO and WHO. After 10 years, reflection on JEMRAs work to date aims to determine how, in the decade ahead, it can effectively contribute to the establishment and implementation of food safety standards that are firmly rooted in sound science.

Purpose: The purpose of this study was to evaluate the impact of JEMRA work on the microbiological food safety standard development work of its primary customer, the CAC.

Methods: The role of risk-based scientific advice in the development of Codex standards with a microbiological food safety component adopted within the last five years was evaluated. The impact of each piece of scientific advice was assessed independently in relation to the standard it contributed to as well as in comparison to other pieces of scientific advice. This was facilitated through the identification of 8 areas for the comparative evaluation.

Results: JEMRA's approaches to providing scientific advice have evolved over the past 10 years. In parallel, the contribution of the advice to the Codex standard setting process has increased. Factors contributing to this included greater understanding of the role of risk assessment and the relationship between risk assessors and managers as well as the specificity of the issue on which advice was sought. The role of risk assessment in standard setting was found to be expanding from standard development to standard implementation, highlighting the need for risk assessments to be living tools rather than finite pieces of work.

Significance: This study indicates that success over the next decade requires ongoing evolution of JEMRA, making maximum use of new technologies and approaches and meeting the demands for real-time scientific advice.

T7-11 Modeling Logistics in Quantitative Microbial Risk Assessment for Salad Bars

Seth-Oscar Tromp, Hajo Rijgersberg and EELCO FRANZ
RIVM — Centre for Infectious Disease Control, Laboratory for Zoonoses and Environmental Microbiology, Bilthoven, The Netherlands

Introduction: Supply chain logistics are seldom accounted for in microbial risk assessments. However, modeling logistics might be important since time delays potentially allow pathogen growth.

Purpose: The purpose of the present study was to make a quantitative comparison, in terms of pathogen (*E. coli* O157, *S. enterica* and *L. monocytogenes*) growth and estimated disease cases, between modeling logistic (MOD) and assuming fixed time delays (FIX) in the supply chain of lettuce destined for working canteens salad-bars.

Methods: The MOD model time delays in the supply chain by modeling the logistic process (demand, ordering policy and salad bar filling policy). The FIX model uses fixed measured delay times. Temperature profiles, consumption and logistic policy were derived from one specific salad bar and its supply chain. Pathogen growth in fresh-cut lettuce was modeled as a function of the time-temperature profile of the supply chain using ALADIN (Agro Logistics Analysis and Design INstrument). Risk characterization was modeled by means of Monte Carlo risk assessment model in Excel (2003) with @Risk (version 4.5). Growth and prevalence data were obtained from literature.

Results: The average relative growth of *E. coli* O157 and *S. enterica* were respectively 17% and 15% and did not differ between the FIX and the MOD model. The average relative growth of *L. monocytogenes* was 194% with the FIX model and 1156% with the MOD model. When the delivery frequency in the MOD model was increased from 2 to 5 times per week the average relative growth decreased to 514%. The estimated number of disease cases resulting from a consumption of contaminated lettuce-based salad from a canteen salad-bar in the Netherlands as modeled by the FIX model was 166 (95% CI 13—544), 187 (29—520) and 0.34 (0.03—1.06) for respectively *E. coli* O157, *S. enterica* and *L. monocytogenes*. The estimated number of *L. monocytogenes* disease cases increased to 1.43 (0.11—4.60) case per year with the MOD model.

Significance: Modeling the logistic process of a fresh produce supply chain has significant consequences for the estimation of pathogen growth and associated public health risks since the tails of storage time distributions are better described as compared to assuming a fixed delay times or a certain distribution. This accounts especially for psychrotrophic bacteria like *L. monocytogenes*.

Poster Abstracts

P1-01 *Listeria monocytogenes* Survival and Growth on Ready-to-Eat Whole Hams Treated Post Lethality with a Natural Liquid Smoke Extract in Combination with Lauric Arginate under Normal and Deep-chilled Storage

YALE LARY, Gil Bakal, Brandon Baer, Roger Conrad and Lynn Knipe
Sysco Food Corporation, Houston, TX, USA

Introduction: There is an interest in natural antimicrobials, lower sodium and "cleaner" labels as manufacturers and distributors of refrigerated ready-to-eat (RTE) meats seek ways to protect consumers from *Listeria monocytogenes* (LM) while lowering sodium and extending shelf life.

Purpose: The purpose of this study was to test the effect of a purified fraction of liquid smoke combined with Lauric Arginate when applied to LM inoculated RTE hams stored under "normal" and initial "deep chill" conditions.

Methods: Water added smoked hams were formulated without sodium lactate/sodium diacetate and commercially processed. The hams (3 replicates per sampling period) were inoculated with a mixed *Listeria monocytogenes* culture (ATCC:7646,7644,and BAA 751),allowed 10 minutes for attachment and surface treated with a solution of CytoGuard LA (10% Lauric Arginate) and 93% CytoGuard Stat-N (proprietary purified fraction of liquid smoke) to achieve 44 ppm LAE via the SLIC™ method. Samples were vacuum packaged at -950 mBar. Trial 1 ham samples were stored under "normal" 3–4°C temperatures. For trials 2 and 3, samples were "deep chilled" -1–0°C initially the first 15 days then moved to "normal" 3–4°C storage until micro sampling on days 1, 7, 30, 52, 75, 90, 105, and 135. At each sampling period, 3 control and 3 treated hams were removed from storage, opened aseptically, neutralized and the purge plated with Modified Oxoid agar. Plates were incubated at 35°C for 48 hours and CFU results recorded.

Results: In trial 1 under "normal" storage conditions, LM outgrowth was greater than 2 logs after 60 days on samples treated with CytoGuard LA and CytoGuard Stat-N (CGLA/SN) and greater than 2 logs after 7 days for the untreated control hams. In trials 2 and 3 hams treated with CGLA/SN under initial "deep chill" conditions followed by "normal" storage, LM outgrowth was less than 1 log through 91 days in both trials and up to 135 days in trial 3. LM outgrowth inhibition improved 3 weeks when comparing treated hams initially deep chilled to treated hams not deep chilled. All CGLA/SN replicates from the 3 trials showed greater than 1 log reduction of LM by day 1. The inclusion of purified liquid smoke with Lauric Arginate did not diminish the initial LM log reduction.

Significance: SLIC™ application of CytoGuard LA (10% Lauric Arginate) and 93% CytoGuard Stat-N (proprietary purified fraction of liquid smoke) at a target rate to achieve 44 ppm LAE plus an initial "deep chill" period of 15 days prior to normal refrigerated distribution provided LM control on RTE hams equivalent to Alternative 1 classification without addition of lactate or sodium diacetate to the formulas.

P1-02 Antilisterial Activity of Natural Ingredients in a Model Poultry Product System

KATHLEEN A. GLASS, Lindsey M. McDonnell, Roxanne R. VonTayson, Brandon J. Wanless and Jeffrey J. Sindelar
University of Wisconsin, Food Research Institute, Madison, WI, USA

Introduction: Demand for "natural" and organic ready-to-eat (RTE) meat and poultry products have increased substantially in recent years. These products contain no added sodium nitrite, which has been shown to have antilisterial activity. Therefore, additional antimicrobial ingredients need to be identified to ensure safety of these products during extended refrigerated storage.

Purpose: To screen the antimicrobial effect of natural ingredients on *Listeria monocytogenes* growth in meat slurry systems prepared with various nitrite sources.

Methods: Turkey slurries (25% ground turkey breast meat, 2.0% salt, final slurry pH 5.8–6.0) were prepared to yield treatments including: (1) uncured, (2) traditionally cured with 156 ppm added sodium nitrite, (3) indirectly cured using celery powder and a nitrate-reducing *Staphylococcus carnosus*, or (4) pre-converted celery-powder as a natural nitrite source. Treatments were supplemented with 15 different natural ingredients, including natural flavorings, plant extracts, and microbial fermentation by-products. Cooked slurries were inoculated with 3-log CFU/g *L. monocytogenes*, stored at 4°C for 4 weeks, and duplicate samples per treatment assayed weekly for changes in listerial populations by plating on Modified Oxford agar.

Results: No growth of *L. monocytogenes* (<1 log increase for 4 weeks at 4°C) was observed in turkey slurries supplemented with 1.5% vinegar/lemon/cherry powder blend, 2% buffered vinegar, 2.5% vinegar/lemon juice blend, 2.5% grapefruit/lime/vinegar blend, 500 ppm tea tree oil, or 3.0% cultured cane sugar/vinegar blend, regardless of cure status. The combination of any of the nitrite treatments with 0.03% grape seed extract powder, 0.5% cherry powder, or 1% smoke flavor-2 prevented growth for 4 weeks, but listerial growth in the uncured treatment was similar to the positive growth control, which included no antimicrobials (>4 log increase at the 4-week sampling interval). The addition of 0.02% nisin-rosemary blend, 2% cranberry powder, 0.5% herb blend and 1% smoke flavor-1 had slightly greater antilisterial effect in traditional cured treatments than in "naturally" cured product with lower residual nitrite levels and had no inhibitory effect in uncured product. Addition of 0.08% rosemary-tocopheral blend or the 0.1% green tea extract did not inhibit pathogen growth compared with the controls without the antimicrobials.

Significance: This study identified several ingredients which may be useful to inhibit growth of *L. monocytogenes* in cured or uncured RTE meats. However, certain ingredients are more effective in the presence of nitrite, regardless of whether the nitrite source is natural or traditional.

P1-03 Effect of Lactate and Diacetate-based Bacteriostatic Agents on *Listeria monocytogenes* in Ready-to-Eat Meat Products

RICO SUHALIM, Darren Toczco, Vladimir Svidenko, Tam Mai and Mansour Samadpour
IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA

Introduction: Listeria monocytogenes continues to be a pathogen of concern in Ready-to-Eat (RTE) meat products. Efforts to keep food free from *Listeria* sp. contamination include adequate product handling, sanitation practices and use of bacteriostatic agents approved for meat products.

Poster

Purpose: Survival and growth of *L. monocytogenes* on RTE meat products formulated with a combination of lactate and diacetate-based bacteriostatic agents was investigated.

Methods: Cured (large diameter) and cured and smoked (small diameter) commercial RTE meat containing varying levels of sodium lactate/sodium diacetate, potassium lactate/sodium diacetate, sodium-potassium lactate/sodium diacetate were surface inoculated with approximately 100 CFU/100 g of a five-strain mixture of *L. monocytogenes*. Products were vacuum-packaged and stored at 4 ± 2°C for up to 12 and 14 weeks for large and small diameter products, respectively. *L. monocytogenes* was recovered by surface plating method. A 2-log outgrowth of *L. monocytogenes* over the study period was used as a threshold of rejection.

Results: Growth of *L. monocytogenes* was inhibited in cured chicken products containing sodium diacetate/sodium lactate at 1.46/0.10%, cured pork product with 2.07/0.148%, pork product with high water content containing 2.4/0.17%, cured turkey breast containing 2.24/0.16%, and cured chicken-beef-pork combination with 1.12/0.08 percent sodium lactate/sodium diacetate. Similarly, cured-smoked products with sodium lactate/sodium diacetate levels of 1.90/0.136%, 1.68/0.12% (beef-pork-chicken products), and 1.29/0.09% (chicken-pork-beef skinless sausage) showed suppressed growth of *L. monocytogenes*. One cured-smoked beef product containing 1.12% sodium and potassium lactate and 0.08% sodium diacetate also maintained the level of *L. monocytogenes* below 2 logs. In contrast, a cured chicken product containing 1.90% potassium lactate and 0.136% sodium diacetate had more than 2 logs outgrowth of *L. monocytogenes*.

Significance: This microbial challenge study suggested sodium lactate and sodium diacetate combination was effective in inhibiting growth of *L. monocytogenes* to less than 2 logs outgrowth throughout the shelf life the company uses on its product packaging.

P1-04 Surface Application of Bio- and Chemical Preservatives to Inhibit *Listeria* on Vacuum-packaged Wieners

DENISE R. CARLSON, Michael E. Stiles, David C. Smith and Lynn M. McMullen
CanBiocin Inc., Research and Development, Edmonton, AB, Canada

Introduction: In past investigations we evaluated the use of a bacteriocin-based biopreservative incorporated into a ready-to-eat meat formulation to inhibit the growth of surface-contaminated *Listeria*. *Carnobacterium maltaromaticum* CB1 (Micocin®) produces three bacteriocins responsible for its antimicrobial activity against a broad range of *Listeria monocytogenes* strains.

Purpose: To evaluate the surface application of bacteriocin-based biopreservative component of Micocin®, in combination with chemical preservatives, to inhibit the growth of surface-inoculated strains of *L. monocytogenes* on wieners.

Methods: Three separate batches of wieners were manufactured in a pilot plant. After processing, the wieners were surface inoculated to give approximately 1×10^3 of a cocktail of *L. monocytogenes* (including serotypes 1/2a, 1/2b, 3a and 4b) per gram. The inhibitory agents (bacteriocin-based biopreservative in combination with different concentrations of sodium lactate and/or sodium diacetate) were added to the surface of the product after processing and before vacuum packaging. The wieners were stored at 4°C and *Listeria* were enumerated on PALCAM agar during storage.

Results: In the control samples without the bio- and chemical preservatives, *Listeria* spp. increased to a population of 10^4 CFU/g within 28 days and 10^8 CFU/g within 60 days. In contrast, in the presence of the preservative agents the *Listeria* did not increase above the levels of inoculation (10^3 CFU/g) for the first 28 days of the evaluation, but after 60 days the inhibitory effect of all of the preservatives was lost.

Significance: In this study we demonstrated that the combination of the Micocin® bacteriocin-based biopreservative and chemical preservatives applied to the surface of meat products after processing controlled the growth of *L. monocytogenes* during the early stages of storage but it also showed that for extended inhibition of *L. monocytogenes* the presence of viable *C. maltaromaticum* CB1 is necessary.

P1-05 Viability of *Listeria monocytogenes* on Pork Scrapple Formulated with and without Antimicrobials during Extended Refrigerated Storage

ANNA C. S. PORTO-FETT, Lisa Yoder, Kelly M. Gartner, Linda Tufft, Steve Campano, Alan Oser, Bradley Shoyer, Jeff Call and John B. Luchansky
USDA-ARS, Microbial Food Safety Research Unit, Wyndmoor, PA, USA

Introduction: Scrapple is a Ready-to-Eat (RTE) specialty/ethnic meat. There are no reported illnesses attributed to scrapple, but there has been a recall of scrapple due to contamination with *Listeria monocytogenes*.

Purpose: Evaluate food grade chemicals as ingredients to control *L. monocytogenes* on scrapple during refrigerated storage.

Methods: Loaves (ca. 11 cm wide × ca. 6 cm high × ca. 64 cm long; ca. 5 kg each) of scrapple were formulated, with or without citrate-diacetate (0.64%; Ional-Plus), by a commercial processor to contain various solutions/blends of the following antimicrobials: i) lactate-diacetate (3.0 to 4.0%; UltraLac KL 564), ii) lactate-diacetate-propionate (2.0 and 2.5%; e(Lm)inate), and iii) levulinate (2.0 and 2.5%). The resulting scrapple was sliced (ca. 1.9 cm thick) and surface inoculated on both the top and bottom faces of each slice to a target level of ca. 2.5 log CFU/g with a five-strain cocktail of *L. monocytogenes*. The inoculated slices were placed into nylon-polyethylene bags that were vacuum-sealed and held at 4°C for up to 60 days.

Results: Regardless of whether or not citrate-diacetate was included in the scrapple formulation, without the subsequent addition of the targeted antimicrobials, pathogen levels increased from ca. 2.5 log CFU/g to ca. 9.0 log CFU/g over 60 days of refrigerated storage. When scrapple was formulated without citrate-diacetate, but lactate-diacetate, lactate-diacetate-propionate, or levulinate were included in the formulation, pathogen levels increased from ca. 2.5 log CFU/g to ca. 8.1 and 7.3, 7.2 and 3.9, or 7.8 and 6.5 log CFU/g, respectively, within 60 days. In contrast, when scrapple was formulated with citrate-diacetate in combination with lactate-diacetate or with levulinate, pathogen levels increased from ca. 2.5 log CFU/g to ca. 3.6 and 2.6 or 4.9 and 3.5 log CFU/g, respectively, whereas, in combination with lactate-diacetate-propionate, pathogens levels decreased to ca. 2.0 log CFU/g during refrigerated storage.

Significance: These data validated that inclusion of lactate-diacetate, lactate-diacetate-propionate, or levulinate as ingredients in pork scrapple formulated with citrate-diacetate would effectively control the outgrowth/survival of *L. monocytogenes* in the event of post-process contamination.

P1-06 Antimicrobial Efficacy of Phosvitin Alone or Combined with Nisin against *Listeria monocytogenes* in a Laboratory Broth Medium at 35°C

SHECOYA B. WHITE, Aubrey F. Mendonca, Aura Daraba and Dong U. Ahn
Iowa State University, Food Science and Human Nutrition, Ames, IA, USA

Introduction: There is a growing trend towards the use of natural antimicrobials in food products. Both phosvitin and nisin are natural antimicrobials from animal and microbial source, respectively.

Poster

Purpose: The aim of the present study was to determine the antilisterial effect of phosvitin alone or combined with nisin in brain heart infusion broth (BHI).

Methods: BHI with phosvitin (10, 20, 40, 60, 80 or 100 mg/ml), nisin (125, 250, 500 or 1000 IU/g) or combinations was inoculated with a 5-strain cocktail of *Listeria monocytogenes* at 5.0 log CFU/ml. BHI without added antimicrobials served as control. Growth of *L. monocytogenes* at 35°C for 24 h was monitored by measuring the optical density (OD600nm) using a Bioscreen C turbidometer.

Results: Control cultures grew faster and reached higher OD values than cultures with added antimicrobials. Both lag phase and growth rate decreased with increasing phosvitin or nisin concentrations. Minimum inhibitory concentrations (MIC) of phosvitin and nisin were 80 mg/ml and 1,000 IU, respectively. Absolutely no increase in OD was observed for cultures containing 80 or 100 mg/ml phosvitin or 1,000 IU nisin. Relatively low levels of phosvitin (20 mg/ml) or nisin (250 IU) were only slightly inhibitory to growth of *L. monocytogenes*. In contrast, the combination of those same levels of antimicrobials was completely bacteriostatic.

Significance: Based on these results combined sub-MIC levels of phosvitin (20 mg/ml) and nisin (250 IU) exhibit good potential as a natural antimicrobial system for controlling growth of *L. monocytogenes*. Evaluations of the antimicrobial effect of phosvitin and nisin combinations in actual food systems are warranted.

P1-07 Influence of Nisin or Selective Meat Additives on the Antimicrobial Effectiveness of Ovotransferrin against *Listeria monocytogenes*

AUBREY F. MENDONCA, Sun Hee Moon, Aura Daraba, Shecoya B. White, Hyun-Dong Paik and Dong U. Ahn
Iowa State University, Ames, IA, USA

Introduction: Ovotransferrin (OTF) is the main component in the antimicrobial defense system of hens' eggs. Like some antimicrobials from natural sources OTF is limited in its range of activity and exhibits effectiveness at relatively high concentrations in food products. One possible way to circumvent this problem might be the use of combinations of antimicrobials. The application of antimicrobials in combination may produce additive, synergistic, or antagonistic results.

Purpose: The aim of the present study was to determine the effect of nisin or selected meat additives (salt, lactate, lactate/diacetate combination, and polyphosphate) on growth inhibition of a 5-strain mixture of *Listeria monocytogenes* by OTF.

Methods: A Bioscreen C turbidometer was used to evaluate the effect of various concentrations of nisin (250 to 2,000 IU) or individual meat additives on the antilisterial activity of OTF in brain heart infusion (BHI) broth (35°C) over a 24-hour period. Concentrations of OTF or nisin alone or combined that proved most inhibitory to *L. monocytogenes*, were applied to frankfurters inoculated with the pathogen (~6.0 $\log_{10}$ CFU/frankfurter) and held under vacuum at 4, 10, or 25°C. OTF (40 mg/ml) strongly suppressed growth of *L. monocytogenes* in BHI broth.

Results: A combination of OTF (40 mg/ml) and nisin (1,000 IU) completely inhibited growth of *L. monocytogenes* in BHI and in frankfurters held at 25°C; however, the antimicrobial effect of OTF alone was not observed in frankfurters at all temperatures used in the present study. Neither salt (0.5%, 1%), lactate (0.78%, 1.56%), nor lactate (1.56%) + diacetate (0.01%), altered the inhibitory effect of OTF against the pathogen in BHI. Only salt (2%) or polyphosphate (0.05%) negated the growth inhibitory effect of OTF on *L. monocytogenes*.

Significance: To optimize the use of OTF in meat products further research is needed to identify additional factors that alter the antibacterial effectiveness of this natural antimicrobial in foods.

P1-08 Testing for Synergy Mixtures of Carvacrol, Eugenol, and Thymol as Antilisterial Agent

REBECA M. GARCÍA-GARCÍA, Enrique Palou and Aurelio López-Malo
Universidad de las Americas Puebla, Departamento de Ingeniería Química, Alimentos y Ambiental, Cholula, Mexico

Introduction: The use of antimicrobials is one of the oldest food preservation techniques. Only one antimicrobial agent was traditionally used to preserve foods; however, it has become a trend in recent years to use mixtures of agents for such a purpose. In theory, using mixtures of antimicrobial agents provide a wider range of activity, which results in the increase of antimicrobial activity.

Purpose: Our objective was to assess whether mixtures of antimicrobial agents (carvacrol, eugenol, and thymol) were consistently synergistic proposing an experimental design using *Listeria innocua* to evaluate it.

Methods: The tested design is based on the minimal inhibitory concentrations (MIC) of individual antimicrobials. The design comprise 14 experiments as follows: three experiments with individual MICs, three experiments with binary mixtures formulated with 1/2 MIC of each agent, one experiment with a ternary mixture formulated with 1/3 MIC of each agent, and for the rest of the experiments combinations of 2/3 of the MIC of an agent and 1/6 of the MIC of the other two agents, 1/6 of the MIC of each agent, or 1/3 of the MIC of one agent and 1/12 of the MIC of the other two agents. Culture media were formulated with the antimicrobials, inoculated with *L. innocua*, and incubated for 72 h at 35°C. Microbial counts of every studied formulation was determined every 24 h.

Results: The most effective individual antimicrobial was carvacrol, followed by thymol and eugenol with MICs of 150, 250 and 450 mg/kg, respectively. It was observed that the most effective binary mixture was 75 mg/kg of carvacrol and 62.5 mg/kg of thymol. Several ternary mixtures corroborate synergism when including at least one phenolic in a fraction higher than its 1/12 MIC. 75, 31.25, and 56.25 mg/kg of carvacrol, thymol, and eugenol, respectively, was the most effective ternary mixture for *L. innocua* inhibition.

Significance: Mixtures of these tested natural antimicrobial agents worked adequately to inhibit *L. innocua* growth.

P1-09 Listericidal Activity of Bacteriophage P100 against Biofilms Cells of *Listeria monocytogenes* Serotypes

Kamlesh Soni and RAMAKRISHNA NANNAPANENI
Mississippi State University, Mississippi State, MS, USA

Introduction: The persistence of *Listeria monocytogenes* biofilms on food and non-food contact surfaces is the major source of this pathogen's environmental spread and subsequent contamination of this pathogen in Ready-to-Eat food products. To date, a number of laboratory-based studies have been conducted to identify the measures to effectively control and eradicate the *L. monocytogenes* cells that are present in a biofilm matrix. Most of these studies focused on the chemical-based treatments where degree of biofilms removal is largely dependent on factors such as biofilm age, the biofilm forming surface, and the substrate in which biofilms are produced. In addition, the repeated exposure to such compounds has also been reported to lead to resistance developments in *L. monocytogenes* cells.

Purpose: Recently, we have demonstrated that GRAS (generally recognized as safe) bacteriophage P100 is an effective anti-listerial agent on both fresh catfish and fresh salmon fillets. However, there are currently no reports on the efficacy of this approved phage on *L. monocytogenes* cells present in a biofilm matrix.

Methods: In this study, we evaluated the efficacy of bacteriophage P100 against 21 different *L. monocytogenes* strains that represent 13 serotypes. Phage P100 efficacy was tested against: (a) *L. monocytogenes* biofilms formed in polystyrene 96-well microtiter wells by a quantitative crystal violet staining assay; and against (b) *L. monocytogenes* biofilm cells formed on the stainless steel coupon surfaces. The viable *L. monocytogenes* cells were recovered from the stainless steel coupons and serially diluted aliquots were spread plated on PALCAM agar plates for enumeration.

Results: L. monocytogenes strains tested showed considerable differences in their ability to form biofilms with strains of serotype 1/2a showing maximum biofilm formation. Irrespective of the serotype, growth conditions or biofilm levels, the phage P100 treatment significantly reduced *L. monocytogenes* cell populations under biofilm conditions. The phage P100 treatment of the stainless steel coupons resulted in a significant reduction of *L. monocytogenes* biofilm population. On this stainless steel coupon surface, there was a 5.4 log/cm^2 reduction in *L. monocytogenes* biofilm counts after phage treatment.

Significance: These findings reveal the potential of phage treatment as an alternative strategy for *L. monocytogenes* biofilm control. Our future experiments will therefore determine the ability of phage cocktail against the removal of mixed *L. monocytogenes* biofilms that occur with other normal microflora on the food contact surfaces.

P1-10 Antimicrobial Activity of Edible Packaging Film Incorporated with Oleic Acid and Nisin against Nalidixic Acid-resistant *Salmonella*

AMRITA PATHANIA and Manpreet Singh
Auburn University, Auburn, AL, USA

Introduction: Microbial contamination reduces shelf life of food and increases the risk of foodborne illnesses. Incorporating antimicrobial substances into packaging materials is a promising form of active packaging to control bacterial spoilage and inhibit and/or eliminate pathogens on contact.

Purpose: The objective of this study was to determine the *in-vitro* antimicrobial efficacy of active packaging material against Nalidixic acid-resistant *Salmonella*.

Methods: A five-strain cocktail of *Salmonella* (Typhimurium, Heidelberg, Enteritidis, Thompson and Kentucky) resistant to 60 µgm of Nalidixic acid were inoculated (ca. ~ 6 log_{10} CFU/ml) onto a thin film of each active packaging material separately. Four active packaging materials used in this study were (1) hydroxy-propyl methyl cellulose [HPMC], (2) HPMC incorporated with Oleic acid [HPMC+OA], (3) HPMC incorporated with Nisin, and (4) HPMC+OA+Nisin. Inoculated active packaging material were maintained at 4 and 10°C for up to 48 h. Samples of the inoculated film were taken at 0, 3, 6, 12, 24 and 48 h, spread plated onto XLT4 agar and incubated for 24 h at 37°C. Analysis of variance was done to compare the efficacy of active packaging materials on survival populations of *Salmonella* over time.

Results: Significant differences ($P < 0.05$) in the survival populations of *Salmonella* were observed as a result of a three way interaction between packaging material, time of exposure to the packaging material, and the temperature of storage. Significantly lower ($P < 0.05$) populations of *Salmonella* were observed on the HPMC+OA+Nisin packaging film over the 48-h period as compared to the other three packaging films.

Significance: Antimicrobial compounds added to packaging films cannot only be used to inhibit spoilage and pathogenic microorganisms but also have a residual effect over time during transport, distribution and storage of food.

P1-11 Use of Natural Antimicrobials to Increase Antibiotic Susceptibility of Drug-resistant Bacteria

KAVITHA PALANIAPPAN and Richard A. Holley
University of Manitoba, Food Science, Winnipeg, MB, Canada

Introduction: Plant-derived antibacterial compounds may be of value as a novel means for controlling antibiotic resistant zoonotic pathogens which contaminate food animals and their products.

Purpose: To study the individual and combined activity of natural antimicrobials (eugenol, thymol, carvacrol, cinnamaldehyde, allyl isothiocyanate) with antibiotic(s) to which test bacteria were highly resistant. Bacteria tested were chosen because of their resistance to at least one antibiotic which had a known genetic basis.

Methods: Individual natural antimicrobial activity was assessed using the broth microdilution method. Fractional inhibitory concentration (FIC) values were calculated to characterize any interaction between the inhibitors using a checker board assay. FIC values ≤ 0.5 indicated that inhibitory agents acted synergistically against the test bacteria.

Results: These bacteria were susceptible to the natural antimicrobials and a considerable reduction in the minimum inhibitory concentrations (MIC) of antibiotics was noted when paired combinations of antibiotic and antimicrobial were used. In the interaction study, thymol and carvacrol were found to be highly effective in reducing the resistance of *Salmonella* Typhimurium SGI 1 to ampicillin, tetracycline, penicillin, bacitracin, erythromycin and novobiocin (FIC < 0.4) and resistance of *Streptococcus pyogenes ermB* to erythromycin (FIC < 0.5). With *Escherichia coli* N00 666, thymol and cinnamaldehyde were found to have a similar effect (FIC < 0.4) in reducing the MIC's of ampicillin, tetracycline, penicillin, erythromycin and novobiocin. Carvacrol, thymol (FIC < 0.3) and cinnamaldehyde (FIC < 0.4) were effective against *Staphylococcus aureus blaZ* and in reducing the MICs of ampicillin, penicillin and bacitracin. AIT was effective in reducing the MIC of erythromycin (FIC < 0.3) when tested against *S. pyogenes*.

Significance: The natural antimicrobials synergistically enhanced the antimicrobial efficacy of the antibiotics to which these bacteria were normally resistant. Trials in an animal intestinal model are to be conducted to verify the potential value of this observation in livestock production.

Poster

P1-12 Molecular Characterization of Fluoroquinolone and Tetracycline-resistant *Salmonella enterica* Isolated from Imported Food Samples

ASHRAF KHAN, Chorng-Ming Cheng, Mohamed Nawaz and Carl E. Cerniglia
U. S. Food and Drug Administration, Microbiology Division, Jefferson, AR, USA

Introduction: Salmonellosis remains a major public health problem world-wide. The emergence and spread of antimicrobial-resistant *Salmonella* has become a major concern in recent years. A variety of foods have been implicated as vehicles transmitting salmonellosis to humans, including poultry, beef, eggs, fruits, vegetables and seafood.

Purpose: Genes responsible for fluoroquinolone resistance, tetracycline and class 1 integrons were characterized among the *Salmonella enterica* serovars isolated from imported food to monitor the prevalence of the multidrug-resistant genes.

Methods: A total of 430 *Salmonella* isolates, representing 64 different serovars, were isolated from imported food samples, and 72/430 isolates were found to be resistant to at least one antibiotic. Fourteen *Salmonella* isolates resistant to fluoroquinolone and tetracycline were further characterized by pulsed-field gel electrophoresis (PFGE), plasmid profiles, antibiotic susceptibility, quinolone resistance-determining region (QRDR) of the gyrA gene, tetA gene and class 1 integron.

Results: All fluoroquinolone and tetracycline resistant *Salmonella* were resistant to several other antibiotics. Most strains were resistant to streptomycin, ampicillin, and kanamycin. Six *Salmonella* strains were resistant to more than six antibiotics including ciprofloxacin and trimethoprim-sulfamethoxazole. Class 1 integrons from six strains resistant to trimethoprim-sulfamethoxazole, tetracycline and ciprofloxacin were characterized. Of 14 isolates, two isolates did not carry any plasmid and eighteen isolates harbored several small and mega-plasmids. For pulsed-field gel electrophoresis (PFGE) analysis, *Salmonella* strain plugs were made and digested with *XbaI* and subjected to 18-h electrophoresis. The PFGE patterns were different for each *Salmonella* strain, although some strains originated from the same country and belong to the same serovar. Eighty percent of strains amplified tetA genes. Quninolone resistance was associated with mutations of the QRDR of the gyrA gene in all strains. To determine the incidence of gyrA mutations, the QRDR region of gyrA gene was amplified and sequenced.

Significance: Our result indicates that imported food could be a reservoir for *Salmonella* isolates resistant to multiple antibiotics and may have potential to transfer these genes to other bacteria.

P1-13 Antibiotic Resistance Profiles of Cattle-associated *Salmonella* Serotypes in Mexico

PRAVEENA SUNKARA, Guy Loneragan, Markus Miller, Alejandro Echeverry and Mindy Brashears
Texas Tech University, Dept. of Animal and Food Sciences, Lubbock, TX, USA

Introduction: Antibiotic resistant *Salmonella* infections have been increasing and account for 20–30% of the *Salmonella* isolated from humans. Patients infected with multidrug resistant *Salmonella* have a fatality rate of 4.2 percent compared to 0.2 percent for antimicrobial sensitive infections. Because the antibiotic resistance patterns vary from region to region in the US they may vary globally; thus investigating the patterns in Mexico and other countries could give insight into the factors contributing to the emergence of the resistance.

Purpose: To determine prominent serotypes of *Salmonella* isolates from Mexican cattle, carcass and retail beef samples and their antibiotic resistance profiles.

Methods: A total of 202 *Salmonella* isolates from Mexico were collected during a similar time period from a feedlot and at the abattoir (hide, fecal grab, carcass at pre-evisceration and in cooler) and retail outlet and serotyped. NARMS antibiotic testing was used. Minimum Inhibitory Concentrations (MIC) of 16-panel antimicrobial drugs were determined using sensititre broth micro dilution technique (TREK Diagnostic systems).

Results: The most common antimicrobial resistance was to sulfisoxazole (85.3%), tetracycline (55.9%), streptomycin (19.6%), trimethoprim/sulfamethoxazol(13.7%), chloramphenicol (12.8%). The remaining antimicrobials have less than 10%. The most common MDR phenotype, observed 14 times, was co-resistant to streptomycin, sulfisoxazole, tetracycline, trimethoprim and chloramphenicol. Antimicrobial resistance was more evident in retail store isolates compared to commercial cattle processing system isolates and feedlot. *Salmonella* muenster was the most prominent serotype followed by *S.* Reading, *S.* Kentucky, *S.* Anatum and *S.* Mbandaka in cattle processing system isolates. *Salmonella* Kinshasha was the most prominent followed by *S.* Derby, *S.* Cubana, *S.* Typhimurium and *S.* Give in the feedlot isolates.

Significance: This study indicates that *Salmonella* serotypes isolated from Mexico have multi-drug resistance patterns. Those associated with retail stores have more resistance compared to the commercial cattle processing system and feedlot.

P1-14 Use of Antimicrobials as Ingredients to Reduce *Salmonella* Populations in Raw Non-intact Poultry Intended for Use in the Manufacture of Frozen, Not Ready-to-Eat Entrees

JARRET STOPFORTH, Damien Wach, Diana Visser and E. W. Bontenbal
Purac, Gorinchem, Netherlands

Introduction: Frozen, breaded chicken products containing raw poultry that appear ready-to-eat but in fact are partly-cooked or browned, specifically raw, frozen chicken nuggets, strips, and entrees, have been identified as sources of recent salmonellosis outbreaks. There has been very little work investigating interventions that can be applied to raw non-intact poultry to reduce the risk of *Salmonella* in frozen, not ready-to-eat entrees.

Purpose: The purpose of this study was to investigate the effectiveness of antimicrobials that may be added as ingredients to raw non-intact poultry intended for use in manufacturing frozen, not ready-to-eat entrees in reducing *Salmonella* populations.

Methods: *Salmonella* was inoculated into raw, cubed chicken and allowed to attach for up to 1 h at 5°C before antimicrobials were added to the meat, mixed and left for 15 min at 5°C. Antimicrobials (ranging from 0.1 to 2%) tested include the following: oregano oil, grapefruit oil, allyl isothiocyanate, carvacrol, peracetic acid, caprylic acid, malic acid, citric acid, polylysine, sodium lactate, and sodium citrate. Following treatment (n = 6), meat was placed into sterile bags containing sterile diluent, homogenized, serially diluted, plated onto tryptic soy agar and violet red bile agar with glucose, and incubated for 48 h at 37°C before surviving populations were enumerated.

Results: Reduction in *Salmonella* populations in raw non-intact chicken ranged from 0 to 5.8 log CFU/g. At a 0.5% (w/w) level (maximum level to be considered a processing aid) antimicrobials able to reduce *Salmonella* > 0.5 log CFU/g were: oregano oil = carvacrol (by 5.8 logs) > peracetic acid (by 4.5 logs). Caprylic acid (1%) and allyl isothiocyanate (2%) resulted in reductions of 5.5 and 4.5 logs, respectively.

Significance: These data suggest that oregano oil, carvacrol, or peracetic acid may be used as processing aids to significantly reduce the risk of *Salmonella* in raw, non-intact chicken.

Poster

P1-15 Efficacy of Fumaric Acid to Reduce *Salmonella* spp. at Various Stages of Poultry Processing

IRYNA SYBIRTSEVA, Sophia Kathariou, Dana J. Hanson, Douglas P. Smith, John B. Luchansky, Karina G. Martino and Fletcher M. Arritt
North Carolina State University, Food, Nutrition and Bioprocessing Sciences, Raleigh, NC, USA

Introduction: The microbiological safety of fresh eviscerated poultry has continued to be a major concern of the public and poultry industry due to frequent foodborne illnesses caused by *Salmonella* spp. Mandated *Salmonella* testing and consumer demand for "natural" antimicrobials to control bacterial contamination during processing have created a need for alternative interventions to reduce pathogens in poultry processing operations.

Purpose: Fumaric acid (FA) was evaluated as an antimicrobial treatment for post-chill dipping, scalding, and chilling by using processing equivalent time and temperature combinations for reduction of *Salmonella* spp. attached to raw chicken skin.

Methods: Irradiated chicken skin samples were inoculated with a four-strain cocktail of *Salmonella* spp. In order to mimic common commercial processing practices, 0.25 and 0.5% FA treatments were applied at 22°C for 40 and 20 s to simulate post-chill dipping, 0.5, 1.0,

and 1.5% FA at 53°C for 3, 2 and 1 min to simulate scalding and 0.05, 0.1 and 0.25% at 3°C for 60, 45 and 30 min to simulate chilling of broiler carcasses. Treatment samples were stomached in buffered peptone water, serially diluted and plated on XLD agar using the thin agar layer technique for acid-injured cells.

Results: Post-chill dipping showed lower reduction in *Salmonella* spp. compared to chilling and scalding treatments. Simulated chilling with 0.25% FA for 45 min resulted in 0.63 *Salmonella* log reduction. The number of recoverable cells decreased as treatment time increased, thus a 0.72 log reduction was observed at 60 min contact time. Overall, the most significant *Salmonella* reductions were obtained when 1.5% FA was applied during scalding, accounting for 1.47 and 1.53 log reductions at 2 and 3 min contact times, respectively.

Significance: Even though, FA at tested parameters resulted in less than 2 log reduction of *Salmonella* counts on carcasses following processing, it may still have the potential for use as antimicrobial agent to reduce *Salmonella* spp. during poultry processing. This could decrease the risk of illness associated with contaminated poultry and the subsequent economic losses related to foodborne illnesses and regulatory recalls.

P1-16 Antibiotic Resistance Patterns in *Escherichia coli* and *Salmonella* Isolates Recovered from Commercially Available Compost

SEAN E. FERGUSON, David T. Ingram, Patricia D. Millner, Cheryl L. Mudd and Manan Sharma
USDA-ARS, EMFSL, Beltsville, MD, USA

Introduction: Compost is used by both conventional and organic farming practices as an eco-friendly means to enhance soil properties and to reduce fertilizer inputs. Inadequate composting may lead to residual human pathogens (e.g., *Escherichia coli* O157:H7 and *Salmonella* spp.) in the final product. Since compost is often prepared from manures and biosolids from therapeutically-treated animals and humans, residual microbial pathogens may exhibit resistance to these antibiotics.

Purpose: We determined the prevalence of resistance to selected antibiotics in *E. coli* and *Salmonella* isolates from commercially available biosolids and yardwaste-based composts.

Methods: Individual *E. coli* (n = 184) and *Salmonella* (n = 53) isolates were selected from composts obtained from eight commercial operations (four yardwaste and four biosolids-based) across the U.S. For each isolate, the Kirby-Bauer method was used to determine antibiograms to the following antibiotics (n = 14): amoxicillin/clavulinic acid, ampicillin, chloramphenicol, ciprofloxacin, doxycycline, erythromycin, kanamycin, nalidixic acid, novobiocin, oxytetracycline, rifampin, streptomycin, sulfamethoxazole/trimethoprim, and tetracycline.

Results: Resistance to one, two, three, four and five antibiotics was observed in 19%, 7.6%, 2.2%, 1.6% and 1.1% of the *E. coli* isolates, respectively. For *Salmonella* isolates, 18.9%, 15.1%, 17.0% and 3.8% were resistant to one, two, three and four antibiotics, respectively. Source-type of compost did not affect the prevalence of antibiotic resistance profiles among *E. coli*, however, *Salmonella* was only recovered from biosolids-based compost. *E. coli* and *Salmonella* isolates were most frequently resistant to the tetracyclines (doxycycline and tetracycline) followed by the penicillins (ampicillin and amoxicillin) and aminoglycosides (streptomycin).

Significance: Commercially available composts may contain low numbers of antibiotic-resistant *E. coli* and/or *Salmonella*. Use of compost in certain pre-harvest environments may constitute a contamination risk for fresh produce. Antibiotic resistance in pathogens has the potential to complicate treatment in cases of foodborne illness.

P1-17 High Throughput, Small Molecule Screening Reveals Diverse Compounds That Inhibit the Growth of *Escherichia coli* O157:H7

JESSICA L. CORRON and Kendra K. Nightingale
Colorado State University, Animal Sciences, Fort Collins, CO, USA

Introduction: *Escherichia coli* O157:H7 causes about 62,500 foodborne illnesses in the U.S. each year. A fraction of individuals infected by *E. coli* O157:H7 develop hemolytic uremic syndrome (HUS), a serious life-threatening condition that can lead to kidney failure and death.

Purpose: Treating individuals infected by *E. coli* O157:H7 with certain antibiotics may lead to an increased incidence of HUS; as a result, few treatment options are available at the clinical level. This demonstrates a clear need to identify novel antimicrobials to be used along the food chain to reduce the load of this pathogen in the human food supply and as therapeutic interventions.

Methods: We thus developed a high-throughput turbidometric assay to identify novel chemical compounds that inhibit *E. coli* O157:H7 growth. Specifically, pin transfers were performed to introduce small molecule libraries into 384-well plates, where each well contained approx. 5 $\log_{10}$ CFU *E. coli* O157:H7. Plates were incubated for 18 h at 37°C and optical density 600 was measured to determine the effect of each small molecule on *E. coli* O157:H7 growth inhibition.

Results: Altogether 64,562 compounds were screened in duplicate for bactericidal properties and 43 compounds inhibited *E. coli* O157:H7 growth. Thirty-eight are known bioactive compounds, and the other five are novel commercially available compounds. Bioactive compounds that inhibited *E. coli* O157:H7 growth were most frequently classified as cephalosporin (n = 13) fluoroquinolone (n = 12) and tetracycline antibiotics (n = 6). One carbapenem, two other antibiotics, an anti-viral and a compound that disrupts the citric acid cycle also inhibited *E. coli* O157:H7 growth. Lastly, two structurally related known bioactives, both common disinfectants used in non-clinical applications, inhibited *E. coli* O157:H7 growth.

Significance: In conclusion, we identified a number of novel chemical compounds, which are both diverse structurally as well as in their modes of action, that effectively inhibit *E. coli* O157:H7 growth. Known bioactive compounds used for non-clinical applications shown to inhibit *E. coli* O157:H7 growth have potential to be applied as dipping or spray-wash interventions to reduce *E. coli* O157:H7 along the food chain.

P1-18 Antimicrobial Effects of Commercial Spices and Plant Extracts against *Escherichia coli* O157:H7 in Uncooked and Grilled Ground Beef

SUMEDHA RAVISHANKAR, Sirtaj Singh, Libin Zhu, Mendel Friedman and Sadhana Ravishankar
Empire High School, Tucson, AZ, USA

Introduction: *Escherichia coli* O157:H7 is reported to be responsible for several foodborne illness outbreaks and fatalities via contaminated ground beef. Reducing or eliminating the pathogen using safe and edible natural products is one approach that can be used to overcome this problem. It has been reported that spices have antimicrobial activity. However, to our knowledge, a systematic survey of the effects of relative antimicrobial activities of commonly used spices in controlling *E. coli* O157:H7 in ground beef has not been performed.

Poster

Purpose: The effects of the following commercially available spice powders and plant extracts against *E. coli* O157:H7 in ground beef were investigated: cardamom, paprika, garam masala, curry powder, Tikka Boti BBQ mix, Meat and Vegetable Spice Mix, olive extract, nutmeg and ginger. The most effective antimicrobials were also tested in combination with heat.

Methods: Antimicrobials (7% wt/wt) were mixed in ground beef which was then inoculated with *E. coli* O157:H7, mixed well and incubated at 4°C for 5 days. Sampling and enumeration was done on days 0, 3, and 5. Beef samples were treated with olive extract and Tikka Boti mix, inoculated with *E. coli* O157:H7, flattened into patties, cooked to an internal temperature of 70°C, and sampled for enumeration of survivors.

Results: Among the antimicrobials tested in ground beef, olive powder showed the highest reduction of *E. coli* (about 2.5 logs) followed by Tikka Boti BBQ Mix (slightly >1 log), and Meat and Vegetable mix (about 1 log), at 5 days of storage. The other spices induced only limited reductions. Compared to the control, heated samples with olive extract induced an additional 2 log reduction of *E. coli* O157:H7.

Significance: Our results suggest that olive extract in cooked or non-cooked ground beef can reduce *E. coli* O157:H7 upon storage and thus, can potentially be used to improve the microbiological safety of ground beef products.

P1-19 Effect of Marinade Ingredients with Antimicrobial Properties against *Escherichia coli* O157:H7 in a Beef Homogenate

HUA YANG, Ifigenia Geornaras, Keith E. Belk, Gary C. Smith and John N. Sofos
Colorado State University, Animal Sciences, Fort Collins, CO, USA

Introduction: Injection of beef muscle cuts with a marinade solution may result in transfer of *Escherichia coli* O157:H7 contamination from the surface to the interior of the product. The activity of natural antimicrobials against pathogenic bacteria *in vitro* is well known, yet few studies have addressed the effects of addition of natural antimicrobials to marinades for the control of *E. coli* O157:H7.

Purpose: This study screened the effect of selected natural antimicrobials as ingredients of marinades against *E. coli* O157:H7 inoculated in a beef homogenate.

Methods: A beef homogenate was prepared by blending beef knuckle (< 5% fat, 100 g) with distilled water (200 ml) for 2 min and then filtering the mixture through cheesecloth. Red wine (10%), (-)- gallocatechin-3-gallate (0.005%), rosemary (1.0%), commercially-available PROTECTA™ Super (with ingredients of salt, sodium acetate and flavorings; 0.5%), and oregano oil, carvacrol and cinnamaldehyde (0.1, 0.2, 0.4, 0.8 and 1.0%), in combination with 1.2% sodium chloride or 0.3% acetic acid, were tested for their effectiveness against rifampicin-resistant *E. coli* O157:H7 inoculated (8-strain composite, 4 log CFU/ml) in the beef homogenate (with 4 to 5 log CFU/ml natural flora). Surviving populations were determined immediately (0 h) and after 48 h of incubation at 15°C. Samples (two replications/three samples each) were analyzed for total bacterial counts on tryptic soy agar (TSA) and pathogen counts on TSA with rifampicin (100 µg/ml).

Results: In samples containing red wine or (-)-gallocatechin-3-gallate, populations (log CFU/ml) increased to 7.8 to 8.0 (total bacteria) and 4.9 to 5.4 (*E. coli* O157:H7) after 48 h at 15°C, and were not different to the control (no ingredients). PROTECTA™ Super, acetic acid, and rosemary inhibited the growth of *E. coli* O157:H7 (counts of 3.7—4.1 log CFU/ml), and acetic acid inhibited the growth of total bacteria (count of 4.8 log CFU/ml). After 48 h incubation, pathogen counts of samples containing 0.1 to 0.2% oregano oil, car-vacrol or cinnamaldehyde were 1.2 to ≥ 5.5 log CFU/ml lower than the control, and at 0.4 to 1.0%, the same ingredients reduced *E. coli* O157:H7 to below the detection limit (1 CFU/ml). At 0.8 to 1.0%, oregano oil and carvacrol caused 2.8 to 5.0 log CFU/ml immediate (0 h) reductions in total bacterial counts. The efficacy of the antimicrobials decreased in the following order: oregano oil, carvacrol and cinnamaldehyde at 0.4% each > acetic acid (0.3%) > PROTECTA™ Super (0.5%) and rosemary (1.0%) > red wine (10%) and (-)-gallocatechin-3-gallate (0.005%).

Significance: Modified marinades containing antimicrobials could improve the safety of non-intact beef products.

P1-20 Surveillance of Antimicrobial Resistance in *Escherichia coli* Isolated from Raw Meat in Korea

HYUN-JUNG PARK, Hee-Soo Lee, Sung-Hwan Wee, Eun-Jeong Heo and Suk-Kyung Lim
National Veterinary Research and Quarantine Service, Livestock Product Safety Inspection, Anyang, Republic of Korea

Introduction: Large quantities of antimicrobials are used for growth promotion and prophylactic purposes and therapy in animals. The occurrence of antimicrobial resistance from livestock is closely related to food additives.

Purpose: In Korea, in order to control the antibiotic resistant bacteria originating from livestock, a national monitoring program has been operational since 2003. Antibiotic resistance data on of *E. coli* isolated from raw meat of slaughterhouses over a three-year period are presented.

Methods: From 2007 to 2009, a total of 1300 samples were collected for *E. coli* isolation in 94 Korean slaughterhouses (437 beef, 445 pork, 418 poultry). The samples were inoculated into Brilliant Green Lactose Bile broth and streaked onto eosin methylene blue agar. Suspected *E. coli* colonies were transferred to MacConkey agar and confirmed by biochemical testing. Tests for resistance to 15 antimicrobial agents and minimum inhibition concentration were conducted by broth microdilution (Sensititre; Trek Diagnostic System).

Results: A total of 602 *E. coli* strains (159 from beef, 171 from pork, 272 from poultry) were isolated from raw meat samples. For 3 years, resistance to tetracycline was found to be the most frequent in beef isolates (43%) and in poultry isolates as 88% on average. But in pork, streptomycin resistance (78%) was the most frequently detected. Resistance to ceftiofur and colistin was rare in all species. The emergence rate of multi-drug resistance (≥ 3 antimicrobial subclass) in poultry isolates (83%) and pork isolates (70%) was much higher than that in beef isolates (26%).

Significance: This result suggests that the prevalence of antimicrobial resistance in poultry meat is higher than that in beef and pork in Korea. Further studies are needed to understand the transmission route of antimicrobial resistant *E. coli* in poultry, including a survey of handlers and any point of contamination during processing in slaughterhouses.

P1-21 Effect of Neutral Electrochemically Activated Water on the Viability and Biofilms of Foodborne Pathogens on Stainless Steel Surfaces

HONGSHUN YANG, Francisco Diez-Gonzalez and Joellen Feirtag
University of Minnesota, Food Science and Nutrition, St. Paul, MN, USA

Introduction: Neutral electrochemically activated water (NECAW) is a sanitizer for food processing equipments which could reduce the risk of foodborne outbreaks. However, little is known about its effectiveness and broad-spectrum activity against pathogenic bacteria on surfaces and biofilms.

Purpose: The objectives of this study were to determine the antimicrobial effect of NECAW on *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* cultures and biofilms.

Poster

Methods: Forty-one pathogenic bacterial strains were tested. Bacterial cultures dried on stainless steel (SS) coupon surfaces and biofilms grown on SS coupon surface were treated with NECAW containing 100 mg/L free available chlorine (FAC) for 1 min and survivors from initial inoculums of 5 to 6 log CFU/coupon were determined by standard plate count on tryptic soy agar. The sanitizing efficacy of NECAW was compared with three other commercially available electrolyzed water sanitizers. Three dimensional morphology images of bacterial biofilms were analyzed by atomic force microscopy (AFM).

Results: The culture viability of most strains on coupon surface was reduced more than 4 log CFU by treatment of NECAW. NECAW was at least 2 log CFU more effective than the other electrolyzed water sanitizers. However, several strains had significant number of survivors after 1 min of NECAW treatment. *E. coli* O157:H7 ATCC 43895 was the most resistant strain to NECAW. NECAW treatment of biofilms reduced bacterial counts by 1 to 3 log CFU per coupon. Reduction of bacterial survivors was increased when NECAW containing higher concentrations of FAC was applied. AFM images of biofilm surface showed tree-like structures. The biofilm structures on SS were destroyed by the treatment with NECAW.

Significance: Different species and strains of foodborne pathogens had variable sensitivity to NECAW. NECAW could be effective in controlling surface contamination with pathogenic bacteria and biofilm growth.

P1-22 Group- and Strain-specific Antimicrobial Activities of Select Plant Essential Oils

DEBJANI MITRA, Hans van Leeuwen and Byron F. Brehm-Stecher
Iowa State University, Ames, IA, USA

Introduction: There is substantial consumer demand for and industrial interest in development of more “natural” antimicrobials for use in disinfection and cleaning of food contact surfaces, for treatment of foods themselves or for use as topical antimicrobials, as alternatives to antibiotics. Plant essential oils are a promising botanical source for such natural antimicrobials.

Purpose: The goal of this work was to screen the antimicrobial activities of 16 essential oils against model Gram-negative and Gram-positive bacterial cell types, represented here by *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. Recent studies indicating the prevalence of traditionally “clinical” pathogens such as methicillin-resistant *Staphylococcus aureus* or *Candida albicans* in food production animals or in foods suggests the potential for non-traditional routes of transmission for these pathogens. Therefore, we also included *C. albicans* ATCC 10231 and a methicillin-resistant strain of *S. aureus* (MRSA, strain BAA-44) in our screen.

Methods: Two indices of antimicrobial activity - Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (or Fungicidal) Concentration (MBC/MFC) were determined in Mueller-Hinton (MH) broth using a Bioscreen C Microbiology Reader and plating onto MH agar.

Results: All 16 oils showed some inhibitory activity against one or more test organisms. However, we noted interesting group- or strain-specific effects. For example, bergamot oil (0.5%), Dalmation sage oil (0.5%), sandalwood oil (0.25%) and Manuka oil (0.0625%) were inhibitory only against *S. aureus* ATCC 25923. Cassia oil and redistilled (high thymol content) oregano oil inhibited MRSA at 0.0625%. Redistilled oregano oil also had the highest activity against *E. coli* (0.0625%). For *C. albicans*, mountain savory (0.125%), lemongrass and lemon myrtle (0.0625%), and redistilled oregano (0.03%) oils were antifungal. Although most oils were effective only at relatively high concentrations, cinnamon and cassia oils inhibited *C. albicans* at only 0.0078%.

Significance: The group- or strain-specific antimicrobial activities shown by these oils against both ”garden variety” and antibiotic-resistant microbes suggest their potential for targeted use as natural antimicrobials, with possible food-related or allied uses.

P1-23 Inhibitory Effect of Apple Phenolic Compounds on the Growth of Selected Bacterial, Yeast and Mold Cells

YAO ZHOU, Xianjia Bai and Jinru Chen
Tianjin University of Science and Technology, College of Food Engineering and Biotechnology, Tianjin, China

Introduction: Phenolic compounds are widely distributed in foods of plant origin such as fruits, vegetables, nuts, seeds, flowers, and barks. Apple has been shown as one of the natural sources of phenolic compounds.

Purpose: This study was undertaken to evaluate the efficacy of apple phenolic compounds on the growths of *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Saccharomyces carlsbergensis*, and *Mucor pusillus* on microbiological media.

Methods: Phenolic compounds in pureed apple (40 g) were extracted with 100 ml of absolute alcohol at 80°C for 3 times, and each for 2 h. The extracts were pooled and vacuum dried (Extract A). The preparation was fractionated using ethyl acetate. The organic and aqueous phases of the extract were separated, dried, and the obtained preparations designated as Extract B and C, respectively. The inhibitory effects of aqueous solutions of Extract A, B, and C (0.01–1.00%) were determined using the agar diffusion assay. The minimal inhibitory concentrations (MIC) of the extracts as influenced by pH (3–8) and salt concentration were also determined.

Results: It was found that the extracts significantly inhibited the growths of tested bacterial cells. However, the growth of tested yeast and mold cells were not affected by the extracts. Extract A and B had stronger inhibitory effects than Extract C. The MIC of Extract A and B for *B. subtilus, E. coli*, and *M. luteus* were 0.10, 0.10, and 0.03%, respectively. The MIC of Extract C for the three bacteria were 0.50, 0.50, and 0.1%, respectively. The strongest and weakest inhibitions of apple phenolic compounds to the tested bacterial cells were observed at pH 5–6 and pH 3–4, respectively. The inhibitory effect of apple phenolic compounds did not seem to be affected by salt concentration.

Significance: The results of the study suggest that apple phenolic compounds could potentially be used as preservatives to control bacterial growths.

P1-24 Evaluation of Different Solvent Solutions for Extraction of Antibacterial Compounds in Jalapeno Peppers

KARLEIGH HUFF, Renee Boyer, Sean O’Keefe, Cynthia Denbow and Robert Williams
Virginia Tech, Food Science and Technology, Blacksburg, VA, USA

Introduction: A small number of studies have indicated that chill peppers (*Capsicum* spp.) possess antibacterial properties, although the compound most commonly investigated from peppers, capsaicin, appears not to be responsible. The complete chili pepper extract should be evaluated for its antibacterial effect. The type of solvent used for extraction can affect the compounds that are isolated.

Purpose: The purpose of this research was to identify which solvent is most successful at extracting unknown antibacterial compounds from jalapeno peppers.

Methods: Fresh jalapeno peppers were chopped, weighed, and placed in a blender with a solvent (either sterilized hot water, 100% methanol, or 100% ethanol) at a 1:1 ratio (g/g) and blended until mixture was completely homogenized followed by shaking for 15 min. The slurry was centrifuged; supernatant was removed and immediately used for antibacterial testing using disk diffusion assay against *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica*. The diameter of growth inhibition was measured and statistically evaluated using ANOVA to determine effectiveness of antimicrobial extraction. Solvents were tested alone as a control.

Poster

Results: There were statistically significant differences ($P < 0.05$) in the diameter of inhibition between extracts, as well as significant differences in diameter of inhibition between each solvent extract and its control. There was greater bacterial inhibition from methanol and ethanol extracts than hot water extracts. *L. monocytogenes* and *S. enterica* were most inhibited by extracts using methanol, while *E. coli* was most inhibited by ethanol extracts. The strongest inhibition was seen from the ethanol extract against *E. coli*.

Significance: Use of methanol or ethanol in the extraction process isolates compounds with greater antibacterial activity. Once an appropriate solvent for antimicrobial extraction has been established, further research can be performed to fractionate the extract and identify exact antibacterial compounds. This research could possibly lead to identification of new natural antibacterial treatments for foods.

P1-25 Inactivation of Foodborne Pathogens by Roselle (*Hibiscus sabdariffa*) *In Vitro* and on Romaine Lettuce and Alfalfa Sprouts

DIVYA JARONI and Sadhana Ravishankar
Southern University Ag. Research and Extension Center, Agricultural Research, Baton Rouge, LA, USA

Introduction: The increased trend in fresh produce consumption has been accompanied with increased outbreaks of fresh produce-associated foodborne illnesses, which suggests a need for better intervention strategies. *Escherichia coli* O157:H7 and *Salmonella enterica* are responsible for about 61% of produce-related illnesses. The limitations of chemical sanitizers used by the produce industry have caused an increased interest in natural interventions as an alternative. Roselle (*Hibiscus sabdariffa*) is an edible plant used in various food applications whose antimicrobial properties, especially against foodborne pathogens, are relatively unclear.

Purpose: To investigate the fate of *E. coli* O157:H7, *S. enterica* and *Listeria monocytogenes* in roselle calyx or leaf aqueous extracts (RCE, RLE) and the bactericidal effects of roselle calyx concentrate (RCC) and tea (RT) against *E. coli* O157:H7 on romaine lettuce and that of RCC against *S.* Newport on alfalfa sprouts.

Methods: Effects of RCE or RLE against *E. coli* O157:H7, *S.* Newport, and *L. monocytogenes* at 4, 8, and 25°C were investigated over 72 h. Effects of RCC and RT against *E. coli* O157:H7 on lettuce and that of RCC against *S.* Newport on alfalfa sprouts were investigated at 4°C over 24 h.

Results: For both *E. coli* O157:H7 and *S.* Newport, no survivors were detected in both extracts at 24 h. At 48 h, *L. monocytogenes* was reduced by > 5 log at 4 and 8°C, and to undetectable levels at 25°C. RCC and RT reduced *E. coli* O157:H7 to undetectable levels on lettuce within 24 h. *Salmonella* Newport was reduced by 1 log in alfalfa sprouts upon exposure, and no organisms detected at 24 h.

Significance: The strong bactericidal activity of roselle calyces and leaves against foodborne pathogens may find applications as antimicrobial treatments during pre- and post-harvest processing of fresh produce and as marinades or salad dressings for fresh-cut produce.

P1-26 Molecular Characterization of Antimicrobial Drug-resistant *Campylobacter* Isolated from Conventional and Antimicrobial-free Swine Production Systems and Their Environment

SIDDHARTHA THAKUR and Macarena Quintana
North Carolina State University, Raleigh, NC, USA

Introduction: Campylobacter is one of the leading pathogens causing foodborne illnesses in the US. Antimicrobial-free (ABF) pork products are gaining wider acceptance in the US. Therefore, we need to understand how the ABF production practices affect the diversity of *Campylobacter* from a food safety perspective.

Purpose: To compare and characterize the prevalence of antimicrobial-resistant *Campylobacter* in the conventional and ABF production systems at farm, slaughter and environment.

Methods: We collected samples from ABF (pigs: 775; environment: 498) and conventional (pigs: 334; environment: 262) farms. At slaughter, we collected swabs from ABF (carcass: 438; environment: 95) and conventional pigs (carcass: 72; environment: 15). A total of 726 isolates from the ABF and 170 isolates from conventional farms were tested for resistance by the broth microdilution method to a panel of nine antimicrobials. Isolates exhibiting tetracycline and erythromycin resistance were screened for the *tet*(O) gene and the A2075G mutation in the 23S rRNA genes, respectively.

Results: Majority of the isolates were speciated as *Campylobacter coli* (n = 1197) followed by *Campylobacter jejuni* (n = 4). There was no significant difference in the prevalence of *Campylobacter* between the ABF (60.6%) and the conventional pigs (61.6% ($P = 0.78$). At slaughter, *Campylobacter* was isolated from all the stages including post chill. The prevalence of *Campylobacter* in the ABF and conventional environment was 23.6% (n = 593) and 26.7% (n = 277), respectively. The highest frequency of resistance was exhibited to tetracycline (ABF 31.9%; conventional 97.8%). A total of five isolates were Ciprofloxacin resistant. Tetracycline resistance was encoded in 86% of the isolates by the *tet*(O) gene while erythromycin resistance was predominantly due to A2075G mutation (92.6%).

Significance: We detected isolates with similar resistance profile between the pigs and their environment on farm and slaughter. Our results highlight the important role played by environment in transmission of antimicrobial resistant *Campylobacter* to ABF pigs.

P1-27 Surveillance of Antimicrobial-resistant *Salmonella* in Antimicrobial-free and Conventional Pigs at Farm and Slaughter

SIDDHARTHA THAKUR and Shivaramu Keelara Veerappa
North Carolina State University, Raleigh, NC, USA

Introduction: Critical knowledge gaps exist in the complex chain of events that leads to dissemination and persistence of antimicrobial resistant *Salmonella* in the antimicrobial-free (ABF) and conventional swine production systems

Purpose: To determine and compare the dynamics of antimicrobial-resistant *Salmonella* in pigs and their environment in the ABF and conventional production systems at farm and slaughter.

Methods: A cohort of 30 pigs per farm was sampled in five ABF and six conventional farms (farrowing, nursery and finishing) and slaughter (post evisceration, post chill and mesenteric lymph nodes-MLN). A total of 1,378 fecal samples were collected at ABF (n = 774) and conventional farms (n = 604). At slaughter, we collected 539 carcass swab samples (ABF n = 407 and conventional n = 132). We also collected 977 environment samples from the farm (feed, water, soil and truck) and 80 from the slaughter (lairage floor). *Salmonella* was isolated and characterized for their antimicrobial resistance profile to a panel of 15 antimicrobials by the broth microdilution method as per CLSI recommendations.

Results: Salmonella prevalence on conventional farm was significantly higher in pigs (5.2%; n = 32) and environment (13.9 %; n = 63) compared to the ABF pigs (0.25%; n = 2) and environment (1.14 %; n = 6) ($P < 0.01$). At slaughter, *Salmonella* was isolated only from ABF carcasses including 10.1% of post evisceration and 3.4% post chill swabs. The highest frequency of *Salmonella* were isolated from the

Poster

slaughter lairage 31.2% (n = 25; ABF n = 20 and conventional n = 5). The isolates exhibited highest frequency of resistance to tetracycline including from conventional farm environment (95.16%) and pigs (69.5%) followed by ABF pigs (28.57%) and its environment (26.6%).

Significance: The prevalence of antimicrobial resistant *Salmonella* in pigs raised on the ABF production system in the absence of selection pressure is concerning. The results highlight the potential role played by the environment in *Salmonella* transmission to the pigs at farm and slaughter.

P1-28 Grape Seed Extract and Malic Acid Effectively Inhibit the Growth of *Campylobacter jejuni* in Broth Culture

NAVAM HETTIARACHCHY, Amara Venkata S. Perumalla, Michael Slavik and Geetha S. Kumar
University of Arkansas, Food Science, Fayetteville, AR, USA

Introduction: *Campylobacter jejuni* is a major foodborne pathogen that may be transmitted through poultry and poultry products and is a leading cause of bacterial gastroenteritis. In the U.S. approximately 1 million symptomatic *Campylobacter* infections are reported annually.

Purpose: The main objective of this study was to determine the effects of grape seed extract (GSE) and malic acid (MA) alone or in combination to inhibit the growth of *Campylobacter jejuni*.

Methods: Broth cultures inoculated with *C. jejuni* at a concentration of 10^5 CFU/mL were used in this study. To study the antimicrobial activity, a total of 3 treatments (1.0% GSE, 1.0% MA, and 1.0% GSE + 1.0% MA) and 1 control (no antimicrobial) were used. Broth cultures with or without the treatment were incubated at 42°C in microaerobic atmosphere with samples taken at 0, 6, 12, 24, 48, 72, and 96 hours. Samples were serial plated onto *Campylobacter* enrichment agar plates with horse blood enrichment and incubated at 42°C for 48 h microaerobically.

Results: By 12 h of incubation control treatment was found to have 10^8 CFU/mL, whereas the treatment groups except the combination treatment showed significant reduction. GSE (1.0%) treatment had its maximum effect (6.36 log CFU/mL reduction) as compared to the control at 48 h of incubation. MA (1.0%) inhibited all the *Campylobacter* population within 6 h of incubation. Combination of GSE (1.0%) and MA (1.0%) did not have appreciable effects against *C. jejuni*.

Significance: These data suggest that natural grape seed extract or malic acid can be used as alternative to conventional chemicals and could have a significant impact on the poultry processing industry.

P1-29 Prevalence, Antibiotic Resistance and Pathogenicity of *Vibrio parahaemolyticus* Isolated from Raw Fish in Korea

Jeong Hwan Cheon, Ji-Hyeon Hyeon, CHANG-HYEON SUNG, In-Gyun Hwang, Hyo-Sun Kwak, Jung-Ah Han, Kwang-Young Song and Kun-Ho Seo
Konkuk University, College of Veterinary Medicine, Seoul, Republic of Korea

Introduction: *Vibrio parahaemolyticus* is a Gram-negative, halophilic pathogen that can contaminate seafood and result in foodborne illness. Prevalence and antibiotic resistance patterns of *V. parahaemolyticus* isolates from raw fish should be monitored.

Purpose: The purpose of this study was to investigate the prevalence, antibiotic resistance, and pathogenicity of *V. parahaemolyticus* in raw fish harvested in Korea.

Methods: A total of 64 samples of raw fish purchased from traditional seafood markets in Seoul were evaluated. Samples of intestine, gill and fin were collected from the raw fish. All samples were incubated into alkaline peptone water at 37°C for 24 h and then streaked onto thiosulfate citrate bile sucrose agar. Suspected colonies were inoculated in triple sugar iron agar for screening test, followed by a biochemical test performed with API 20NE strip. Pathogenicity was evaluated by real-time PCR targeting *tdh* and *trh* genes. Antibiotic susceptibility tests were performed with the disk diffusion method following in accordance with National Committee for Clinical Laboratory Standard protocol.

Results: Thirty-three *V. parahaemolyticus* isolates were recovered from raw fish (33 out of 64, 51.5%) and all isolates were lacking *tdh* and *trh* genes. Sixteen isolates (16 out of 33, 48.5%) were resistant to ampicillin and seven isolates (7 out of 33, 21.2%) were resistant to amikacin. However, 90% of the isolates were sensitive to other antibiotics such as amoxicillin, sulfamethoxazole, trimethopenem, ciprofloxacin, cefotaxime, and cefepime.

Significance: The prevalence of *V. parahaemolyticus* was high in raw fish from traditional seafood markets in Korea. However, no pathogenic strains were found and the antibiotic resistance rate of the isolates was relatively low. These results could be useful information for risk assessment of *V. parahaemolyticus* in Korean seafood.

P1-30 Antilisterial and Antiviral Properties of Bacteriocin Produced by *Pediococcus acidilactici* ST3Ha, a Strain Isolated from Norwegian Smoked Salmon

BERNADETTE D. FRANCO, Svetoslav D. Todorov, Monika B. Wachsman and Manuela Vaz-Velho
University of São Paulo, Food and Experimental Nutrition, São Paulo, Brazil

Introduction: Bacteriocins of lactic acid bacteria (LAB) are ribosomally synthesized antimicrobial peptides. The mode of action indicates an electrostatic interaction with bacterial surfaces, leading to pores formation and dissipation of the proton motive force, resulting in an efflux of amino acids, potassium ions and inorganic phosphate.

Purpose: The purpose of this study was to characterize the bacteriocin produced by the strain *Pediococcus acidilactici* ST3Ha, isolated from smoked salmon, and check for its antilisterial and antiviral properties.

Methods: The production of bacteriocin was studied at 30°C and 37°C. Resistance of the bacteriocin to enzymes, detergents, various pH and temperature was evaluated. The molecular weight was estimated using tricine-SDS-PAGE. The effect on HSV-1 virus and cytotoxity to Vero cells were determined. The partial bacteriocin operon was sequenced.

Results: *P. acidilactici* ST3Ha produces a pediocin-like bacteriocin active against several LAB, *Listeria* spp., other human and food pathogens and remarkably against HSV-1 virus. Bacteriocin ST3Ha was produced at high levels in MRS broth at 30°C and 37°C. The maximum activity (1.64×10^6 AU/ml) against *Listeria ivanovii* ATCC19119 was reached after 27 h. Addition of bacteriocin ST3Ha to a 3-h-old culture of *L. ivanovii* ATCC19119 inhibited the growth of this strain for 24 h. The molecular weight was estimated to be 4.5 kDa. Strain *P. acidilactici* ST3Ha harbors a 1.044 kb DNA fragment fitting in size and presenting high DNA homology (98%) to pediocin PA-1/AcH. The combined application of levels below MIC of ciprofloxacin and bacteriocin ST3Ha resulted in a synergistic effect in the inhibition of *L. ivanovii* ATCC19119. Bacteriocin ST3Ha displayed antiviral activity against HSV-1, an important human pathogen, with a CE50 (50% effective concentration) of 800 µg/mL and CC50 (50% cytotoxic concentration) of 4030 µg/mL.

Significance: To the best of our knowledge, this is the first report on a strain of *P. acidilactici* capable to produce a pediocin-like bacteriocin with activity against HSV-1.

P1-31 Control of *Listeria monocytogenes* in Cheese by Bacteriocinogenic Strains of *Enterococcus mundtii* CRL35 and *Enterococcus faecium* ST88Ch

BERNADETTE D. FRANCO, Esteban V. Pingitore, Danielle N. Furtado, Svetoslav D. Todorov, Maria Elena F. Nader and Fernando J. Sesma
University of São Paulo, Food and Experimental Nutrition, São Paulo, Brazil

Introduction: Bacteriocins are antimicrobial peptides produced by several bacteria. For control of *Listeria monocytogenes* in foods, they can be supplemented with bacteriocins, or inoculated with bacteriocinogenic strains for *in situ* production of bacteriocins.

Purpose: The aim was to evaluate the capability of two bacteriocinogenic strains *Enterococcus mundtii* CRL35 (bac+) and *Enterococcus faecium* ST88Ch (bac+), isolated from cheeses, to control the growth of *Listeria monocytogenes* 426 in experimentally contaminated fresh bovine cheese over 10 days of storage at 8°C.

Methods: Cheeses were prepared with pasteurized milk, lactic acid, calcium chloride and commercial rennet. Four sets were prepared: two made with milk containing one of the two bac+ strains (10^6 CFU/ml), one with milk containing a bac- strain (*E. faecium* ATCC19443) and one added of nisin (12.5mg/l). Other appropriate control cheeses were also prepared. *L. monocytogenes* 426 was added to cheeses during the manufacturing, in order to obtain 10^3 CFU/g. Counts of lactic acid bacteria and *L. monocytogenes* in the cheeses stored at 8°C were done every other day for 10 days.

Results: After 10 days at 8°C, counts of *L. monocytogenes* 426 in the cheeses containing the bac+ and the bac- strains of *E. faecium* decreased in a similar manner (2.63-log and 2.87-log, respectively), showing that inhibition might have occurred due to another factor than the production of bacteriocin. In the cheeses prepared with *E. mundtii* CRL35 bac+, the counts of *L. monocytogenes* decreased 4.69-log, and in those containing nisin, only a 0.75-log reduction was observed.

Significance: The study showed that the strain *E. mundtii* CRL35 bac+ was more effective than *E. faecium* ST88Ch bac+ for the control of growth of *L. monocytogenes* in cheeses stored at 8°C. *E. mundtii* CRL35 bac+ was even more effective than nisin. This research underlines the potential of application of different bacteriocins in the control of nisin-resistant variants of *L. monocytogenes* in cheese.

P1-32 Antimicrobial Effects of Cranberry Extract, Vanillin and Vanillic Acid against *Clostridium perfringens*

PASCAL DELAQUIS, Moussa S. Diarra, Heidi Rempel and Dania Saeed
Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, Canada

Introduction: Clostridium perfringens is a Gram-positive spore-forming, toxigenic, anaerobic bacterium found in soil, dust, feed, the gastrointestinal tract and feces of animals. The species is important in food safety, public and animal health. Whereas cranberry (*Vaccinium macrocarpon*) fruit extracts, vanillic acid and vanillin from the vanilla bean or pod (*Vanilla planifolia*) are known to inhibit several foodborne pathogens, their activity against *C. perfringens* has not been described.

Purpose: The purpose of this study was to evaluate the antimicrobial activity of cranberry extract, vanillin and vanillic acid against 11 *C. perfringens* isolates recovered from broiler chickens.

Methods: Minimum inhibitory concentrations (MICs) were determined using an agar dilution method. Time-kill assays were performed in liquid media supplemented with 0 to 16 mg/ml cranberry extract and 0 to 20 mM vanillic acid. Viable cell populations were estimated using the spread plate method after 2, 4, 8 and 24 h incubation. All assays and analyses were carried out at 37°C under anaerobic conditions.

Results: The MIC of cranberry extract was 8 mg/ml for 10 of the 11 isolates, and 32 mg/ml for the remaining isolate. In contrast, MIC values of 40 mM and 10 mM were measured for vanillin and vanillic acid, respectively, for all the isolates. In the time-kill assays cranberry extract induced a > 3 log CFU/mL decrease in viable cells over 24 h in four of five *C. perfringens* isolates examined. Vanillic acid induced > 3 log CFU/mL decrease in viable cells for all isolates.

Significance: The data indicates that cranberry extract and vanillic acid exert bactericidal effects against *C. perfringens* and that further examination of the antimicrobial potential of these compounds is warranted.

P1-33 Effect of *Mentha spicata* and *Mentha pulegium* Essential Oils on *Debaryomyces hansenii* in an Iranian Yogurt Drink (Doogh)

GUITY KARIM, Mahzad Aghazadeh Meshgi, Rahim Karimi Ababil and Saeed Bokaie
University of Tehran, Faculty of Veterinary Medicine, Food Hygiene, Tehran, Islamic Republic of Iran

Introduction: Particular interest has focused on the potential application of plant essential oils for maintenance or extension of food products shelf life. *Mentha spicata* and *Mentha pulegium* essential oils and some herbs are traditionally used as flavor ingredients in Doogh, a popular yogurt drink in Iran. Blowing and spoilage of Doogh by yeasts are very common defects when this product is not refrigerated.

Purpose: The purpose of this study was to evaluate the antimicrobial effects of *Mentha spicata* and *Mentha pulegium* essential oils on *Debaryomyces hansenii*, which causes spoilage and off-flavor in Doogh.

Methods: A lyophilized culture of *Debaryomyces hansenii* (DSM 70590, obtained from Deutsche Sammlung Von Mikroorganism und Zellkulturen GmbH, Germany) was grown twice in tubes containing Yeast Extract Dextrose Chloramphenicol broth (Merck, KGaA) at 25°C for five days, followed by streaking on YEDC agar slants (Merck, KGaA) and incubating under the same conditions. The cultures were stored at 4°C and sub-cultured every two weeks. *D. hansenii* inocula were prepared by transferring cells from working culture to tubes of YEDC broth and sub-cultured twice. The yeast broth culture was adjusted to an optical density which gave a cell concentration of 1×10^8 CFU/ml for the microorganism. Doogh was prepared according to the national standard and essential oils (EO) were added to the samples according to the study design and inoculated with yeast inocula of 3×10^6 CFU/ml. A sample with no EO was used as control. The study design included six levels of *Mentha spicata* EO (0.05, 0.1, 0.25, 0.5, 1, 1.5%), six levels of *Mentha pulegium* EO (0.025, 0.05, 0.1, 0.5, 1, 1.5%), two storage temperatures (25°C and 4°C) and five intervals (0, 7, 14, 21, and 28 days). All experiments were conducted in independent triplicate. The effect of EO's concentrations on *D. hansenii* in each storage temperatures were evaluated using ANOVA.

Results: The growth of *Debaryomyces hansenii* was significantly ($P < 0.01$) decreased by EO concentrations in all samples containing both essential oils stored at 25°C. *Mentha spicata* EO in 0.25, 0.5, 1, and 1.5, *Mentha pulegium* EO in 0.5, 1, and 1.5% concentrations significantly ($P < 0.01$) decreased the growth of the yeast at 4°C. Increasing storage time decreased growth rate of the microorganism.

Significance: Essential oils of *Mentha spicata* and *Mentha pulegium* not only have antimicrobial effects against *D. hansenii*, but also in low concentrations, may improve sensory properties of Doogh.

Poster

P1-34 Prevalence and Antibiotic Resistance of *Enterococcus faecalis* and *Enterococcus faecium* from Retail Meat and Seafood in Korea

JEONG HWAN CHEON, Ji-Yeon Hyeon, Chang-Hyeon Sung, Yun-Gyeong Kim, Kwang-Young Song, In-Gyun Hwang, Hyo-Sun Kwak and Kun-Ho Seo

Konkuk University, College of Veterinary Medicine, Seoul, Republic of Korea

Introduction: Resistance of pathogenic bacteria against to antibiotics resulting in limited therapeutic options has become a serious concern around the world. Especially antibiotic resistant *Enterococcus* spp. such as vancomycin-resistant enterococci (VRE), are now the most significant nosocomial pathogen.

Purpose: In this study, *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) isolated from retail meat and seafood were analyzed for the pattern of antibiotic resistance.

Methods: A total of 146 retail meat products and seafood purchased from retail market in Seoul were used. Samples were incubated in tryptic soy broth with 6.5% of NaCl at 42°C for 24 h and then streaked onto Slantz-Bartley agar followed by incubating at 37°C for 24 h. Suspicious colonies were screened by oxidase test and finally confirmed by API 20 strep kits. Antibiotic susceptibility tests were performed with disk diffusion methods in accordance with the National Committee for Clinical Laboratory Standards.

Results: Sixty *E. faecalis* (60 out of 146, 41.1%) and six *E. faecium* (6 out of 146, 4.1%) were ultimately isolated. Among the combined 66 isolates of *E. faecalis* and *E. faecium*, fifty-five (55 out of 66, 83.3%) and twenty-eight (28 out of 66, 42.4%) isolates were resistant to tetracycline and rifampin, respectively. However, all isolates were sensitive to chloramphenicol, penicillin, amoxicillin, and gentamycin. Although there were no VRE, fifty percent of the isolates were vancomycin-intermediate enterococci, namely VIE.

Significance: Overall antibiotic resistances of the isolates of *Enterococcus* were relatively low and no vancomycin-resistant enterococci strain was recovered. However, it appears a potential risk of VRE still exists because of the high prevalence of VIE.

P1-35 Production of Antifungal Compounds by *Lactobacillus plantarum*

ANDREIA BIANCHINI and Lloyd B. Bullerman

University of Nebraska-Lincoln, Lincoln, NE, USA

Introduction: Increased concern over chemical food additives has prompted searches for more natural antifungal agents. One source for these agents are lactic acid bacteria, which have been used to ferment foods for years. Different mechanisms have been suggested for this antimicrobial activity, including competitive growth, metabolites, pH, or a combination of these factors.

Purpose: This research was designed to evaluate factors that may influence the production of antifungal compounds by *Lactobacillus plantarum*.

Methods: Lactobacillus plantarum isolated from sauerkraut was evaluated under different conditions for the production of antifungals. The effects of incubation temperature, time, pH, aeration and sugars were investigated. Cultured broths obtained under the different conditions were evaluated using a 96 well plate assay, where *Aspergillus parasiticus* was the test organism for antifungal activity. The plates were prepared with a combination of potato dextrose broth, modified MRS broth cultured with *L. plantarum*, and mold spores, and incubated at 25°C for 7 days. Measurements of optical density were taken daily at 620 nm and the inhibition was determined based on mold growth in the presence and absence of the cultured broth. Statistical analyses of the results were done by analysis of variance.

Results: The production of antifungals increased with incubation time up to 96 hours and the best temperatures for production of active compounds were 30 and 35°C. The pH of the broth during bacterial growth affected the production of antifungals, with best results observed at pH 7.0 ($P < 0.01$). None of the sugars tested improved the production of active compounds when compared with the use of dextrose. Aeration also showed an effect on the production of antifungals, with cultures grown under agitation showing higher antifungal activity than those grown under static conditions ($P < 0.05$).

Significance: Information provided by this research can be used to enhance the production of antifungals by *Lactobacillus plantarum*.

P1-36 Prevalence and Antimicrobial Susceptibility of *Enterobacteriaceae* Isolates on Condiment Jars and Containers in Domestic Refrigerators

AGNES K. KILONZO-NTHENGE, Emily C. Rotich, Sandria Godwin, Fur-chi Chen and Cindy Thompson

Tennessee State University, Nashville, TN, USA

Introduction: Antibiotic resistance is a global food safety and a public health challenge. Resistance to antimicrobial drugs is becoming a serious problem in the United States. It costs lives, money, and threatens the ability to treat infections in humans.

Purpose: The purpose of this study was to determine the occurrence of antibiotic-resistant *Enterobacteriaceae* on condiment jars and containers found in domestic refrigerators.

Methods: An assortment of containers stored in domestic refrigerators (n = 90) were swabbed and analyzed for bacterial contamination. Biochemical methods were used for bacteria identification and characterization. All presumptive *Enterobacteriaceae* was identified by commercially available identification kits. Characterized isolates were afterward subjected to a panel of antibiotics. The antimicrobials susceptibility was determined according to Kirby-Bauer technique.

Results: Contamination levels on jars and containers ranged from not detected to 3.68 log CFU/sample. Jars and containers of salad dressings, miracle whip, jalapeno pepper, and mayonnaise had contamination levels of 2.38, 2.24, 3.1, 3.02 log CFU/sample, respectively. These jars and containers were also contaminated with a range of antibiotic resistant bacteria including: *Enterobacter sakazakii, Escherichia vulneris, Klebsiella pneumoniae, Klebsiella oxymora, Klebsiella terrigena, Enterobacter cloaca, Serratia liquefaciens,* and *Acinetobacter baumanii*. Most isolates indicated single, double, and triple antibiotic resistance. Resistance was recorded for ampicillin (72.56%), tetracycline (7.6%), erythromycin (100%), streptomycin (12.09%), kanamycin (8.79%, chloramphenicol (1.09), and penicillin (100%) All tested isolates were 100% susceptible to gentamycin.

Significance: This study suggests that condiment jars and containers are contaminated with bacteria and therefore, consumer should frequently clean these jar or containers. Food containers should be added to the list of potential contamination sources when health officials investigate new food poisoning cases.

Poster

P1-37 Mechanisms of Antibacterial Action of Catechin against Bacterial Spores

NAOFUMI SHIGEMUNE, Motokazu Nakayama, Takashi Tsugukuni, Hajime Tokuda, Chihiro Yoshizawa, Youko Mekada, Masahiro Kurahachi and Takahisa Miyamoto
Kao Corporation, Global R&D-Safety Science, Ichikai-Machi, Haga-Gun, Tochigi, Japan

Introduction: Catechins can inhibit the growth of bacterial spores. However the mechanism for antibacterial action of catechins on bacterial spores is not well understood.

Purpose: In this study, we have investigated the antibacterial mechanisms of catechins against bacterial spores.

Methods: The effects of green tea extract (GTE, catechin contents: 82%) on germination and outgrowth of *Bacillus* spores were investigated by the measurement of the number of total viable cells and spores in 50% L-broth with or without GTE. The spores incubated with Epigallocatechin gallate (EGCg) were observed by the transmission electron microscopically (TEM) after the treatment with our polyphenol-visualizing method.

Results: In the early stage of incubation, whether 50% L-broth contained GTE or not, the number of spores decreased, while the number of total cells was almost constant. After a 6 h-incubation, total viable number in the presence of GTE was lower than that without GTE. These results showed that catechins didn't inhibit the germination of spores, but inhibited the vegetative cell growth. TEM observation showed that the adsorbed amount of EGCg was high on the surface of vegetative cells and was extremely low on spores. These results suggest that antibacterial action of catechins on spores is attributed to the inhibition of outgrowth due to adsorption of catechins to vegetative cells after germination.

Significance: Information obtained in this study is useful for control of spore-forming bacteria in food by catechins.

P1-38 Study on the Adsorption of Catechins over the Surface of the Bacterial Cell

MOTOKAZU NAKAYAMA, Naofumi Shigemune, Takashi Tsugukuni, Hajime Tokuda, Tomoyo Matsushita, Youko Mekada, Masahiro Kurahachi and Takahisa Miyamoto
Kao Corporation, Global R&D-Safety Science, Ichikai-Machi, Haga-Gun, Tochigi, Japan

Introduction: The mechanism of antibacterial action of catechins is not well understood. Recently we have developed a novel method for visualizing polyphenols by using cerium chloride. Our method is based on the fact that many polyphenols produce hydrogen peroxide in an alkaline environment and that hydrogen peroxide oxidizes cerium to generate cerium oxide precipitates. In this study, catechin-treated bacterial cells were treated with our method and observed with an electron microscope to know the state of catechins over the surface of bacterial cells.

Purpose: The purpose of this study was to obtain detailed information on the adsorption of catechins to the cell surface and advance the understanding of the mechanism of antibacterial action.

Methods: MIC of Epigallocatechin gallate (EGCg) was determined on *Staphylococcus aureus* after the incubation in 50% L broth containing various concentrations of EGCg for 48 h at 37°C at various pHs. The adsorption of EGCg to *Staphylococcus aureus* was observed by transmission electron microscopy (TEM) after the treatment with our visualizing method.

Results: The TEM observation clearly showed the adsorption of EGCg over the surface of the cell. The layer of EGCg on the surface of the cell was thin but with high density under alkaline pH and thick but with low density under acidic pH. The amount of adsorbed EGCg increased with increases in the concentration of EGCg. After the treatment with EGCg at acidic pH, EGCg was observed inside of cell wall, but not at alkaline pH. Under acidic pH, it seems that EGCg can penetrate the cell wall and reach the outer surface of the cytoplasmic membrane, since EGCg has no electrical charge under the condition.

Significance: The results obtained in this study are very useful in understanding the antibacterial mechanism of catechins.

P1-39 Inhibitory Effect on *Aspergillus niger* and *Penicillium* spp. by Vapor Contact of Essential Oils Added to Edible Films

RAÚL AVILA-SOSA, Cesia Castillo-Arzate, Perla Garza-Sánchez, Enrique Palou and Aurelio López-Malo
Universidad de las Americas Puebla, Departamento de Ingenierìa Quìmica, Alimentos y Ambiental, Cholula, Mexico

Introduction: Edible films can incorporate antimicrobial agents to provide microbiological stability, since they can be used as carriers of a wide number of additives that can extend product shelf life and reduce the risk of microbial growth on food surfaces.

Purpose: The aim of this study was to evaluate inhibition of *Aspergillus niger* and *Penicillium* spp. by vapor contact of selected concentrations of Mexican oregano (*Lippia berlandieri* Schauer), lemongrass (*Cymbopogon citratus*) and cinnamon (*Cinnamomum verum*) essential oils added to amaranth or chitosan edible films.

Methods: Essential oils were characterized by gas chromatography-mass spectrometry (GC/MS) analysis. Amaranth and chitosan edible films were formulated with essential oils concentrations of 0.00, 0.25, 0.50, 0.75, 1.00, 2.00 or 4.00%. The antifungal activities in the gas phase were evaluated by determining the colony diameter and minimum inhibitory concentrations (MIC) on mold growth on the agar inoculated with *A. niger* and *Penicillium* spp. after exposure to vapors arising from essential oils added to amaranth or chitosan films placed on the inverted lids of Petri dishes during incubation at 25°C for 8 days. The modified Gompertz equation was utilized to model mold growth curves.

Results: Chitosan edible films exhibited better antifungal effectiveness (MIC values of 0.25% for Mexican oregano and cinnamon, respectively for *A. niger*, and 0.50% for *Penicillium* spp. and both oils) than amaranth films (MICs for both molds of 2.00 and 4.00% for cinnamon and Mexican oregano, respectively). For lemongrass essential oil MICs were higher than 4%. The modified Gompertz equation adequately described mold growth curves. A significant ($P < 0.05$) change of Gompertz parameters was observed among essential oil concentrations, increasing the lag phase and decreasing radial growth rates as oil concentration increased.

Significance: Chitosan edible films added with Mexican oregano or cinnamon essential oils could improve the quality of foods due to the action of volatile compounds against surface growth of molds.

P1-40 Antifungal Activity by Gaseous Contact of Oregano (*Origanum vulgare*) Essential Oil on the Growth of *Aspergillus flavus*

AÍDA I. GÓMEZ SÁNCHEZ, Enrique Palou and Aurelio López-Malo
Universidad de las Americas Puebla, Departamento de Ingenierìa Quìmica, Alimentos y Ambiental, Cholula, Mexico

Introduction: The potential antimicrobial effect of essential oils when applied in vapor phase is an interesting approach for their application. Also, combination with environmental factors such as incubation temperature is poorly studied.

Purpose: The aim of this study was to evaluate the antifungal activity in vapor phase of oregano (*Origanum vulgare*) essential oil on the growth of *Aspergillus flavus*.

Methods: Different concentrations of oregano essential oil (0, 14.7, 29.4, 58.8, 117.6 or 294.1 µL of essential oil/L air) and selected incubation temperatures (25, 30 or 35°C) in a model system of potato-dextrose agar adjusted to a_w 0.98 and pH 4.0 were evaluated. Petri dishes were inoculated pouring 2 µL of *A. flavus* spore suspension. The inoculated and control dishes were incubated in plastic chambers (1.7 L) with transparent cover and sufficient headspace; at the bottom of the chamber a dish containing the necessary amount of essential oil was placed to obtain the desired concentration. Mold colony diameter was periodically determined during 3 months of incubation and results fitted with the Gompertz model. If no growth was observed after the incubation period, the inoculated plated was further incubated without essential oil in order to determine if the observed antifungal effect was fungistatic or fungicidal.

Results: Mold growth curves were described satisfactorily by the Gompertz model ($0.984 \le R^2 \le 0.999$). Both, temperature and essential oil concentration exhibited significant effect ($P < 0.05$) on growth rate and lag time of *A. flavus*. Higher concentrations of essential oil and lower temperatures, decreased mold growth rate and increased lag time, suggesting that the essential oil works by slowing or inhibiting the mold germination stage. For every studied temperature, the minimal inhibitory concentration was 294.1 µL/L air. Further, it was identified that at 30 or 35°C the antifungal effect was fungistatic rather than fungicidal as observed at 25°C.

Significance: Results suggest that oregano essential oil applied in its gas phase exerts important antifungal activity on *A. flavus* growth.

P1-41 *In Situ* Control of Food Spoilage Fungus Using *Lactobacillus acidophilus* NCDC 291 and Its Ability to Survive Food-processing Conditions

SEEMA GARCHA and Navdeep Kaur
Punjabi University, Biotechnology, Patiala, India

Introduction: A challenge for the food industry today is to produce minimally-processed food, without use of chemical preservatives and little compromise on nutritional status. *Lactobacillus acidophilus* NCDC 291 is a bacteriocin-producing bacteria which can be added directly to food where it has to withstand processing conditions to survive and enhance shelf life or the metabolite be purified and used.

Purpose: The purpose was to evaluate its *in vitro* food processing method's survival capability and ascertain the efficacy of this organism as biopreservative against fungi *in situ*.

Methods: Lactobacillus acidophilus NCDC 291 was subjected to food processing related stresses, (brining, Class I preservative, sodium chloride (1-5% v/v) and thermal stress (60°C–120°C temperature for varying time periods 10 – 60 min). Survivor cells were grown on recommended medium and growth curves were plotted. Commonly encountered food spoilage fungi were isolated from visibly spoiled food and identified. *In situ* biopreservation was studied by co-culturing on fresh fruit (*Mangifera indica*), vegetable (*Lagneria siceraria*), meat and processed food (cheese and fruit beverages).

Results: A decrease of approximately 1-log cycle with every 1% increase in concentration of sodium chloride was observed. Heat treatment at 60°C/10 min. had minimal effect on cell count compared to optimal incubation but higher temperatures lowered the cell count ($P < 0.05$, Bonferroni Holm). Four most commonly obtained spoilage fungi were isolated and identified as *Fusarium, Alternaria, Penicillium*, and *Aspergillus*. All of them were inhibited *in vitro*. *Alternaria* was evaluated *in situ* where its colony count was significantly ($P < 0.05$) reduced in presence of *L. acidophilus*. Surface application on *Mangifera* and *Lageneria* delayed texture deterioration by more than 48 h and microbial spoilage was not observed up to 6 days.

Significance: Bacteriocin producer culture added directly delays food spoilage and extends shelf-life facilitating transportation. It can be used as a tool of hurdle concept, diluting the necessity of cold temperature storage which may not be available everywhere.

P1-42 Microfiber Cloths – A Sanitation Lesson from the Clinical Sector?

JOHN HOLAH and Deb L. Smith
Campden BRI, Gloucestershire, United Kingdom

Introduction: Microfiber cloths were introduced into the healthcare sector on the basis that their very fine fibers could attract soil and microbial particles. They were also said to be capable of repeated laundering and re-use. While little scientific evidence was available to substantiate this, the technology has been widely accepted for the cleaning of floors and patient contact surfaces.

Purpose: This study sought to examine the inherent soil and microbial cleaning properties of commercially available cloths and determine their soil loading capacity and efficacy following repeated laundry cycles.

Methods: Sheets (15 × 60 cm) of stainless steel, laminate and ceramic tile were soiled with a commercially available soiling agent (Browns solution) subsequently inoculated with cultures of *Escherichia coli*, MRSA or *Clostridium difficile* spores. Nine commercial microfiber cloths were assessed for their inherent capability to remove the soil from single sheets by attaching the cloths to a robotic arm programmed to undertake a series of cleaning actions. Microorganisms were recovered from the sheets by swabbing using commercial wipes. A second series of experiments used the same cloth to wipe 9 consecutive, soiled laminate sheets to determine the capacity of the cloth to adsorb a soil/*C. difficile* mixture. A third series of experiments assessed the efficacy of the cloths, after being laundered once, 75 and 150 times, to clean laminate sheets soiled with soil/*C. difficile* mixture. Triplicate replicates were undertaken for each variable (surface or microorganisms) for each trial.

Results: Overall, the cloths demonstrated a 2 to 3 log reduction of microorganisms inoculated into Browns soil and, using statistical techniques to assess practically significant cleaning differences of one log order, all nine cloths performed similarly for all surfaces and microorganisms. There were, however, differences in the cleaning ranking of the cloths between individual surfaces and microorganisms. Capacity for cleaning was reduced with successive sheet cleans, though cleaning efficacy was not affected by laundering the cloths for up to 150 times.

Significance: The work demonstrated that microfiber technology works in principle and is capable of removing clinically relevant soils and microorganisms from a range of surfaces. The technique may thus be an alternative method to decontaminate environmental surfaces in the food industry, particularly where the use of water should be avoided or minimized.

P1-43 Pathogens in California Restaurant Kitchen Towels

Jack Neal, DANIEL HENROID and Rochelle Lacey
University of California, San Francisco, Microbiology and Immunology, San Francisco, CA, USA

Introduction: Used kitchen towels in restaurants may be a significant potential source of foodborne pathogens contributing to cross contamination events in the establishment. Observational studies published by the U.S. Food and Drug Administration in 2000 and 2004, surfaces and utensils were found to be contaminated more than half of the observations. To evaluate the significance of this potential hazard, kitchen towels (n = 12) were collected from a convenience sample of 4 full-service restaurants in San Francisco.

Purpose: The objective of this study was to determine if towels used in restaurants may be contaminated with high levels of bacteria capable of transferring onto food preparation surfaces as well as food items prepared on these surfaces.

Methods: Samples were collected in sterile sample bags, labeled with a random number identifier and shipped with ice packs to retard additional growth to the Food Microbiology Lab at the University of Houston within 24 hours of collection. Additional data were completed for each establishment's samples for the amount of time the towel was used, the work area where the towel was used, activities the towel was used for (e.g., cleaning dirty surfaces) and the condition of the towel at the time of collection. Samples were tested for total aerobic plate counts, coliforms, and generic *Escherichia coli*.

Results: The median APC count was 3.8 log CFU/g. Six samples tested positive for coliforms with a mean of 2.7 log CFU/g. Towels associated with produce preparation, extended use, or with heavily soil did not have detectable levels of *E. coli*. Comparative results indicated that there were no significant differences ($P > 0.05$) between the microbial counts and the towel condition (wet or dry) or the location within the kitchen.

Significance: Results indicate that towels used in restaurants may be contaminated with high levels of bacteria capable of transferring onto food preparation surfaces as well as food items prepared on these surfaces. Restaurants need to focus more attention on proper usage and storage of kitchen towels in the workplace.

P1-44 Handwashing Practices in California and Texas Quick-service and Full-service Restaurants

DANIEL HENROID, Mag Binkley, Rochelle Lacey, Ann Ulmer and Mindy M. Brashears
University of California, San Francisco, Microbiology and Immunology, San Francisco, CA, USA

Introduction: Poor personal hygiene, specifically hand hygiene, has been reported as a significant cause of foodborne illness outbreaks. According to observational studies published by the U.S. Food and Drug Administration (FDA) in 2000 and 2004, employees in fast food and full-service restaurants were found to be out of compliance for proper handwashing practices more than other segments of the retail food industry.

Purpose: The purpose of this study was to assess the back-of-house employee handwashing prevalence by observing the employees during food preparation.

Methods: A convenience sample of 37 chain and independent full-service and quick-service restaurants was selected from San Francisco (urban) and Lubbock, Texas (rural). Employees were observed during specific work activities to see whether or not they washed their hands when indicated. Indications of handwashing, based on the FDA Model Food Code and local health authority food codes, were recorded along with the corresponding action (if any) taken by the employee.

Results: For all restaurants, urban restaurants were out of compliance 65% of the time compared to rural restaurants at 51%. Urban full-service restaurants were out of compliance 68.8% and quick-service 60.7%. For rural restaurants, full-service restaurants were out of compliance more often at 57.9% and quick-service at 44.8%. Examining urban chain and independent establishments, full-service chains (70%) and quick-service independent (69%) were out of compliance more often. Rural chain establishments from both categories were more often out of compliance, 61% for full-service and 49% for quick-service. When employees did wash their hands, only 37% of urban employees, and 29% of rural employees did so adequately. The handwashing indications out of compliance most often for urban were: after engaging in activities that contaminated the hands (74%), before putting on gloves (9%) and during food preparation (6%). For rural the results were: after engaging in activities that contaminated the hands (68%), when switching between working with raw and ready-to-eat food (8%), and during food preparation (8%).

Significance: The study showed that appropriate handwashing is less likely to happen in full-service than quick-service establishments and in chain rather than independent. When employees do wash their hands, the majority do not adequately perform the task. Restaurants need to spend more time training employees on proper handwashing and developing systems to assess the frequency and quality of employees' handwashing in their establishments.

P1-45 Virucidal Activities of Detergents and Enzymes against Feline Calicivirus, a Norovirus Surrogate

TOMOMI HOSHINO, Ryoko Kumakura, Yuka Inagaki, Yukifumi Konagaya, Fumihito Inageta, Hiroshi Kibuse and Hiroshi Urakami
Niigata University of Pharmacy and Applied Life Sciences, Niigata, Japan

Introduction: Only a few of disinfectants are known to be effective against norovirus. We analyzed virucidal activities of various detergents and enzymes against feline calicivirus (FCV), a norovirus surrogate.

Purpose: To develop an effective disinfection measure applicable to food and food-service industries.

Methods: FCV F9 was prepared from infected feline kidney cells by differential centrifugation and suspended in a saline solution. The suspension was mixed with solutions containing detergents and enzymes at a ratio of 1:9 and reacted for 5 minutes at room temperature. The mixtures were then serially diluted and inoculated onto the cells in multiplates. The cells were fixed and stained to determine the infectivity after cultivation for four days.

Results: We analyzed 12 anionic, non-ionic and amphoteric detergents and found that only some of the anionic detergents inactivated FCV by more than 4 logs at 1% concentration. Relationship could not be revealed between the molecular structures of detergents and their efficacy. Proteases widely used for various industrial purposes, such as for dishwasher detergents, also inactivated FCV by more than 5 logs by treatment with proteases at the concentration of 0.1%. RNase, however, showed no effects on the infectivity at any pH of the suspensions. No significant synergic effect was observed in mixtures of the effective detergents and the proteases.

Significance: The anionic detergents and proteases inactivate FCV probably by their denaturing effect on the viral capsid proteins. They probably also effective against norovirus.

P1-46 Monitoring of Korean-style Raw Fish Restaurant for Developing Standard Sanitation Manual

DANHEE JEONG, Aeson Om, Hyunah Shin and Aejung Lee
Hanyang University, Food and Nutrition, Seoul, Republic of Korea

Introduction: The habit of eating raw fish exists in Korea and Japan. Specially, Koreans eat raw fish that are stored in water tanks in restaurants. Eating raw fish, along with a lack of standard sanitation, leads to foodborne disease. Effective education for improving field sanitation is needed in Korea. Thus, an easily understood standard sanitation manual is needed.

Purpose: The purpose of this study was to offer basic data to develop a standard sanitation manual for processing Korean-style raw fish in restaurants.

Methods: Countrywide, 90 Korean-style raw fish restaurant kitchens were monitored for good sanitation habits. Indicator bacteria were measured from kitchen tools (knives and chopping boards for pre-cooking process, knives and chopping boards for cooking process, towels for removing water on surface of raw fish, gloves used to prevent fish slippage and chunsachea used for dish decoration).

Results: Employee knowledge of hygiene and hygiene management were investigated at 90 sushi restaurants in 15 cities. 61.1% of these restaurants showed generally well-managed hygiene. The hygiene management of halls and kitchens showed a higher percentage of 97% and 93% respectively, compared to other types of establishments. However, the execution rate of personal hygiene management, utensil management, process management and ingredients/sub ingredients management was 71%, 63%, 59% and 34%, respectively. Thus, it was concluded that improvement was necessary, especially with chunsachae and moochae ingredients/sub ingredients management. The data from the indicator bacteria revealed weak sanitation conditions on surfaces which came into contact with raw fish.

Significance: Based on the above data, it is concluded that there is urgent need for the development of a manual for management of standard sanitation, especially for workers using the Korean cooking style.

P1-47 Efficacy of Different Dry-cleaning Methods for Removing Allergenic Foods from Food-contact Surfaces

LAUREN S. JACKSON and Fadwa Al-Taher
Food and Drug Administration, NCFST, Summit-Argo, IL, USA

Introduction: Cleaning of shared processing equipment is of paramount importance for reducing the risk of allergen cross-contact. Although wet cleaning methods tend to be effective at removing allergenic residues from food processing equipment, there are circumstances where water can not be used for cleaning. In these instances, dry cleaning methods are used.

Purpose: The objective of this study was to evaluate the efficacy of two dry cleaning methods, high efficiency vacuum and alcohol-moistened wipes, for removing residues of milk, egg, peanut and soy foods from several food-contact surfaces.

Methods: For experiments evaluating the effectiveness of wipes, slurries containing 1000 ppm peanut flour, skim milk powder, whole egg powder, soy flour, soy milk and soy infant formula powder were prepared in HPLC-grade water. One mL aliquots of the slurries were deposited on the surface of stainless steel, Teflon and urethane plates. The plates were heated at 80°C for 1 h to form a cooked food residue. Alcohol-moistened wipes were used to clean the cooked food residues from the plates. For experiments evaluating the use of vacuum, plates were prepared as described above. In addition, approximately 3 g of peanut flour, milk powder, egg powder, soy flour and soy infant formula powder were applied to the surface of the plates without heat. A vacuum was then used to clean the surface of the plates. The efficacy of the cleaning methods was determined using visual evaluation, ELISA, a total protein swab, and ATP swabs (sensitive and conventional). All experiments were done at least four times.

Results: For the three surfaces, the wipes were able to remove all cooked food residues as determined by visual inspection, ELISA, and the protein swab. In contrast, conventional and sensitive ATP swabs detected the presence of residue when the surfaces were clean according to visual, ELISA and protein tests. For all trials, use of the vacuum was unable to remove cooked food residues using a visual inspection or any of the analytical methods. When foods were not cooked onto the surface of the plates, the vacuum was able to remove all visible traces of the foods. However, in some cases, ELISA, the protein swab and both ATP swabs detected the presence of food residues.

Significance: These results indicate that the moistened cloths were effective at removing allergenic food residues from food-contact surfaces under the conditions studied here. ELISA and protein swabs were equally effective tools for detection of food residue. The results suggest that the use of vacuum may not be effective for removing allergenic food residues from food-contact surfaces.

P1-48 Comparison of Specific Immunoassay Techniques to ATP and General Protein Methods for Reliability in the Detection of Common Food Allergens

ANTHONY J. LUPO, Chris D. Roebuck, Anna E. Quain and Monica A. Dutcher
Neogen Corporation, Technical Service, Lansing, MI, USA

Introduction: A study was performed to evaluate the ability of ATP systems and general surface protein systems to detect food allergens when compared to traditional immunoassay techniques and rapid immunoassay detection devices. An ATP system and a general protein detection system currently being marketed as methods for reducing the possibility for food allergen contamination by detecting ATP (adenosine triphosphate) or general proteins and simple sugars on food contact surfaces were evaluated using the common food allergens egg, wheat, milk, and peanuts. After repeated trials were performed comparing to accepted traditional specific immunoassay techniques and rapid immunoassay detection devices, it was concluded that general protein and ATP techniques while appropriate for general cleanliness measurement lacked the appropriate sensitivity and specificity to be an appropriate allergen control tool. Measurable differences in sensitivity between 10 and 1000 fold were observed across multiple common food allergens indicating that specific immunoassays were the best choice for allergen control.

Purpose: The purpose was to evaluate multiple methods used by industry for allergen control for appropriate specificity and sensitivity in the verification of allergen cross contact events.

Methods: Standard microwell enzyme immunoassay, lateral flow immunoassay, ATP Chemiluminescence, and traditional protein chemistry were all challenged with serially diluted allergenic and non-allergenic foods to compare their respective sensitivity and specificity.

Results: Upon comparison of ATP to immunoassay, it was determined that immumnoassay was 10x more sensitive in peanut butter, 1000x more sensitive in milk, and 10,000x more sensitive in egg with full specificity to non-allergenic residues. Respectively, upon comparison of Protein swabs to immunoassay, it was determined that immumnoassay was 10x more sensitive in peanut butter, 100x more sensitive in milk, and 1000x more sensitive in egg with full specificity to non-allergenic residues.

Significance: Based on the supporting data, specific immunoassays were the best choice for allergen control.

P1-49 Effects of Sanitizer Washing on Produce with Different Surface Morphologies

SHIH HUI SUN, Kyung Yoon Kwon and Ki Sun Yoon
Kyung Hee University, Food and Nutrition, Seoul, Republic of Korea

Introduction: Washing fresh produce with sanitizer is one of the most important intervention steps for managing bacterial populations. Thus, effective disinfecting agents are needed for produce washing to ensure the safety of produce.

Purpose: The purpose of this study was to evaluate washing efficacy of sodium hypochlorite on the reduction of *L. monocytogenes* inoculated onto produces with different surface properties.

Methods: Nalidixic acid-resistant strains of *L. monocytogenes* or *S.* Typhimurium were inoculated onto the surfaces of produce including cherry tomatoes, cucumbers, and carrots, which were then washed with 100 ppm sodium hypochlorite. The washed samples were cut into pieces and fixed by primary-fixation (2% paraformaldehyde and 2% glutaraldehyde) and post-fixation (1% osmium tetroxide) at 4ÆC for 2 hours. The fixed samples were dehydrated with graded ethanol [30, 50, 70, 80, 90% (v/v), 3 changes of 100%] at room temperature for 10 min each, and treated with isoamylacetate (100%) for 15 min, 2 times and dried with a critical point dryer. After drying, the samples were attached to metal stubs and examined by a scanning electron microscope (SEM) at 15 Kv.

Results: Washing with 100 ppm sodium hypochlorite removed the wax layer on the surface of the cherry tomatoes and cucumbers, indicating that sanitizer washing with sodium hypochlorite may affect the sensory qualities of cherry tomatoes and cucumbers during storage after washing. In contrast, no wax layer was observed on the surface of the carrots. Compared to the surfaces of the cherry tomatoes and cucumbers, fewer cells of *L. monocytogenes* were observed on the carrots. The unremoved *L. monocytogenes* seemed to penetrate into the surface of the carrots. Thus, washing with sanitizer may affect the sensory qualities of the produce during storage.

Significance: Appropriate washing methods and sanitizers according to the types of produce and pathogens must be used in foodservice operations.

P1-50 Effects of Non-antimicrobial Excipients on Cationic Antimicrobials Used in the Food Industry

GERARDO GUZMAN-GOMEZ and Alvaro García-Ayala
Universidad de Guadalajara, Producción Animal, Zapopan, Mexico

Introduction: Cationic antimicrobials have been recognized for their wide spectrum and low toxicity. Some of them are used in the food industry, but the different efficacy between commercial formulations depends of their non-antimicrobial excipients interaction. Five excipients provided from Viator and five cationic antimicrobials purchased from the chemistry industry were mixed to measure MIC values and to determine their synergy or antagonist effects.

Purpose: To evaluate synergistic and antagonistic effects of non-antimicrobial excipients on cationic antimicrobials used in the food industry.

Methods: Excipients tested with four replicates were a mixture of chelating agent and humectants (emix-1), non ionic surfactant and emulsification agent (emix-2), chelating agent, humectants and emulsification agent (emix-3), amphoteric surfactant and amphoteric surfactant (mix-4), non ionic surfactant and humectants (emix-5). Cationic antimicrobials tested were diamine, polimixin E and three generations of quaternary ammonium compounds (QAC-1st, QAC-4th, and QAC-5th) reaching concentrations of 0.5, 2, 4 and 20 ppm respectively, and blended with each excipient inside of tubes with 10 mL brain heart infusion broth with fenol red as a pH indicator. Inoculums with *S. aureus* or *E. coli* reaching 10^6 cells/mL were added to each tube, incubating at 35°C for 48 h. Bacterial growth was confirmed in brain heart infusion agar.

Results: Only excipents emix-1 and emix-3 were synergist with all cationic antimicrobials ($P < 0.05$), with MICs for *E. coli* and *S. aureus* from ≤ 0.5 to 4 ppm. Excipients emix-2, emix-4 and emix-5 were antagonist with all cationic antimicrobials (*E. coli* MICs >20 ppm and *S. aureus* MICs from 2 to >20 ppm).

Significance: Two excipients had synergistic effects on the five cationic antimicrobials tested and three had am antagonistic effect. Practical evaluation of MICs may be useful in the validation process of commercial sanitizers in the food industry.

P1-51 Efficacy of Non-chlorine Biodegradable Sanitizers in Artificially-contaminated Poultry Water Lines

GERARDO GUZMAN-GOMEZ and Alvaro García-Ayala
Universidad de Guadalajara, Producción Animal, Zapopan, Mexico

Introduction: Water lines are recognized as a contamination source in the food and poultry industry because microbes can adhere and colonize them, causing adverse effects in the food chain. Alternative non-corrosive and friendly environment sanitizers could substitute Sodium hypochlorite as water line sanitizer in the poultry production premises. Readily biodegradable Amino acid-surfactants-like had been reported to be effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. Therefore, a product formulated with alkyl betaine seems to be a good alternative as water line sanitizer.

Purpose: To evaluate the efficacy of biodegradable non-chlorine sanitizers on artificially-contaminated water lines .

Methods: Miniature water lines of 6-foot long with four replicates for each product, were filled with contaminated water (APC 6.5 log CFU/mL, TC 6.0 log CFU/100 mL) that had been collected from poultry farm drinkers and then allowed to sit for seven days. After seventh day, the lines were flushed with two gallon of product, allowed to sit in the lines for two hours, and then, the lines were flushed five times with sterilized tap water, allowed to sit in the lines for other seven days to determine aerobic plate count (APC) and total coliforms (TC). Solutions of products tested were Efiplak® (0.5% alkyl betaine), Efisham® (0.2% Sodium lauryl sulphate), Sodium hypochlorite (50 ppm) and sterilized tap water.

Results: The most effective sanitizer was alkyl betaine ($P < 0.05$) (APC 2.3 log CFU/mL, TC 2 NMP/100 mL) followed by Sodium lauryl sulphate (APC 3.6 log CFU/mL, TC 170 NMP/100 mL) and Sodium hypochlorite (APC log 4.6 CFU/mL, TC 0.5 NMP/100 mL). Tap water was not effective (APC 4.6 log CFU/mL, TC > 2,400 NMP/100 mL).

Significance: Non-chlorine biodegradable sanitizers are a good alternative for the sanitation of poultry water lines.

P1-52 Evaluation of the Sanitation Process by the Use of Swab PRO-Clean™ in Equipment/Utensil Surfaces Used in Food and Nutrition Units

SILVANA M. SREBERNICH, Kátia Regina M. Rodrigues, Daiane V. Camargo and Ludmila M. Lima
PUC-Campinas, College of Nutrition, Campinas, Brazil

Introduction: The sanitation process in Food and Nutrition Units must be performed by following rules, standards and procedures for the safe food production. The efficiency of the sanitation process should be followed by rapid methods of control so that corrective measures can be taken immediately.

Poster

Purpose: The purpose of this study was to evaluate the sanitation process by the use of swab PRO-Clean™ rapid method in equipment/utensil surfaces of six food and nutrition units.

Methods: To verify if the units had SOPs, GMP and HACCP Manuals, types of cleaning used and frequency of employee training, questionnaires were developed. The next step was the evaluation of the cleaning process through the use of swab PRO-CleanTM in meat board, meat counter, deli slicer, vegetable board and vegetable chopper. Evaluations were performed twice with an interval of one month between them. In this period employee training on cleaning methods was performed.

Results: All units had SOP and BPM Manuals while only two units had HACCP (33.3%). Chlorine as a sanitizer was used in 83.3% of units. However there was no uniformity related to concentration level and action time used by the units. The training of the employees was performed once a month for fifteen minutes, with exception of one unit (3 month interval/120 minutes). The approval ratings before and after training of the employees were from 33.3% to 66.7% for meat boards, from 66.7% to 83.3% for meat counters, from 50.0% to 33.3% for deli slicers, from 33.3% to 66.7% for vegetables board and from 16.7% to 50.0% for vegetable chopper showing a marked improvement with the exception of the deli slicer.

Significance: The results obtained with the swab PRO-clean™ test could immediately show the need for continuous training of employees and also for systematic monitoring by professional food people.

P1-53 Ozone: A Novel Disinfectant for End-of-Production and Wholeroom Decontamination Applications

John Holah, ALICJA MALINOWSKA and Karen Middleton
Campden BRI, Gloucestershire, United Kingdom

Introduction: Traditional sanitation approaches to controlling environmental contamination in the food industry have been sufficient to maintain day-to-day control. In some instances, however, microbial strains have become persistent in food factories, surviving for several years.

Purpose: This work assessed the use of ozone (O_3) to control microorganisms under two scenarios. First, could ozone be used on a day-to-day basis to replace chemical disinfectants and secondly, could high levels of ozone be used to decontaminate food processing areas for specific pathogens, an approach termed 'wholeroom' disinfection.

Methods: Microbial suspensions dried onto stainless steel coupons were placed at a range of heights and orientations in a specifically designed aerobiology containment rooms. Decontamination with O_3 at concentrations of 8 to 25 ppm was undertaken to simulate continuous daily operational and single use decontamination strategies, as used in the UK. Three single-use ozone decontamination trials in factories were assessed together with one 4 week, continuous factory based trial.

Results: In laboratory trials, microbial log reduction was directly related to O_3 concentration and contact time. A 0.50, 0.90 and 0.80 log reduction of *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively, was achieved with 8 ppm gaseous O_3 after 30 min exposure and 5 min decay while 20 ppm for 30 min with natural decay to 15 ppm for 1.50 h and manually removal using fans for 2 h produced a 4.30, 3.40 and 1.40 log reduction respectively. Coupon room height or orientation was not significant. The relationship between dose (ozone concentration in ppm x contact time in h) and microbiological log reduction suggested that appropriate treatments to generate desired microbial reductions for specific microorganisms may be possible. During three factory visits, gaseous O_3 showed its effectiveness on environmental surfaces to reduce general microbial contamination. A 4-week continuous field trial demonstrated an average 0.69 log reduction on food contact surfaces and 0.56 log reduction on environmental surfaces total viable count when O_3 gas replaced chemical disinfection, a performance equivalent or better than previously obtained with quaternary ammonium compounds.

Significance: Low levels of ozone (~8 ppm) applied every day may be equivalent to chemical disinfectants for end-of-production disinfection against a general microbial flora. Higher levels (~20 ppm) for several hours may be appropriate for wholeroom disinfection of *Listeria* spp.

P1-54 Cleanabilty of a Newly-developed Hinged Conveyor Belt under Normal Cleaning and Sanitation Conditions

ZHINONG YAN, Gary Larsen and Elliot T. Ryser
Thermodrive LLC, Grand Rapids, MI, USA

Introduction: Smooth, seamless plastic conveyor belts that can be easily cleaned and sanitized provide a hygienic solution for the food industry. However, since installation of these belts using splicing and welding devices has proven difficult, other types of hygienic and easy-to-assemble conveyor belt seams need to be developed.

Purpose: The objective of this study was to determine if a newly developed conveyor belt with a hinged seam can easily be cleaned in place under normal cleaning and sanitation conditions.

Methods: Cocktails of *Listeria monocytogenes* (6 strains) or *Pseudomonas fluorescens* (5 strains) were inoculated onto a ThermoDrive polyurethane conveyor belt (Intralox, Harahan, LA) containing 12 newly-developed pinned hinges (12×15 cm) by passing the belt through a 10% (w/v) slurry of delicatessen turkey meat that was previously inoculated to contain 10^8 CFU/ml of the target organism. After air-drying the belt for 30 min, a standard commercial cleaning protocol was used which included spraying with detergent (Foam-Pro, 3%, Ecolab, St. Paul, MN) and chlorine-based sanitizer (XY-12, 200 ppm, Ecolab). Two hinged belting pieces were each removed before and after cleaning, placed in neutralizing buffer and sonicated in a waterbath (40 kHz) for 5 min. Two top unhinged areas (12×15 cm) were swabbed with 1-ply composite tissues which were placed in neutralizing buffer and stomached for 2 min. Appropriate dilutions were plated before or after membrane filtration on Modified Oxford Medium overlayed with trypticase soy agar containing 0.6% (w/v) yeast extract to recover injured *Listeria* cells or S1 medium to recover *P. fluorescens*. Microbial reductions were then calculated based on log CFU/piece before and after cleaning.

Results: After cleaning and sanitizing, *L. monocytogenes* populations decreased 5.5 log on hinged and unhinged pieces of the belt with *P. fluorescens* decreasing 5.5 and 5.0 log on these same pieces, respectively.

Significance: Based on these findings, this newly developed and readily cleanable hinged belt can serve as a viable alternative to seam-welded belts which are difficult to install.

P1-55 Validation of Electrostatic Spray as a Low-volume Sanitization Method for Food-processing Surfaces

SHAWN M. LYONS, Mark A. Harrison and Edward Law
University of Georgia, Food Science and Technology, Athens, GA, USA

Introduction: The incorporation of an electrostatic force field allows atomized droplets of liquid to be strongly attracted to a surface and to create a uniform film when deposited. Electrostatic force field also allows small droplets to overcome the force of gravity to attach to obscured targets such as the undersides of surfaces in a "wrap-around" effect.

Poster

Purpose: This study experimentally evaluated air-assisted electrostatic spray application as a means to enhance deposition of sanitizers onto food contact surfaces encountered in the fresh produce industry. Spray deposition of sanitizer was then evaluated microbiologically for population reduction of *Salmonella* spp. and *Listeria monocytogenes*.

Methods: Three spray methods were used, an air-assisted electrostatic nozzle (ESS Spraying Systems) with charging on and charging off, and a hydraulic nozzle (TeeJet Disc-Core Full Cone Spray Tips; D1 disc; DC31 core). Target deposition was quantified via fluorometric-tracer technique for the three methods of application. Population reduction of pathogens on food contact surfaces, in biofilm and nonbiofilm, was assessed after sanitizer spray treatment for each method using a repeatable robotic arm. Sanitizer treated surfaces were neutralized, swabbed, and plated in nutrient-rich plating medium for recovery of damaged cells. Target deposition and microbiological analysis were done for both target frontside and backside with respect to the incoming spray vector.

Results: Air-assisted charged spray showed 3.8-times greater deposition of liquid than air-assisted uncharged spray for frontside and 2.5-times greater deposition for backside targets. Air-assisted charged spray showed 1.8-times greater deposition than hydraulic spray for frontside targets and 55.6-times greater deposition for backside targets per milliliter liquid dispensed.

Significance: Enhanced spray deposition by air-assisted electrostatic spray can reduce the pathogen population on a food contact surface while using a lower volume of sanitizer solution than conventional means such as hydraulic sprays. Finely atomized droplets produced and propelled by air-assisted electrostatic spray nozzle can be applied to moisture sensitive materials such as waxed cardboard and are also able to penetrate into narrow spaces.

P1-56 Effectiveness of Ultrasound in Desoiling *Bacillus anthracis* Embedded in Complex Food Matrices Attached to Various Contact Surfaces

SHANTALA R. PAMARTHI, Kerri C. Cooper, Lisa Wang, Fleischman G. Gregory, Claudia Rodriguez and Alvin Lee
Illinois Institute of Technology, National Center for Food Safety and Technology, Summit Argo, IL, USA

Introduction: Although the deadly anthrax attack in 2001 was not food related, it brought awareness to the vulnerability of our food supply to terrorist attack. Since that time there has been a growing concern that decontamination processes, previously developed for dealing with the unintentional introduction of pathogens in food processes, must now deal with the deliberate introduction of pathogens. Previously we demonstrated a detergent followed by a sanitizer yields an improved reduction of 3 to 4 log compared to a single step sanitizing process. Ultrasound may enhance the efficacy of the wash step.

Purpose: To determine the effectiveness of ultrasound in desoiling *Bacillus* spores embedded in peanut butter attached to stainless steel, teflon, polypropylene, buna N and glazed tile.

Methods: Bacillus anthracis (sterne strain 34F2) spores were used for the microbial analysis. Spores were inoculated into peanut butter, spread onto contact surfaces and dried. These dried contact surfaces were subjected to high power ultrasound for various combinations of time (1–5 min) and amplitude (10–100%). A 400 Watt high power ultrasound unit fitted with a titanium sonotrode (Ø3mm diameter, 100 mm length) provided ultrasonic energy. Microbial analysis of spores was conducted using serial dilutions and direct plating method after placing the tubes in 80°C water bath for 20 min.

Results: Ultrasound exposure time, wash temperature and amplitude were varied to establish the conditions necessary to remove the attached peanut butter to a condition of non detectable increase in weight loss (2 min, 30°C and 100% amplitude). Process treatment resulted in a 99% by weight removal of peanut butter from stainless steel, teflon, polypropylene, buna N and glazed tile and an approximate 2 log removal of *B. anthracis* from each coupon material. Ultrasound enhanced the removal of the peanut butter but did not enhance the inactivation of the spore.

Significance: The above result shows that ultrasonication helps in dislodging the *B.anthracis* spores from various contact surfaces.

P1-57 Evaluation of an Alcohol-based Sanitizer Spray's Bactericidal Effects on *Salmonella* Inoculated onto Stainless Steel and Shell Egg Processing Equipment

MICHAEL MUSGROVE
USDA-ARS, Egg Safety and Quality Research Unit, Athens, GA, USA

Introduction: Safety regulations are being drafted for the shell egg industry. Sanitation standard operating procedures are an important precursor to HACCP regulations. *Salmonella* is the pathogen was most often associated with egg-borne outbreaks. Developing effective sanitation procedures that will reduce *Salmonella* contamination of equipment or other surfaces in the processing environment may help to reduce consumer exposure even if eggs are not handled or cooked properly.

Purpose: Experiments were conducted to determine the ability of a alcohol-quaternary ammonium sanitizer delivered in a mist to reduce *Salmonella* inoculated onto stainless steel and shell egg processing equipment.

Methods: A nalidix acid-resistant *Salmonella* Typhimurium was grown on agar plates at 37°C for 18–24 h. Cells were harvested and added to phosphate buffered saline to generate inoculum with a density of ~100 billion cells/mL in each of two repetitions. Inoculum was added to a sterile spray bottle. Each of two experiments were repeated twice. In the first experiment the inside of four stainless steel beakers was inoculated by spraying 10 mL of inoculum, respectively. Excess liquid was decanted and the beaker was allowed to dry for 15 min. Two of the beakers were sprayed with 20 mL of water and the other two were sprayed with a sanitizer solution (70% isopropyl alcohol and 200 ppm quaternary ammonium. Sanitizer was delivered in a mist fine enough to spray onto water sensitive equipment. After five min and 24 h each beaker was swabbed with a sponge moistened with phosphate buffered saline. After swabbing, sponge diluent was enumerated by plating serial dilutions onto BGS supplemented with 200 ppm nalidix acid. In the second experiment, the 10 mL of inoculum was sprayed onto each of two brushes used to transport washed shell eggs into cartons or flats. After drying for 15 min, one brush was sprayed with water for five min and the other was sprayed with the sanitizer for 30 s. Each brush was sampled by swabbing three times at the same time intervals as described previously.

Results: After 5 min, on average 4.2 and 2.0 log CFU/mL *Salmonella* were recovered from stainless steel sprayed with water and sanitizer, respectively. After 24 h, 3.2 and 1.2 log CFU/mL were recovered, respectively. Packer head brush average results were 4.7 and 3.1 log CFU/mL *Salmonella* after 5 min and 4.0 and 0.00 after 24 h.

Significance: Often, spraying with water is the sanitizing treatment after eggs are washed. This sanitizer solution and delivery system were 100 to 10,000 times more effective than water in reducing *Salmonella* numbers.

P1-58 Method Comparison for Detection of *Salmonella* spp. from Stainless Steel Surfaces

VERAPAZ A. GONZALEZ and Meredith Sutzko
Strategic Diagnostics Inc., Technical Support, Newark, DE, USA

Introduction: Exposure to pathogens on surfaces may take place either by direct contact with contaminated objects or indirectly through airborne particles. The hygiene of surfaces, instruments and equipment in the food industry essentially affect the quality and safety of the final product. Testing surfaces after cleaning and disinfection gives information on efficacy of the sanitation procedure applied.

Poster

Purpose: Method comparison demonstrates the effectiveness of RC SELECT *Salmonella* 24-h method in recovery and detection of *Salmonella* spp. on stainless steel inoculated surfaces.

Methods: Stainless Steel coupons were inoculated with *Salmonella* Typhimurium ATCC 19585, *Listeria innocua* ATCC 33090 and *Pseudomonas aeruginosa* isolated from chicken in 10% skim milk. Surfaces were allowed to dry. The sampling area was wiped with D/E pre-moistened sponges. Samples were evaluated by both the FDA/BAM and RC SELECT *Salmonella* method. All samples were confirmed biochemical and serological according to BAM procedure.

Results: One hundred fifty (150) samples were tested by both the test method and the FDA/BAM reference method. The RapidChek SELECT *Salmonella* presented a 0% false negative rate and 0% false positive rate, 100% specificity and 100% sensitivity, no significant difference between RC SELECT *Salmonella* and FDA/BAM reference method was found, calculated Chi square was zero. Results from present study indicate *Salmonella* stressed cells can be successfully recovered and detected by LFD in the minimum 24-h enrichment protocol.

Significance: Although food particles usually are cleaned from surfaces when good hygienic practices are applied, bacteria attached to these surfaces are not visible to the eye and may therefore not be removed. Proper cleaning and sanitizing should prevent the spread of microorganism and cross-contamination to ready to eat food. The use of reliable and fast methods for detection of pathogens can provide a powerful tool in the verification of efficiency in sanitation practices and promote improvements in the HACCP program.

P1-59 Effect of *Listeria monocytogenes* Acid-tolerance Response on Its Adherent Survival under Food Processing-related Stresses and on the Subsequent Disinfection of Attached Cells with Standard Acid and Natural Sanitizers

Nick Chorianopoulos, Stathis Giaouris, PANAGIOTIS SKANDAMIS, John Sofos and George Nychas
AUA, Food Science and Technology, Athens, Greece

Introduction: Attachment to surfaces is believed to be important for survival and persistence of *Listeria monocytogenes* in food processing environments. Thus, attached *L. monocytogenes* cells are more resistant to sanitizers than their free-living counterparts. Note-worthily, adaptation to hostile environmental conditions has been demonstrated to alter cellular physiology of a number of foodborne pathogens such that they become resistant to further stronger stresses.

Purpose: The aim of this study was to investigate the potential effect of adaptive stationary phase acid tolerance response (ATR) on the survival of attached to stainless steel (SS) *L. monocytogenes* cells under common food-relevant stresses and on the subsequent resistance of sessile cells to disinfection with standard acid and natural sanitizers.

Methods: Common stresses tested in this study were: (i) refrigeration, 5°C; (ii) acidity, pH 4.5; (iii) salinity, 5.5% w/v NaCl; (iv) cells adjusted at pH 2 with HCL or lactic acid and (v) with essential oil or hydrosol of the Mediterranean spice *Satureja thymbra*. The biofilm was developed for 10 days.

Results: Results revealed that acid adaptation of *L. monocytogenes* cells during their planktonic growth slightly increases the subsequent survival of these cells once become attached to SS under extended exposure (at 16°C for 10 days) to mild acidic conditions (pH 4.5), while it significantly improves the resistance of sessile cells to strong otherwise lethal acid treatments (pH 2). The trend observed with viable count data agreed well with conductance measurements, used to indirectly quantify remaining attached bacteria via their metabolic activity. The essential oil of *S. thymbra* (1%), as well as its hydrosol fraction (100%), presented sufficient bactericidal effect on attached cells, regardless of attachment conditions and ATR induction.

Significance: To sum, ATR should be carefully considered when applying acidic decontamination strategies to eradicate attached to food processing equipment *L. monocytogenes*. Additionally, the use of some natural antimicrobial agents could provide alternative or supplemented ways for the disinfection of microbial contaminated SS surfaces.

P1-60 Inactivation of *Listeria monocytogenes* within a Pork Biofilm Matrix Using Chlorine Dioxide Gas Treatment

RICHA VAID, Richard H. Linton and Mark T. Morgan
Purdue University, Food Science, West Lafayette, IN, USA

Introduction: *Listeria monocytogenes* (LM) is a foodborne pathogen with the highest mortality rate and listeriosis mainly afflicts immunocompromised individuals. LM can adhere and grow on different surfaces causing biofilm formation and leading to post-process contamination of Ready-to-Eat meats. Chlorine dioxide (ClO_2) gas is more effective as compared to conventional chlorine in inactivating cells in a biofilm, and, there is an increasing interest to study ClO_2 gas inactivation for biofilm cells in the presence of animal fat and protein residues.

Purpose: To evaluate factors that affect inactivation of LM within a biofilm matrix in the presence of pork fat, pork sarcoplasmic proteins residues and their combination, using treatment of ClO_2 gas.

Methods: A mixture LM (Scott A, N1-227, 103M, 82 and 311) was used to develop four-day-old biofilms on SS304 coupons using a 100% relative humidity dessicator for incubation at room temperature (22 ± 2°C). After biofilm development, coupons were rinsed, dried, pork fat and pork sarcoplasmic protein (15 mg/ml) residues were applied at various levels (low - 40 µl/coupon, medium – 280 µl/coupon and high - 600 µl/coupon), and dried again before treatment with 2 mg/l chlorine dioxide @ 75% RH. The treated coupons were neutralized; vortexed for 1 min. and serial dilutions were pour plated using tryptic soy agar + 0.6% Yeast extract + 1% sodium pyruvate. A full 3 × 3 factorial design was used to identify significant factors.

Results: The log reductions/duplicate coupons (log CFU/cm^2) of LM biofilm cells using 2 mg/l ClO_2 @ 75% RH with residue/coupon of 40 µl fat was approximately 1.5 (2 min treatment time) and, 2.0 (10 min treatment time), and for 40 µl protein, was 1.6 (2 min treatment time) and 2.7 (10 min treatment time). For coupons with 600 µl fat, log reduction was 0.4 (2 min treatment time) and 0.9 (10 min treatment time), and 600 µl protein was 0.6 (2 min treatment time) and 1.1 (10 min treatment time).

Significance: Limitations of ClO_2 gas treatment to inactivate LM biofilm cells with pork fat and sarcoplasmic protein residues.

P1-61 Effect of Sanitizer and Disinfectant Treatments for Reduction of *Arcobacter butzleri*

BYUNG-JIN NA and Sang-Do Ha
Chung-Ang University, Food Science and Technology, Ansung-Si, Republic of Korea

Introduction: Members of the genus *Arcobacter* are often described as "emerging pathogens," and *A. butzleri* is the species most often linked to outbreaks of disease in humans. Sodium hypochlorite and ethyl alcohol are generally used to reduce *A. butzleri* ATCC 49616

Purpose: The bactericidal effects of sodium hypochlorite and ethyl alcohol for reducing contamination of *A. butzleri* ATCC 49616 were examined.

Methods: The concentrations of sodium hypochlorite and ethyl alcohol used were 25–200 mg/l and 10–70 % (v/v), respectively. The efficacy of sanitizers and disinfectants was estimated by the European EN 1276 method based on quantitative suspension testing. The Korea

Poster

Food and Drug Administration (KFDA) use the EN method as an official method. Initially, the inoculated contamination level was 5.49 log CFU/ml.

Results: The reduction of *Arcobacter butzleri* after treatment with 25, 50, 75, 100, 125, 150, 175, 200 mg/l sodium hypochlorite were 0.14, 0.31,0.11, 0.24, 0.27,1.00, 3.89, 5.10 log CFU/ml, respectively. Reductions of *A. butzleri* after treatment with 10, 20, 30, 40, 50, 60, 70 % ethyl alcohol were 0.28, 0.33, 1.08, 4.47, 5.34, above 5.49, above 5.49 $\log_{10}$ CFU/ml, respectively. Consequently sanitizers and disinfectants containing 200 mg/l sodium hypochlorite and more than 50 % ethyl alcohol reduced *A. butzleri* ATCC 49616 by more than 5 $\log_{10}$ CFU/mL in vitro.

Significance: Sodium hypochlorite and ethyl alcohol treatments showed greater efficacy against *A. butzleri* and could be applied on animal farms, slaughterhouses and food contact surfaces of food manufacturing industries.

P1-62 Thermal Destruct and Z Values for Human Pathogens on Finfish

KATHLEEN RAJKOWSKI
USDA, Wyndmoor, PA, USA

Introduction: In the National Advisory Committee on Microbiological Criteria for Food (NACMCF) report, it was determined that the cooking process (time/temperature) requirement for seafood would be different than for meat products.

Purpose: NACMCF identified a need to determine the time/temperature requirements to adequately cook finfish to assure the thermal inactivation of the human pathogens: *Salmonella* spp. and *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus*.

Methods: Thermal inactivation, D-values, was obtained for the four pathogens inoculated on raw catfish and tilapia at 55, 60 and 65°C. The Z-values were calculated from the D-value data.

Results: The D-values at 60°C ranged from 1 min for *Salmonella* and *E. coli* to 5 min for *L. monocytogenes* and *S. aureus* on the finfish, which is lower that the reported values for meat products. The Z-values (6.0–6.2°C) for *L. monocytogenes* and *S. aureus* was higher than those Z-values (4.3–5.0) for *Salmonella* and *E. coli* on the finfish. In this study we found that the fat content of the finfish did not make a difference.

Significance: This data can be used to determine the cooking guideline for finfish to assure the safety of cooked product.

P1-63 Comparison of Gamma and Electron Beam Irradiation on the Survival of Natural Microflora in Seafood

HYUN-JOO KIM, Ji-Hyoung Ha, Ju-Woon Lee and Sang-Do Ha
Chung-Ang University, Food Science and Technology, Ansung-Si, Republic of Korea

Introduction: Contamination levels of various pathogens in seafood are being issued. Food irradiation can be used to improve the microbiological safety and extend shelf-life of a wide range of foods. However, the information on the reduction effectiveness against foodborne pathogens and radiation sensitivity of electron beam in seafood have not been documented.

Purpose: The objective of this study was to evaluate and compare the efficacy of gamma and electron beam irradiation to reduce the natural microbial contamination in seafood.

Methods: The seafood samples such as oyster (*Crassostrea gigas*), manila clam (*Ruditapes philippinarum*), mussel (*Mytilus coruscus*) and squid (*Todarpdes pacificus*) were purchased from a Korean local market. The applied doses were 0, 0.1, 0.3, 0.5, 1.0 and 2.0 kGy using a Co-60 gamma irradiator and a 2.5 MeV-linear electron beam RF accelerator.

Results: Total aerobic bacteria ranged from 10^2 to 10^5 log CFU/g, initially. However, coliform bacteria were not any population. Irradiation of 2 kGy for oyster and manila clam and 1 kGy for mussel and squid were sufficient to reduce the total aerobic bacteria to the under detection limit (10 CFU/g).

Significance: Gamma irradiation was evaluated as a more effective method than electron beam irradiation to reduce contamination levels of natural microflora in seafood.

P1-64 Concentration of Male-specific Bacteriophage MS2 from Marine Water Using Tangential Flow Filtration

ROBERTO MARRERO-ORTIZ and William Burkhardt
U.S. Food and Drug Administration, Health and Human Services, Dauphin Island, AL, USA

Introduction: Human enteric viruses can enter estuarine and marine environments through municipal wastewater streams or certain non-point sources. Contamination of these environments can impact recreational activities and seafood safety. The presence of enteric viruses (e.g., norovirus, hepatitis A) in seafood harvesting areas is difficult to assess, and traditional indicator microorganisms are considered unreliable.

Purpose: The objective of this project was to evaluate tangential flow filtration (TFF) for concentrating enteric viruses from marine waters, using MS2 bacteriophage as a surrogate.

Methods: Polysulfone membrane TFF cartridges (0.2 µm and 500 kDa cut-off) were pre-treated with 250 mL of 1% or 3% polyethylene glycol (PEG 8000) to minimize adsorptive losses. Ten liters of seawater were seeded with 1 mL of 10^5 PFU/mL MS2 and passed through the cartridges using a peristaltic pump (1 L/min). Cartridges were back-flushed with retentate (~250 mL), followed by 250 mL each of 0.01% sodium polyphosphate (NaPP) and 0.01% NaPP/0.01% Tween-80 solutions, to improve recovery of MS2. Bacteriophage was enumerated by a modified double agar overlay method.

Results: Recovery of MS2 from seeded marine water was <25% without back-flushing of TFF cartridges. Recoveries of MS2 were 47 and 53% after back-flush with retentate alone, for membranes blocked with 1 and 3% PEG, respectively. With additional back-flushes (0.01% NaPP and 0.01% NaPP/Tween-80), overall mean recovery was improved to 67 and 70%, respectively.

Significance: These results demonstrate that TFF is a promising economical and efficient approach for the concentration of enteric viruses from marine waters. This method may allow for their detection and enumeration by traditional and molecular techniques.

Poster

P1-65 Low-temperature Treatment to Reduce the Risk of *Vibrio* Species in Shucked Oyster

RAVIRAJSINH P. JADEJA and Marlene E. Janes
Louisiana State University, Food Science, Baton Rouge, LA, USA

Introduction: Oysters from the estuarine environments are well known to be carriers of *Vibrio* species. Due to the foodborne illnesses caused by consuming raw oysters, the FDA may require all raw oysters to have a post-harvest treatment to reduce *Vibrio* species.

Purpose: The current study was conducted to develop a post-harvest treatment to reduce the levels of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in shucked oysters by using low temperature treatments.

Methods: Shucked oysters (300 g) were collected in sterile Whirl-Pak bags and spiked with *V. vulnificus* or V. *parahaemolyticus* to achieve about 10^6 CFU/g. Inoculated oyster meat samples were placed in water baths for 12 min at four different temperatures 40, 45, 50 or 55°C.

After heating oyster samples were rapidly cooled by placing the oyster meat on ice and immediately storing at 4°C. The following samples were analyzed for bacterial counts for all the four temperature treatments, inoculated oyster samples or control, heat treated inoculated oysters without cooling, inoculated oysters heated then cooled on ice. Bacterial counts were determined at day 0, 1 and 2 by plating on nutrient agar with 2% NaCl, incubating plates at 37°C for 24 h and calculating CFU/g.

Results: *V. vulificus* was more sensitive to the temperature treatments reaching non-detectable levels at 50°C on day 0, 1 and 2. *V. parahaemolyticus* was more heat resistant at 50°C with average bacterial counts of 2.26×10^2 CFU/g after 1 day at 4°C. The most effective treatment was 55°C that reduced the bacterial counts for both *Vibrio* species to non-detectable on day 0, 1 and 2.

Significance: Application of low heat treatment to shucked oyster meat can be a promising post-harvest treatment for control of *Vibrio* species in oysters.

P1-66 Boiling and Steaming Temperatures for Safe Consumption of *Callinectes sapidus*

NICOLE W. HAZARD and Marlene E. Janes
Louisiana State University, Food Science, Baton Rouge, LA, USA

Introduction: Although always cooked before consumption, Louisiana blue crabs (*Callinectes sapidus*) are still a vehicle of foodborne illness outbreaks, particularly in private residences. Outbreaks of *Vibrio cholerae, Vibrio parahaemolyticus*, and *Vibrio vulnificus* are the pathogens most associated with *Callinectes sapidus*.

Purpose: The purpose of this research was to determine the minimum temperatures necessary, either with boiling or steaming, for the safe consumption of Louisiana blue crab by consumers.

Methods: Callinectes sapidus was inoculated with *V. cholerae* (ATCC 14035), *V. parahaemolyticus* (ATCC 33847), or *V. vulnificus* (ATCC 27562) and subjected to boiling or steaming with the internal temperatures recorded by ACR SmartButton® temperature loggers. Once the crab cooled, it was picked and diluted with an equivalent of PBS. The homogenized samples were plated in duplicate on TCBS (*V. cholerae* and *V. parahaemolyticus*) or VVA (*V. vulnificus*) plates, incubated overnight at 37°C or 35°C, respectively, for 24 h, and log CFU/g was determined.

Results: Statistics showed significant differences between increasing temperature and bacterial reduction for each *Vibrio* species tested. Temperatures that achieved non-detectable levels during boiling or steaming were: *V. cholerae* for two min at 53.5°C or five min at 47.5°C; *V. parahaemolyticus* for three min at 50.5°C or six min at 51.5°C; and *V. vulnificus* for three min at 60°C or five min at 53.5°C.

Significance: Based on the data, it is recommended that a single blue crab be either boiled for three min at 60°C or steamed for five min at 65°C with an additional eight min to cool while picking to ensure that any *V. cholerae, V. parahaemolyticus*, and *V. vulnificus* present will be killed, with steaming being preferred over boiling.

P1-67 Use of γ-Irradiation to Inactivate *Listeria monocytogenes* in Fresh Blue Swimming Crab Meat *(Portunas pelagicus)*

KANNAPHA SUKLIM, George Flick and Kanit Vichitphun
Valaya Alongkorn Rajabhat University, Agricultural Technology Dept., Pathumthani, Thailand

Introduction: Listeria monocytogenes is ubiquitous in some food processing facilities and could result in a foodborne illness through product contamination after thermal processing especially in ready-to-eat foods.

Purpose: The objective of this study was to identify γ-irradiation doses that did not adversely affect the physical properties of freshly-picked blue swimming crab meat and would result in a listericidal process.

Methods: Lump crab meat was exposed to 2, 4, and 6 kGy γ-irradiation, and evaluated for color and textural properties using a colorimeter and a TA.XT plus texture analyzer. Irradiation doses causing no changes in physical properties were determined and subsequently applied to *L. monocytogenes* DMST 1753 and 4553 inoculated crab meat (~10^7 CFU/g) in order to inactivate the organisms. Log reductions were calculated.

Results: There was no statistically difference ($P > 0.05$) in texture between non-irradiated (0 kGy) and irradiated meat (2, 4, and 6 kGy) according to the following forces required to shear samples: 6.60, 6.20, 6.40 and 6.00 N/g. CIEL*a*b* values of 6 kGy irradiated meat was not statistically different from the control ($P > 0.05$), whereas 2 and 4 kGy meat had lower a* (more green) and b* (less yellow). Doses of 4 and 6 kGy resulted in a complete inactivation of *L. monocytogenes* DMST 1753 and 4553 after irradiation treatments and during subsequent storage at 4°C for 28 days. At 2 kGy, a considerable inactivation of *L. monocytogenes* DMST 1753 and 4553 was observed i.e., 5.35 and 4.19 log reductions respectively. During storage at 4°C, recovery of injured cells of both strains was achieved shown.

Significance: These results suggest that listericidal processes with a 5-log reduction were achieved through 4 and 6 kGy dose γ-irradiation with no textural changes. There was variability in sensitivities of *L. monocytogenes* DMST 1753 and *L. monocytogenes* DMST 4553 to 2 kGy irradiation, yielding different inactivation levels.

P1-68 Inactivation of *Vibrio parahaemolyticus* in Hard Clams (*Mercanaria mercanaria*) by High-pressure Processing

GABRIEL K. MOOTIAN, Mukund K. Karwe and Donald Schaffner
Rutgers University, Food Science, New Brunswick, NJ, USA

Introduction: Vibrio parahaemolyticus is a leading cause of gastroenteritis associated with consumption of raw or undercooked molluscan shellfish in the US. The current post-harvest treatment methods (relaying and depuration) used by the shellfish industry have been found ineffective in reducing naturally occurring *Vibrio* spp. and viral pathogens such as norovirus. High pressure processing (HPP) is gaining popularity as an alternative processing method. HPP has been shown to inactivate viral and bacterial pathogens in oysters with minimal effect on the flavor and freshness of raw meat, but this technology has never been used on clams

Purpose: The objective of this research was to demonstrate the potential of HPP against *V. parahaemolyticus* in New Jersey hard clams (*Mercanaria mercanaria*).

Methods: Live clams were inoculated through bioaccumulation with up to 8 log CFU/g of a cocktail culture of five outbreak strains of *V. parahaemolyticus*. Inoculated clams were processed at pressures ranging from 250 MPa to 450 MPa and hold times of 2 to 6 minutes. The double layer plate method (DLPM) was used to recover pressure injured cells. Experiments were performed at room temperature and the highest temperature attained after adiabatic heating was 30°C so temperature had negligible effect on inactivation

Results: Pressure and times conditions of 450 MPa and 6 minutes, 450 MPa and 4 minutes, 350 MPa and 6 minutes reduced concentrations of *Vibrio parahaemolyticus* to below the limit of detection.

Significance: This demonstrates that HPP is a potentially useful technology in reducing the risk of illness associated with consumption of raw clams.

P1-69 Assessing the Shelf Life of Retail Shrimp Using Real-time Microrespirometer

FAHAD ALDEREES and Yun-Hwa P. Hsieh
Florida State University, Nutrition, Food, and Exercise Sciences, Tallahassee, FL, USA

Introduction: Shrimp is the most consumed seafood item in the United States (U.S.). Currently 90% of the shrimp consumed in the U.S. is imported mainly from Asia. Majority of import refusals are due to bacterial contamination and prohibited drug residue. The FDA is constantly detecting unapproved veterinary drug residue upon inspecting seafood imports and has issued refusal warnings, penalties, and sanctions against violators. Seafood inspection requires methods that are simple, accurate and rapid to allow fast decision making regarding the seafood microbial quality.

Purpose: The objectives of this study are to rapidly determine the microbial quality of domestic and imported shrimps, and to examine the shelf life of chloramphenicol (CAP) treated and non-treated shrimp samples stored at 4°C using a novel microrespirometer.

Methods: Domestic wild-caught and imported farmed shrimp samples were purchased from local seafood suppliers. Samples were divided into four treatment groups (no treatment, 0, 10 and 30 ppm CAP treatments) and tested daily for CO_2 evolution rate (CER) of contaminated microorganisms associated with the samples using the microrespirometer. Results were compared with those of the Aerobic Plate Count (APC) cultural method. Sensory evaluation was also conducted to determine the onset of sample spoilage.

Results: The CER values were strongly correlated with APC for all treatments (r^2 = 0.785 to 0.875). Organoleptic detection of shrimp spoilage ranged from 5 to 8 days and found to be associated with a CER value of >27 µl/h/g. CAP treated samples had a longer shelf life of 1–2 days at 4°C compared to the untreated samples. Domestic shrimps had a similar shelf life as the imported samples tested.

Significance: This study demonstrated that the microbial quality of both imported and domestic shrimp samples, drug-treated or non-treated, as well as their predicted shelf lives can be effectively determined in real-time from the CER value using a non-instrumental microrespirometer.

P1-70 Microbial Risk of Raw Fish Consumption Determined by PCR-DGGE and Fingerprinting Approach

CHONGTAO GE, Jiyoung Lee and Changsoo Lee
The Ohio State University, Food Science and Technology, Columbus, OH, USA

Introduction: The raw fish diet is becoming popular around the world. However, it may pose health risks due to possible spoilage and pathogenic microbial contamination from environmental and human factors.

Purpose: The most popular fish consumed as raw are salmon, tuna, and red snapper. Those fresh and frozen fish samples were collected from different markets to compare the profile of microbial community between these two storage types using metagenomes analyzed by PCR-DGGE and fingerprinting tools.

Methods: The microbial community DNA was directly extracted. Bacterial DNA was separated from fish DNA using somatic cell releasing agent (SRA), which removed fish cells. The use of SRA improved the quality and quantity of the bacterial DNA. Bacterial 16s rRNA was amplified by PCR using GC-338f/518r primers. The PCR products were separated by denaturing gradient gel electrophoresis (DGGE). The band patterns were analyzed with band-search algorithm and the DNA were sequenced.

Results: The sequencing showed spoilage bacteria, *Carnobacterium maltaromaticum, Serratia ureilytica*, and pathogens, *Lactococcus garvieae, Shewanella* spp.,*Vagococcus salmoninarum*, were prevalent in all the samples. Human pathogen, *Acinetobacter baumannii, Plesiomonas shigelloides*, and fish pathogen, *Edwardsiella ictaluri*, were found in fresh samples. *Aerococcus viridans*, a famous nosocomial pathogen, was detected in frozen samples. *Shewanella, Serratia* and *V. salmoninarum* are known as waterborne bacteria. *A. baumannii, A. viridans, C. maltaromaticum, E. ictaluri, L. garvieae* and *P. shigelloides* may come from environmental contamination. Furthermore, the collected fish samples showed certain indication of fecal contamination. Frozen fish showed less microbial diversity, which may imply that freezing could act as a selection pressure by inactivating some microorganisms while favoring others that are more resistant to temperature stress. The band pattern analysis showed that, within the same fish type, either frozen or fresh samples clustered each other. This may indicate that the microbial communities are more influenced by after-harvest practices rather than the original environmental contamination.

Significance: Spoilage and pathogenic bacteria were detected in fish. The metagenomic fingerprinting analysis was useful for comparing the difference in microbial community between frozen and fresh fish. It is a promising tool to understand the sources of microbial contamination.

P1-71 Development of a Real-time PCR Assay with an Internal Amplification Control for Detecting Gram-negative Histamine-producing Bacteria in Fish

KRISTIN BJORNSDOTTIR-BUTLER, Susan A. A. McCarthy, Jessica L. Jones, Ronald Benner and William Burkhardt
FDA Gulf Coast Seafood Laboratory, Dauphin Island, AL, USA

Introduction: Scombrotoxin (histamine) fish poisoning (SFP) is a primary cause of seafood-associated illness worldwide. Prompt detection of the bacteria responsible for histamine production can aid in the identification of potentially toxic fish products and prevent the occurrence of illness. Real-time PCR is uniquely suited for rapid detection of bacteria as it is sensitive, specific, and provides analytical results in less than an hour.

Purpose: The objective of this study was to develop a real-time PCR method for detecting Gram-negative histamine-producing bacteria (HPB) in fish.

Methods: A degenerate primer set and a TaqMan MGB fluorogenic probe were developed from conserved regions of the histidine decarboxylase gene. The specificity of the real-time PCR assay was tested against 150 HPB and non-HPB isolates. The efficiency of this assay was determined from standard curves of four high-HPB and of a HPB mix in the presence of common background microflora or a 1:10 (fish:1% NaCl) dilution of Spanish mackerel (*Scomberomorus maculatus*). Finally, the real-time PCR assay was applied in the detection of HPB from yellowfin tuna (*Thunnus albacares*), bluefish (*Pomatomus saltatrix*) and false albacore (*Euthynnus alletteratus*) samples decomposed at 35°C for 24 h.

Results: The real-time PCR assay detected all 73 high-histamine producing (>1,000 ppm) isolates and none of the six low- (125–500 ppm) or 71 non-histamine producing (<125 ppm) isolates. The efficiency of the assay, using DNA from each of the HPB (*Morganella morganii, Raoultella planticola, Enterobacter aerogenes, Photobacterium damselae*) and a mix of the four HPB (with/without internal amplification control), was 102/104%, 97/98%, 98/100%, 96/101%, and 101/98%, respectively. The efficiency of the assay in the presence of 10^6 CFU *Shewanella putrefaciens* or *Pseudomonas aeruginosa*/rxn, or the Spanish mackerel matrix was 95%, 92%, and 96%, respectively. HPB were detected in one decomposed false albacore (muscle, skin, intestine), one tuna (muscle), and three bluefish (muscle, skin) samples. *P. damselae* was subsequently isolated from the false albacore and bluefish; *M. morganii* was isolated from the tuna.

Poster

Significance: These results indicate that the real-time PCR assay developed in this study is a rapid and sensitive assay for detecting high-HPB. The assay has the potential to detect HPB before toxic concentrations of histamine accumulate and could be used to investigate mitigation strategies for HFP.

P1-72 Bacteriological Quality of Oysters Cultured in Sea Farms Located at the South Bay in Santa Catarina's Island – Brazil

Roberta J. Ramos, Leticia A. Miotto, Luiz F. Bleyer de Faria and CLEIDE R. VIEIRA
Federal University of Santa Catarina, Food and Science Technology, Florianópolis, Brazil

Introduction: Oysters are frequently ingested raw or partially cooked, and had to this, the control of the sanitary problems that affect the public health is essential for the development of the aquaculture as economic activity.

Purpose: The purpose of this study was to monitor the hygienic and sanitary quality of oysters (*Crassostrea gigas*) from sea farms located in six different geographical regions at South Bay in Florianópolis, Santa Catarina State – Brazil, over a period of 9 months, totaling 12 collections, from June 2006 through February 2007.

Methods: 144 samples were analyzed; each sample consisted of a pool of 12 oysters. They were collected directly from their cultivation sites. Microbiological assays were carried out for counts of coliforms at 35°C, coliforms at 45°C, *Escherichia coli*, coagulase-positive Staphylococci, besides detection of *Salmonella* sp., using methods described in the APHA, 2001.

Results: All the samples analyzed showed absence of *Salmonella* sp., and 12.5% (18/144) of the samples showed presence of *Escherichia coli*. The counts of coagulase-positive Staphylococci varied from <10 to 1.9×10^2 CFU/g, whereas the counts of coliforms at 35°C, 45°C and *E. coli* ranged from <3.0 to 4.6×10^2 MPN/g, <3 to 1.5×10^2 MPN/g and <3 and 4.3×10 MPN/g, respectively. Positive correlations were found among counts of coliforms at 35°C, 45°C and *E. coli* in the oysters. Based on the results of the microbiological assays, the samples analyzed showed acceptable bacteriological quality, i.e., within the parameters foreseen in the Brazilian Legislation, by the RDC 12/2001—ANVISA, which establishes limits of up to 10^3 CFU/g for coagulase-positive staphylococci and absence of *Salmonella* sp. in 25 grams.

Significance: However, importance of the basic sanitation in regions of bivalve mollusks culture stood out as well as the constant bacteriological monitoring of quality indicators, such as for the culture water as for the cultivated oysters.

P1-73 Preliminary Results of the Incidence of *Vibrio* spp. in Oysters Harvested in Florianópolis, Santa Catarina— Brazil

Roberta J. Ramos, Leticia A. Miotto, Luiz Fernando B. Faria, Marília Miotto, Helen S. Silva, Andréia Cirolini, Andressa M. Baseggio, Gracieti V. Mottana and CLEIDE R. VIEIRA
Federal University of Santa Catarina, Food and Science Technology, Florianópolis, Brazil

Introduction: Vibrios are very common in marine and estuarine water environments and some may cause infections in humans which were exposed to seafood or sea water. Several *Vibrio* species are pathogenic to humans and may be present in shellfish, raw or partially cooked.

Purpose: The purpose of this study was to assess the incidence and level of contamination for *Vibrio* spp. in oysters (*Crassostrea gigas*) harvested in the South Bay of Florianópolis, Santa Catarina State – Brazil, over a period of seven months, totaling 10 collections, from October 2008 through April 2009.

Methods: 60 samples were analyzed; each sample consisted of a pool of 12 oysters. They were collected directly from their cultivation sites. Microbiological assays were carried out for counts of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and detection of the *Vibrio cholerae* and *Vibrio alginolyticus*, using methods described in the BAM /FDA, 2001.

Results: Of the 60 samples analyzed, 28 (46.7%) contained one or more *Vibrio* species. The most frequently isolated species were *Vibrio parahaemolyticus* (22 isolates, 36.7%) and *Vibrio vulnificus* (6 isolates, 10%). All the samples analyzed showed absence of *Vibrio cholerae* and 4 (6.7%) samples were positive for *Vibrio alginolyticus*. The counts of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in the samples ranged between < 3 and 2.1×10^2 MPN/g and 1.2×10^2 MPN/g, respectively. December and January showed the highest counts of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in samples of oysters.

Significance: These results indicate the necessity to improve shellfish-borne disease control strategies focusing attention also in pathogenic *Vibrios*. Therefore, only classical fecal indicators are not suitable for oyster quality control. The significance of this for public health is dependent on the health status of the consumer as well as on the concentration and on the pathogenicity of the pathogen.

P1-74 Center for Food Safety and Applied Nutrition Outbreak Surveillance Database and Seafood Associated Outbreaks That Involve Food and Drug Administration, 2004–2009

BABGALEH B. TIMBO, Katherine A. Vierk, Marianne P. Ross, Erin Gudger and Patrick V. McCarthy
Center for Food Safety and Applied Nutrition, FDA, Health and Human Services, College Park, MD, USA

Introduction: Each year multiple illness events associated with FDA regulated products are reported. The Center for Food Safety and Applied Nutrition (CFSAN) Outbreak Surveillance Database was created in 2004 to capture, maintain and retrieve data on foodborne and cosmetic-related outbreaks that involve FDA-regulated products. Seafood associated outbreaks accounted for the highest number of outbreaks captured in the CFSAN Outbreak Surveillance Database from 2004 to 2009.

Purpose: The purpose of study is to illustrate the usefulness of the database by highlighting the seafood outbreaks that involve FDA from 2004 to 2009.

Methods: The CFSAN Outbreak Surveillance Database collects and maintains data on foodborne and cosmetic-related outbreaks associated with FDA-regulated products. The data is gathered from FDA district offices, Center for Disease Control and Prevention (CDC), and state and local health departments; from emails, conference calls and consumer complaints and entered into an Epi Info database. For an outbreak to be entered in the CFSAN database, the outbreak must be associated with illness that involved an FDA-regulated product that has been in interstate commerce.

Results: From 2004–2009, there were 63 seafood-associated outbreaks and 1335 illnesses that involve FDA. Among the outbreaks, 65.1% were due to finfish, 31.7% were due to shellfish and 3.25% were due to "other seafood" category. Seventy-six percent of the illnesses were due to shellfish, 23.7% were due to finfish, and 0.3% were due to the "other seafood" category. Tuna was the most frequent (46.3%) vehicle responsible for finfish-associated outbreaks and it accounted for 51.4% of the finfish-associated illnesses, whereas oysters were responsible for 70% of the outbreaks and 59.1% of the illnesses that are due to shellfish-associated outbreaks.

Significance: The CFSAN Outbreak Surveillance Database is an important contributing tool in FDA's food regulatory responsibility and food safety decisions. The study identifies tuna and oysters as the vehicles most frequently responsible for seafood-associated outbreaks that involve FDA from 2004 to 2009.

P1-75 Isolation and Identification of the Causative Agent for a Salmonellosis Outbreak from White Pepper

Lourdes Tapay, GREGORY MA, William E. Keene and Mansour Samadpour
IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA

Introduction: In March 2009, epidemiologists in several western states (California, Nevada, Oregon and Washington) noted a spike in the number of cases of an extremely rare *Salmonella* serotype—*Salmonella* Rissen (mean for U.S. < 10 cases/year). A coordinated multi-state investigation ensued, but despite intensive structured interviews, no common source became apparent. Spices were considered as a vehicle from the outset because a matching isolate had been recovered from a sample of imported black pepper tested by the FDA in 2006.

Purpose: The purpose of this study was to isolate and quantify *Salmonella* in epidemiologically outbreak-associated food products.

Methods: Spice and other food samples collected from restaurants visited by cases were tested using a multiplex polymerase chain reaction assay and confirmed following the FDA-BAM method (Chapter 5). *Salmonella* loads in positive samples with sufficient mass were determined by FDA-BAM. *Salmonella* isolates were analyzed by pulsed-field gel electrophoresis (PFGE) on restriction DNA digests using *Xba*I and *Bln*I enzymes.

Results: Thirty-eight samples were received for analysis: 13 of ground black pepper, 11 ground white pepper, and 14 other spices and condiments (mungbean sprouts, basil, Jalapeno peppers; and powdered curry, garlic, and ginger). Seven of the white pepper samples (all brand X) tested positive for *Salmonella*. By PFGE, all the white pepper isolates were indistinguishable from clinical isolates of *S.* Rissen from an eventual total of 87 outbreak-associated human cases. MPN enumeration of *Salmonella* in five samples yielded counts from 2.8 to 1,490,000 MPN/100 g.

Significance: Although not infrequently contaminated, spices are extremely difficult to identify as outbreak vehicles due to poor recall and unrecognized exposures. In some circumstances, “brute force” product testing can help focus or accelerate epidemiological investigations. Our test results, corroborated by subsequent testing at other laboratories, quickly led to a recall of all spice products processed at brand X’s parent California facility, which was found to be heavily contaminated with *S.* Rissen. The outbreak ended shortly after the implicated products were removed from wholesale and retail channels.

P1-76 Nonviral Gastrointestinal Outbreaks in Alberta 2003 — 2009

CHRISTINA FERRATO, Sheila Cook, S. Drews, B. Lee, X. Pang, L. Chui, G. Tyrrell and M. Louie
Alberta Health Services, Provincial Laboratory for Public Health (Microbiology), Edmonton and Calgary, AB, Canada

Introduction: Outbreaks due to gastrointestinal infections are not uncommon. ProvLab (PL) is a provincial public health laboratory providing reference identification and food testing for enteric and foodborne pathogens.

Purpose: This study describes the laboratory investigations of nonviral human gastrointestinal outbreaks in Alberta.

Methods: Public health officials declare an outbreak (OB) when clustering of two or more epidemiologically linked cases occur. All laboratory investigations associated with human gastrointestinal OBs in Alberta are performed by PL. The spectrum of tests against viral, bacterial, parasitic, and other targets is OB specific depending on the suspected agents.

Results: Between 2003—2009, of the 402 gastrointestinal OBs with unknown or suspected bacterial agents investigated, the following etiologies were identified: 183 (45.5%) bacterial; 13 (3.2%) parasitic; 94 (23.4%) unknown; and 112 (27.9%) viral. Of 183 bacterial OBs, the most common pathogens included *Salmonella* (67; 36.6%), *Escherichia coli* O157:H7 (47; 25.7%), *Campylobacter* (16; 8.7%), *Clostridium perfringens* (18; 9.8%); and *Bacillus cereus* (13; 7.1%). Bacterial OBs occurred in a variety of settings including food establishments (72/183; 39.3%), private/community gatherings (39; 21.3%), daycares (17; 9.3%); long-term care/hospital facilities (13; 7.1%); camps/schools (5; 2.7%) and other miscellaneous settings (37; 20.2%). Of the 72 OBs in food establishments, the two most common pathogens were *Salmonella* species (21; 29.2%) and *E. coli* O157:H7 (17; 23.6%). Viral and bacterial OBs occurred primarily in the winter and summer months respectively ($P < 0.05$).

Significance: Confirmed bacterial pathogens accounted for 45% of gastrointestinal outbreaks of suspect bacterial or unknown origin with *Salmonella* and *E. coli* O157:H7 being the most common. Seasonal difference was noted for bacterial and viral outbreaks. The most common source of exposure for bacterial OBs were in food establishments and restaurants.

P1-77 Reporting of Cases of Toxinfections That Have Occurred in the Last Five Years in the State of São Paulo, Brazil

KATIA REGINA M. RODRIGUES, Beatriz M. Meciano, Paula C. Breda and Silvana M. Srebernich
PUC-Campinas, College of Nutrition, Campinas, Brazil

Introduction: Outbreaks of foodborne illnesses are defined by the appearance of at least two similar cases of a group of symptoms, usually gastric-intestinal, resulting from the same food source, on which the toxinfection is the result of the ingestion, through the food, of high amount/quantity of bacteria on its vegetative form that in the intestine will release toxins as they sporulate.

Purpose: The purpose of this study was to survey cases of toxinfections registered in epidemiological data base identifying the microorganisms responsible for the outbreaks.

Methods: Data collection of cases of toxinfections was carried out by searching Brazilian scientific and governmental epidemiological data bases over the last five years.

Results: It was verified the occurrence of 878 outbreaks of foodborne illnesses with 20,471 cases. Most contaminations occurred in restaurants, cafes, bakeries and bars: food served in these establishments has caused 34% of outbreaks. In second place, with 22%, are the foods prepared at home. However, these data represent only part of the cases since a great part of them are not reported. The main microorganisms responsible for most outbreaks reported were *Salmonella* spp., *Escherichia, Shigella*, *Staphylococcus* sp. and *Bacillus cereus*. These results show the need to educate the owners of the food services about the importance of preventive hygienic measures in addition to a Public Health Surveillance more active. On the other hand, the population must be aware about its right of having a safe food and also, bad producers and food handlers must be denounced.

Significance: The incidence of outbreaks of foodborne illnesses shows the importance of preventive hygienic measures in food services.

P1-78 A Review of Enteric Outbreaks in Prisons: Effective Infection Control Interventions

MARILYN LEE, Judy D. Greig and Janet Harris
Ryerson University, School of Ocupational and Public Health, Toronto, ON, Canada

Introduction: Prison kitchens are frequently staffed by inmates who, although not trained in food safety, prepare meals for fellow inmates. Confined living space coupled with poor sanitary behaviors allow enteric disease agents to be easily transmitted by direct or indirect means.

Purpose: The purpose of this study was to review documented outbreaks of enteric illness in prisons, a topic not well reviewed, in the last ten years to identify etiology, mode of transmission, the number of inmates affected, morbidity and mortality patterns, and interventions to control and prevent outbreaks.

Methods: Computer-aided searches of databases and systematic searches through government web sites were completed to identify outbreak reports. Reference lists were hand searched to validate the electronic search methodology. The search also included reports identified through personal communications with public health officials.

Results: Of the 72 reported outbreaks that met inclusion criteria, 76% were associated with bacterial and 21% with viral agents. The majority of outbreaks were associated with *Salmonella* (20), *Clostridium perfringens* (14), norovirus (14), pathogenic *Escherichia coli* (10), and *Campylobacter* spp. (5). Routes of transmission were primarily foodborne (67%). Control measures most frequently undertaken during the outbreak included limiting the movements of ill inmates and staff, and excluding them from kitchen duty. The most frequently mentioned recommendation made retrospectively to prevent future outbreaks was monitoring food temperatures (18% of 171 recommendations). Other significant recommendations included effective infection control procedures (16%) such as enhanced handwashing, excluding ill food handlers, having adequate hand sinks and handwashing supplies. Educating food handlers represented another 11% of recommendations

Significance: To control enteric outbreaks in prisons emphasis should be placed on monitoring food temperatures such that pathogens are inactivated or do not have opportunity to multiply. Inmates who work with food need to be offered safe food handling training which could be offered internally by the correctional facility, the local health department, or even on-line. Once they occur, enteric outbreaks can be best controlled by effective infection control practices (enhancing handwashing; excluding ill food handlers; having adequate hands sinks, soap, and paper towels/dryer). Having active surveillance for early diagnosis may also prevent further spread of illness.

P1-79 An Interval-accumulation Based Predictive Modeling Tool to Evaluate Multi-stage, Dynamic-temperature Profiles

Greg M. Burnham, Emma Hartnett, Todd Ruthman, CHERYL A. BAXA, Florence Feeherry, Donald W. Schaffner and Steven C. Ingham
United States Army Natick Soldier Research, Development and Engineering Center - DoD Combat Feeding Directorate, Food Safety and Defense Team, Natick, MA, USA

Introduction: Information on pathogen behavior in food is needed by risk managers in making sound food safety decisions. Ideally, this information would be obtained through controlled inoculation studies using the pathogen/food combination of concern during actual processing. Alternatively, this information can be obtained by analyzing processing conditions using computer generated predictions of pathogen behavior in food. There is an ever increasing acceptance of this approach in food safety risk analysis decision making and there are many existing models and tools. A predictive modeling tool that offers simplicity, flexibility, and an easy-to-understand results-reporting method is needed.

Purpose: The objective of this work was to develop an easy-to-use web-based predictive modeling tool, the Time-Temperature Pathogen Predictor (T2P2), which makes use of existing predictive models to provide information for use in food safety risk analysis decision making.

Methods: To develop T2P2, we transferred existing predictive models (like those found in THERM and PMP) to the software Analytica®, developed an easy-to-use interface, and expanded its functionality, relative to the other tools, in terms of (1) the scope of calculations performed, (2) the level of user interaction, and 3) results reporting. The qualitative predictive accuracy (growth = ≥ 0.3 log CFU) of T2P2 was then tested against previously published data from the validation of the THERM tool for the behavior of *Escherichia coli* O157:H7 and *Salmonella* serovars in ground beef.

Results: The accuracy of T2P2 for predicting pathogen behavior in ground beef was accurate (58%) or fail-safe (42%) for *E. coli* O157:H7 and accurate (42%), fail-safe (50%), or fail-dangerous (8%) for *Salmonella* serovars.

Significance: T2P2 is an easy-to-use predictive modeling tool that provides risk managers with accurate-to-fail-safe predictions of pathogen behavior in food matrices for the implementation of hazard mitigation strategies and other food safety risk analysis decision making.

P1-80 Development of Computer-assisted Quantitative Risk Assessment — Hazard Analysis Critical Control Point Program for Food Safety in Retail Food Establishments

SANG-WON KIM, Seung-won Jung, Seung-ju Lee and Kwang-geun Lee
Dongguk University, Dept. of Food Science and Technology, Seoul, Republic of Korea

Introduction: Most of restaurants in Korea are retail food establishments. There is always a high potential of foodborne outbreak which requires special sanitary management to prevent.

Purpose: The aim of the this study is to develop the QRA-HACCP program for a quantitative technique to predict contamination levels and perform sensitivity and scenario analyses in retail food establishments.

Methods: Sanitary practices were surveyed and evaluated to develop the computer-assisted QRA-HACCP program in 3 service sites, including Korean style restaurant, Japanese style restaurant and Western style restaurant. All database files and processing programs were created by using visual basic application (VBA). The program has been coded according to steps of exposure assessment. The object linking an embedding (OLE) EXCEL automation method for automatic data processing is used in this program, which is a type of programming technique for the EXCEL control.

Results: The performance test on the basis of reference data has been carried out by using the developed program. In the results of performance test, the final contamination level of *Staphylococcus aureus* and sensitivity value for the identification of the critical control point (CCP) by the developed program were similar to those obtained from the reference data. The critical limit (CL) for the CCP can be quantitatively estimated by scenario analysis in the developed program. In addition, it was revealed that the efficiency of implementation of HACCP plan is increased by using the developed program.

Significance: We expect that this program will play the role of promotion to introduce and implement of HACCP system for each retail food establishment by themselves without special consulting.

P1-81 Developing the Predictive Growth Model of *Staphylococcus aureus* on Cooked Spinach in Commissary School Foodservice

JIYEON PAK, Hyungsu Park, Kyung Ryu and Kihwan Park
Yeungnam University, Food and Nutrition, Gyeongsan, Republic of Korea

Introduction: The commissary school foodservice system has been operated to reduce the operating costs since 1990s in Korea. Foodborne illness has increased dramatically due to cross-contamination and inadequate temperature control during distribution in school foodservice.

Purpose: This study was performed to develop a predictive model in order to evaluate microbiological safety of cooked spinach.

Methods: Diluted culture of *S. aureus* (ATCC 6538) was inoculated into the cooked spinach for an average initial concentration of 3 log_{10} CFU/g. Microbiological analysis was performed at 0, 0.5, 1, 1.5, 2, 3, 6, 12, 24, 48 and 72 hours of storage with different storage temperatures (5, 15, 25 and 35°C). The Gompertz model was used to determine the R^2 values of lag time (LT) and specific growth rate (SGR). The second stage of modeling was used to describe the variation of the parameters of the growth curve as a function of growth condition using response surface model (RSM).

Results: In primary model, R^2 values were obtained as 0.72, 0.94, 0.95 and 0.84 at each storage temperature by GraphPad. Among RSM, the correlation coefficients of observed and predicted values were 0.89 and 0.88. SGR values were 0.04, 0.07, 0.40 and 0.45 for each temperature. The mean square error (MSE), Bf and Af values for LT were 0.007, 1.01 and 0.83 and values for SGR were 0.130, 1.18 and 1.90, respectively.

Significance: These results indicate that the predictive model of *S. aureus* on cooked spinach has the high statistical compatibility and could be applied for quantitative microbial risk assessments.

P1-82 Development of Predictive Growth Models for *Bacillus cereus* on Various Food Matrices

KIM YOOWON, Kang Kyung Ah and Yoon Kisun
Kyung Hee University, Seoul, Republic of Korea

Introduction: *Bacillus cereus* contamination was observed in 240 ready-to-eat (RTE) foods including 118 seafoods, 82 Korean packaged meals, and 40 other RTE foods.

Purpose: The objective of this study was to develop predictive growth models for the growth of *B. cereus* on various food matrices consisting primarily of RTE foods.

Methods: Cooked rice, hamburger patties, tofu, boiled bean sprouts, boiled Chinese noodles, smoked chicken, sliced bread, and baked fish were artificially inoculated with *B. cereus* at an initial concentration of 3 log CFU/g and stored at 13, 17, 24, and 30°C. Growth kinetic parameters were determined at each temperature by the Gompertz equation. Specific growth rate (SGR) and lag time (LT) values were further used in square-root and Davey models as a function of temperature, respectively.

Results: Model performance was evaluated based on bias (Bf) and accuracy (Af) factors. SGR and LT were mainly affected by temperature in the primary models of the various food matrices. At 13°C, *B. cereus* growth was significantly more rapid on protein-based foods such as tofu, boiled bean sprouts, hamburger patties, and smoked chicken than on carbohydrate-based foods such as cooked rice, boiled Chinese noodles, and sliced bread. However, no significant the differences in the growth kinetics of *B. cereus* were observed between the protein and carbohydrate based foods at 17, 24, or 30°C. The developed secondary models in this study showed suitable performances to predict the growth of *B. cereus* on various food matrices.

Significance: These models will be used in the development of tertiary models for RTE foods to quantify the effects of temperature on the growth of *B. cereus*.

P1-83 The Establishment of an Food Safety Objective and Microbiological Criteria Based on QMRA Modeling

Eun-Kyung Ko, Ki-Hwan Park and GYUNG-JIN BAHK
Kunasn National University, Dept. of Food and Nutrition, Gunsan, Republic of Korea

Introduction: Quantitative microbial risk assessment (QMRA) can be to provide scientific advice to the risk managers who will use the information to decide upon the risk management option(s) that will be implemented to achieve the desired level of consumer protection from microbial hazards. This public health goal must be converted into parameters that can be controlled by food producers and monitored by government agency such as FSO (food safety objective). A microbiological FSO is the maximum frequency and/or concentration of a microbial hazard in a food considered tolerable for consumer protection.

Purpose: The objective of this study was to provide FSO and microbiological criteria based on QMRA modeling as an example of *Clostridium perfringens* in an animal product.

Methods: QMRA model was constructed in an Excel spreadsheet. Exposure assessment consisted of three process steps (produced products, retail and consumption as post process) including predictive growth model. A novel dose-response model and serving size etc. included for hazard characterization and risk characterization. In addition, the Negative binomial and Lognormal distribution was used for the set up of FSO and microbiological criteria as three-class sampling plan. The developed model was simulated with @Risk.

Results: Many scenarios were simulated with various assumptions for contamination levels at the point of production and considering with temperature at distribution and storage time, etc. According to simulation results, in Korea, the suggested reasonable FSO was the level of *Clostridium perfringens* in animal products must not exceed 100 CFU/g, and the microbiological criteria also proposed n = 5, c = 1, m = 10, M = 100 as three-class sampling plan at the point of production.

Significance: This study can be used as scientific information and showed the practical possibility using QMRA model for the establishment of an FSO and microbiological criteria in a food.

P1-84 Risk Assessment of Arsenic in the Most Frequently Consumed Fish by Korean Elderly Population

HYUN AH SHIN, Ji Hea Moon, Jin Young Kim, Sang Hwa Yi, Dan Hee Jeong, Ae Jung Lee, Ji Eun Kim and Ae Son Om
Hanyang University, Seoul, Republic of Korea

Introduction: Humans can be exposed to arsenic (As) via the intake of air, water and food. Although food is the main source of As exposure for the general population. Fish and other seafood comprise 90% of total food As exposure with all other foods comprising the remaining 10%.

Purpose: With increasing public concern over the intake of contaminants like As in foods, performed in this study was an evaluation of the risk assessment of As in the most frequently-consumed fish (MFCF) in Korean elderly population.

Methods: Calculations were made on the residual level and total dietary exposure of As in the fish and the other dietary products. First, the residual levels of As were investigated not only in the 8 MFCF but also in all other dietary products and environmental soil, water and atmosphere. Next, the dietary exposure of As in the 8 chosen fish was estimated based on the data from the previous investigations of As consumption in Korea. Then, a hazard index (HI) of As was calculated by the level of daily dietary exposure and the maximum allowable daily body load derived from the provisional tolerable weekly intake (PTWI).

Results: The respective As concentrations ranged from 1.028 to 7.478 μg/g in the different fish. Total dietary exposures of As for elderly male varied from 0 to 6.67×10^{-4} mg/kg/day, while total dietary exposures of As for elderly female, which ranged from 0 to 6.65×10^{-4} mg/kg/day, were less than those for male. All HIs calculated were lower than 1 (HI < 1) with only 0.110 and 0.117, respectively. In other words, the estimated dietary intakes of As for Korean elderly population were well within the safe limits.

Significance: These data suggest that there is no imminent health risk to Korean elderly population from dietary intake of As in the MFCF.

P1-85 Wash Your Paws Handwashing Education Initiative for Youth Audiences

JUDY A. HARRISON
University of Georgia, Dept. of Foods and Nutrition, Athens, GA, USA

Introduction: Handwashing is one of the best ways to prevent illnesses, both infectious diseases such as H1N1 flu as well as foodborne illness. In response to requests for handwashing education in schools, The University of Georgia Cooperative Extension has developed a handwashing education initiative that targets youth through Family and Consumer Sciences and 4-H programs in schools.

Purpose: The purpose of this study was to develop a handwashing education initiative that uses a university mascot and to evaluate the effectiveness of such a campaign in teaching youth the six steps of proper handwashing and when hands should be washed.

Methods: A curriculum, entitled Wash Your Paws, Georgia! was developed to include a bulldog mascot and combine handwashing guidelines developed by the National Sanitation Foundation and the Centers for Disease Control and Prevention. Components of the curriculum include posters, an instructor guide, PowerPoint slides, lesson dialogues, suggested activities, evaluation tools including parent/guardian information letters, and magnets to supplement the educational messages. Extension agents were trained in the use of the materials. IRB approval was obtained for the use of human subjects. Both Family and Consumer Sciences Agents and 4-H Agents statewide were presented with the materials and trained in the use of the educational components and the evaluation components. Education programs using the materials have been initiated throughout the state. A pre-test/post-test design was used to evaluate the effectiveness in increasing awareness of the need for proper handwashing and the knowledge of the six steps of proper handwashing.

Results: In just three months since the program was initiated, more than 600 youth have been reached with the Wash Your Paws, Georgia! Handwashing Education Campaign. Results indicate that materials using a university mascot are very popular and effective in increasing awareness about the need for proper handwashing and educating youth about the proper steps in handwashing.

Significance: This approach can serve as a model for handwashing education initiatives by educational institutions in other states and countries.

P1-86 Development of an Interactive Food Safety Curriculum for Secondary Science Education

ADRIENNE E. SHEARER, O. Sue Snider and Kalmia E. Kniel
University of Delaware, Animal and Food Sciences, Newark, DE, USA

Introduction: Eleven workshops conducted over ten years introduced various food science materials to over 150 secondary educators and students and were evaluated for interest and implementation. Findings indicate applicability of food science across the curriculum, particularly foodborne outbreak case studies. There was a strong desire among teachers for interactive, ready-to-implement, and cost-effective educational materials.

Purpose: To satisfy the needs of secondary educators and enhance food science awareness, an interactive curriculum on microbiological food safety was developed for implementation in basic and applied high school science classes. The use of web-based technology and case studies were utilized for their reported effectiveness in reaching high school students.

Methods: In phase one of this two-phase research project, a microbiology food safety curriculum was developed with guidance of a teacher advisory board representing biology, chemistry, agricultural and family and consumer sciences, and from feedback of recent high school graduates (60 students over 3 yr). Components of the curriculum include a presentation, foodborne illness outbreak case studies, and a virtual laboratory with gaming features on pathogen detection in support of outbreak investigations. Case studies are based on historical outbreaks which demonstrate evolution of scientific understanding and response. Exercises involve scientific reasoning, data handling, and discussion of societal implications.

Results: Data from teachers and students suggests positive use of the case studies which were regarded as engaging and appropriate for ability, approach, and format. Figures and assignments were modified based on responses. Advisory board recommendations included flexibility for use of components and focus on principles rather than technology alone. Teachers acknowledged a need for laboratory education that circumvents safety constraints.

Significance: Curriculum is being disseminated and phase two involves implementation in regional secondary schools. This novel curriculum provides a means to support education on microbiological food safety while meeting science content standards and increasing awareness of career opportunities.

P1-87 Development of a Capstone Food Protection and Defense Computer-based Simulation for Graduate Students and Working Professionals

NIGEL M. HARPER, Tejas H. Bhett, Richard H. Linton, Kelly J. Getty and Alok R. Chaturvedi
Kansas State University, Food Science Institute, Manhattan, KS, USA

Introduction: Kansas State University, Purdue University and Indiana University collaborated to develop a food safety and defense curriculum for graduate students and working professionals. A panel of 13 stakeholders with expertise in food safety/defense and public health participated in a DACUM (Developing A CurriculUM) process and 297 professionals that validated DACUM results identified a Capstone Experience as key component of the curriculum.

Purpose: The objective was to evaluate the effectiveness of a computer simulation as Capstone Experience.

Methods: Purdue University has developed and enhanced a computer-based simulation program focused on assessing economic and public health consequences for intentional contamination scenarios of the food supply. Information was gathered from various food industry areas including supply chain, biological and chemical hazards, hazard testing facilities, and consumer market research. After a two-day workshop on food safety/defense, participants (food defense stakeholders, graduate students, and working professionals — 41 total) were split into nine teams. Teams represented ingredient suppliers, food processors, retailers, and multiple levels of government personnel. To represent a 28-day period, teams were given data involving customer complaints (illness/injury or quality), media reports, and CDC illness data. Teams reviewed this data and reacted by tracing, holding, testing, recalling, or releasing any lot of product for potential contaminants. Teams gathered twice during the simulation to discuss data and actions taken. At the end of simulation, teams were given the scenario of the intentional contamination and the impact of their actions on public health and industry-wide economic losses. Following the one-day simulation, participants rated the value of the simulation on a 1 to 5 scale (1 = strongly disagree and 5 = strongly agree).

Results: Overall, values ranged from 4.12 to 4.77 with a mean score of 4.50.

Significance: The computer simulation of an intentional contamination of the food supply was an effective Capstone Experience for a food safety and defense curriculum.

Poster

P1-88 Impact of Educational Materials on Improving Consumer Food Safety during Extended Power Outages and Other Emergencies

SHERYL CATES, Sandria Godwin, Richard Coppings, Leslie Speller-Henderson, Katherine Kosa and Melanie Ball
RTI, Research Triangle Park, NC, USA

Introduction: Natural disasters can cause extended power outages which can endanger the safety of food. Consumers need to be prepared to keep food safe during such emergencies.

Purpose: To evaluate the effectiveness of educational materials at improving consumers' knowledge, intentions, and behaviors related to food safety during extended power outages and other emergencies.

Methods: We developed a 12-page booklet that provided information on recommended food safety practices during power outages and other emergencies. Internet panel members who had recently experienced an extended power outage were randomized to an intervention group which received the booklet (n = 192) or control group (n = 179). Participants completed surveys at pre-intervention and 3 months post-intervention.

Results: About 60% of intervention participants described the booklet as "very informative" and "very useful," and 90% said they would be very or somewhat likely to refer back to the booklet. At follow-up, awareness of the recommendation to have a 3-day supply of food and water increased for the intervention group (77 to 97%, $P = 0.01$) and control group (76 to 87%, $P = 0.01$); however, the difference in the changes between groups was not statistically significant. Adoption of this recommendation did not increase for either group. Awareness of four other food safety recommendations increased significantly for both groups ($P < 0.01$), with the difference in changes between groups statistically significant for two of the four recommendations ($P < 0.01$) (do not eat refrigerated perishable foods after 4 hours without power and discard food that has partially or completely thawed unless it still contains ice crystals or is 40 degrees Fahrenheit or below). Intentions to adopt these recommendations during the next extended power outage did not improve between the pre- and post-intervention surveys for either group.

Significance: The educational materials helped to improve consumers' knowledge of recommended food safety practices, but did not improve consumers' intentions or behaviors. Additional outreach activities may be needed to improve these outcome measures.

P1-89 Assessing Consumers' Cleaning Practices and Microbiological Contamination in Their Home Kitchens

FUR-CHI CHEN, Sandria Godwin and Agnes Kilonzo-Nthenge
Tennessee State University, Family and Consumer Sciences, Nashville, TN, USA

Introduction: Many foodborne illnesses occur as a result of cross-contamination during food handling and preparation at home. Consumers are instructed to clean kitchen surfaces frequently with sanitizing agents to reduce the potential for cross-contamination. However, few studies have evaluated consumers' cleaning practices and their effectiveness in reducing microbial contamination.

Purpose: This study assessed consumers' reported kitchen cleaning practices and correlated them to microbiological contamination.

Methods: One hundred fifty participants completed in-home surveys which included questions regarding their kitchen cleaning practices. Swab samples were taken from several locations in the kitchens. Additionally, cleaning tools (dishcloth, sponges and dish rags) used in participants' homes were collected. Samples were analyzed for Aerobic Plate Count (APC), *Enterobacteriaceae* (EC), and *Staphylococcus aureus* (SA). Microbiological data were statistically compared to the reported practices.

Results: Kitchen sinks, and faucets/handles were more contaminated than other locations ($P < 0.05$). The average APC, EC, and SA of both locations together were 1.3×10^7, 3.3×10^6, and 2.5×10^4 CFU/sample, respectively. Reportedly, 88% of homes incorporated some sanitizing agents in their cleaning scheme; however, there was no significant difference in microbial load when sanitizers were used. Participants who washed sink handles on a regular basis had significantly lower contamination than those who only reported cleaning it when it looks dirty ($P < 0.05$). Tools used for cleaning the kitchens were the most contaminated, with average APC, EC and SA of 4.0×10^7, 2.5×10^7, and 3.5×10^4 CFU/sample, respectively. The data showed that households with highest incomes had the most contaminated kitchens. People who reported worrying about safety of food eaten at home had cleaner kitchens.

Significance: Our study indicated a significant number of kitchens and cleaning tools are heavily contaminated. Consumers need to be educated on the importance of regular cleaning to reduce microbial contamination and prevent foodborne illness.

P1-90 Preliminary Results of a Food Safety Knowledge Survey of Suburban Chicago Restaurant Food Handlers

Mark S. Dworkin, MINDI MANES and Li Liu
University of Illinois at Chicago, Division of Epidemiology and Biostatistics, Chicago, IL, USA

Introduction: Approximately half of the foodborne outbreaks reported to the CDC are associated with restaurants or delicatessens.

Purpose: The purpose of this study is to assess food safety knowledge gaps among suburban restaurant food handlers and use that information to create and test the efficacy of educational materials that are targeted to the more common knowledge gaps.

Methods: To determine food safety knowledge gaps among restaurant food handlers, we interviewed ~700 suburban Northern Illinois restaurant food handlers among a random sample of restaurants as part of a USDA-funded research project (award 2008–35201—04479). A 50-question survey was administered in English or Spanish by a trained interviewer during June 2009 through January 2010.

Results: The preliminary results of the first 152 interviews revealed the following: 34% of the food handlers spoke Spanish, 34% were sure food was safe to eat if it smells and tastes normal, 26% did not know the difference between cleaning and sanitizing, 15% did not know that raw eggs and 68% did not know that cooked rice can have germs that make people sick, only 13% knew the correct minimum internal temperature to cook chicken, 40% thought that raw meat can be stored anywhere in the refrigerator as long as it is wrapped in plastic, 34% would come to work if they had a sore throat and cough, 17% would come to work if they had diarrhea, and 30% of food handlers had not received any food-safety training at their current job.

Significance: These preliminary results demonstrate substantial and important knowledge gaps among suburban restaurant food handlers that can place restaurant patrons at increased risk of food poisoning. An educational intervention is needed. This project will create and disseminate educational materials (a brochure and a comic book) that target the most substantial knowledge gaps. This study will then determine the relative efficacy of each of these materials to learn if one is more effective overall and examine which materials are more appropriate for English versus Spanish speakers.

P1-91 Food Safety Knowledge among Chicago Food Handlers Differs between Spanish and English Speakers

PALAK PANCHAL, Mark S. Dworkin and Li Liu
University of Illinois at Chicago, School of Public Health, Chicago, IL, USA

Introduction: Foodborne diseases cause millions of diseases and thousands of deaths in the United States, annually. The risk of acquiring foodborne diseases may arise as a consequence, by the level of food-handler knowledge and specific food handling behaviors.

Purpose: The purpose of this survey was to determine food safety knowledge among restaurant food handlers.

Methods: We administered an oral, 51-question, survey to 509 food handlers in 125 randomly sampled restaurants during a 6-month period in the city of Chicago. Surveys were administered in both English and Spanish languages based on the preference of the participants.

Results: Both English-speaking and Spanish-speaking food handlers had significant knowledge gaps concerning food handling, optimal temperatures for cooking, holding, and refrigerating foods, cross-contamination and personal hygiene. Overall, the food handlers scored an average of 32 (71%) out of the possible 45 knowledge points. Some of the important factors affecting the scores included restaurant classification, service classification, food specialization, and entrée price. The most frequently missed question, by 97% of the food handlers, was the range of temperatures at which there is rapid bacterial growth. Ninety-two percent of the food handlers did not know the temperature for cooking ground beef mixtures, and 61% of the food handlers did not know that raw meat cannot be stored on foil-lined shelves to prevent dripping onto other foods. In addition, 78% of Spanish-speaking and 55% of English-speaking food handlers did not know whether beef could be defrosted in a microwave ($P = 0.0001$), 52% of Spanish-speaking and 33% of English-speaking food handlers did not know that if hot roast beef was held in a steam table at a temperature below 135°F (57°C) for more than 4 h, it should be thrown away ($P = 0.0001$) and 30% of Spanish-speaking and 50% of English-speaking food handlers did not know that eating uncooked ground beef could cause bloody diarrhea ($P = 0.0001$).

Significance: This study reveals substantial knowledge gaps among food handlers and provides useful information to create intervention materials. Differences on food safety knowledge were identified between English and Spanish-speaking persons. Attention to these differences may be helpful when providing training to food handlers.

P1-92 Evaluation of Food Safety Compliance for Hot/Cold Self-serve Bars

ANDREA E. DOW, Christine Alvarado, Maragret Binkley and Scott Burris
Texas Tech University, Animal and Food Sciences, Lubbock, TX, USA

Introduction: Self-service bars are quickly becoming a major addition to grocery stores, especially with the amount of dollars spent on food-away-from-home. The addition of self-service bars into this outlet in the food industry presents new challenges to food safety.

Purpose: The purpose of this study was to evaluate the current food safety practices and procedures being used in retail self-service bars areas and determine if manager certification and training improved food safety practices.

Methods: Fifteen grocery stores participated in the evaluation process and, of these, eight stores participated in the food safety training session while seven stores remained untrained control stores. The evaluations were conducted in two parts, a pre-training evaluation and a post-training evaluation approximately three weeks after food safety training. Each evaluation consisted of an audit of the kitchen and preparation areas and an observation of employees that focused on the self-service areas (hot/cold self service bars and coffee bars).

Results: The comparison of the pre- and post-audit scores indicated that the stores significantly increased in performance from 84.83% to 89.02% ($P < 0.05$). The comparison of the pre- and post-observations also displayed a significant increase in performance from 67.86% to 78.46% ($P < 0.05$). SPSS, a t-test was calculated and the means were used to formulate the performance percentages. However, there was not a significant difference between the performance of the stores that attended the food safety training certification class and the stores that did not. This lack of significance could be attributed to the fact that all stores were given back their pre-evaluations prior to their post-evaluations taking place. The availability of this feedback could have motivated the managers to improve their practices, regardless if they went through the food safety training, which would have accounted for their increase in performance on the post-evaluations.

Significance: These results indicated that feedback in the form of audits and observations did have a positive impact on the overall improvement of the food safety practices used by employees of self-service bars.

P1-93 Are Hot/Cold Self-serve Food Bar Employees Food Safety Savvy?

ABIGAIL MCCULLOCH, Scott Burris, Christine Alvarado and Margaret Binkley
Texas Tech University, Lubbock, TX, USA

Introduction: In order to be competitive of the home meal replacement market grocery retailers have incorporated hot/cold self-service food bars for customer convenience.

Purpose: This study sought to measure the current food-safety knowledge of food bar employees and assess if safe handling is implemented.

Methods: A sample of 33 managers and 74 employees was obtained from 15 purposively selected matched stores from two grocery chains. Participants completed a questionnaire concerning food safety knowledge and handling practices. The reliability and validity of the instrument were established through a pilot test and a panel of food industry experts and agricultural education professionals.

Results: The average scores on the safety knowledge food test were 12.18 (SD = 2.05) and ranged from 7.0 to 16.0 for managers and 10.49 (SD = 2.17) for employees and ranged from 4.0 to 15.0. There were five questions which 50% or more of participants missed and one additional question missed by over half of employees. These questions related to food borne illness out break and time and temperature abuse. Food safety practices being used were: no bare hand contact with food (100.0%, n = 33), cleaning and sanitizing once a shift (60.6%, n = 20) with 84.8% (n = 28) using a three-compartment, the implementation of HAACP based food safety plans (78.8%, n = 26), hand sanitizers use (63.6%, n = 21), and temperatures taken every four hours (45.5%, n = 15).

Significance: It is important for grocery stores to provide the safest food possible for their consumers; this study showed that further food safety education of employees is needed. The majority of grocery stores observed were following food safety practices in accordance with Food and Drug Administration standards. Training needs to concentrate on educating employees in time and temperature abuse of food and foodborne illness outbreaks, as well as include information on proper procedures was provided in the training program.

P1-94 Tool for Measuring Food Safety Climate

BRITA BALL, Anne Wilcock and Scott R. Colwell
University of Guelph, Food Science, Guelph, ON, Canada

Introduction: "Food safety culture" has become a buzz phrase in recent years. However, there is little substantive evidence to support the claim that there is a definable "culture" that contributes to appropriate behavior of workers in food plants. Health and safety literature contains validated scales for safety culture and climate; there are no comparable scales to assess food safety climate.

Purpose: The purpose of this study was to develop a measurement tool for identifying key factors that influence meat plant workers to follow food safety behaviors in their plants.

Methods: A written questionnaire containing 65 items each with a 7-point rating scale was administered to all salaried personnel at four medium to large meat plants and all personnel at a small plant in Ontario, Canada. Items were related to five areas: management

commitment to food safety (including leadership and resource allocation), work unit commitment to food safety (including supervisor, co-worker and personal commitment), food safety training, infrastructure for food safety, and worker food safety behavior. The 124 usable questionnaires were analyzed through principal axis factoring using SPSS 17.

Results: The exploratory factor analysis extracted 15 factors that had initial Eigenvalues of greater than 1 and explained 67% of the total variance. As was expected, the factor matrix showed considerable cross loading. Most items loaded on two or more factors, suggesting the potential for higher order factors (i.e., factors that contain several subfactors).

Significance: The development of valid measurement items provides the necessary tools required to research "food safety culture." This tool will allow for quantitative investigation of the key factors that contribute to food safety behaviors. This supports the concept of food safety climate, a component of food safety culture.

P1-95 Weight of Evidence in Foodborne Illness Investigations

Jeffrey M. Farber, MARIA NAZAROWEC-WHITE, Gosia Kozak, Lisa Landry, Davendra Sharma and Celine Nadon
Bureau of Microbial Hazards, Health Canada, Ottawa, ON, Canada

Introduction: A foodborne outbreak investigation is complex, multi-disciplinary, non-linear and dynamic. Data collection from laboratory, food safety and epidemiological investigations is critical. As part of the lessons-learned exercises resulting from the 2008 Canadian deli-meat listeriosis outbreak, a guidance document was developed to provide information on factors to consider in determining the weight of evidence to ensure timely and appropriate actions.

Purpose: For potential use during a foodborne outbreak investigation to ensure the implementation of timely and appropriate risk mitigation strategies.

Methods: A team of representatives from Canadian federal government departments involved in foodborne outbreak investigations including Health Canada, the Public Health Agency of Canada and the Canadian Food Inspection Agency, were assembled. Through discussions and consensus building, a guidance document was developed to examine and determine the type and weight of evidence necessary and/or sufficient to take action.

Results: Decision-diagrams stipulating what information should be gathered during an investigation, as well as guidance to assign "strength" to the evidence gathered, e.g., weak or strong, were developed. A framework was developed to determine the strength of all the evidence gathered. For the strength of the microbiological evidence, criteria, such as, does the organism show suitable PFGE diversity are considered, while for epidemiological evidence factors such as plausibility, consistency, specificity or the strength of statistical association are examined. For the strength of traceback/traceforward information, criteria such as the ability to identify the manufacturer, the point of purchase, distribution channels etc., are included.

Significance: The document provides a systematic approach to decision-making using the overall weight of scientific evidence to decide whether to proceed with a health risk assessment and appropriate risk management actions. It is hoped that this type of systematic approach will prove to be useful by all those involved in foodborne outbreak investigations in Canada.

P1-96 Exploring HACCP Teams across the Dubai Food Industry

ABDULWAHED K. KASSIM and Katherine Clark
University of Salford, Salford Business School, Greater Manchester, United Kingdom

Introduction: The HACCP team is a group of people selected to undertake food safety and tackle all its related issues. This study was based on a standard interview in order to explore the HACCP teams' composition across the Dubai food industry. Quantitative and qualitative techniques were of use to conduct the analysis. There were 61 accessible HACCP-certified businesses from which 11 businesses were selected randomly in order to carry out the interviews. HACCP teams included various disciplines and a multidisciplinary approach existed to a certain extent in some HACCP teams. While an inter-hierarchical approach was unknown to the teams, surprisingly, it appeared to exist in some HACCP teams.

Purpose: The primary purpose of this study was to explore the composition of HACCP teams and to what extent they utilize multidisciplinary and inter-hierarchical methods, having in mind their ultimate goal to implement and maintain HACCP system so as to produce safe food.

Methods: The research method employed in this work was a standard interview. Eleven companies were selected randomly from a sample population of 61 food factories which were obtained from the Food Control Section of the Dubai Municipality database

Results: It is essential to assemble the right blend of expertise and experience, as the team will collect, collate and evaluate technical data and identify hazards and critical control points (FAO, 1998). From the results obtained and from the interviewees' responses, the research revealed the most positive HACCP team experiences and the difficulties encountered by the HACCP teams: 1. Positive experiences that relate specifically to teams •Members' knowledge improved •Members' awareness and commitment increased •Employees' performance improved; 2. Other positive findings about HACCP in general •Business improved •Business secured •Management improved •Compliance with the legislation •Healthy working environment emerged; 3. Negative experiences that relate specifically to teams •The starting steps were a challenge •Lack of time •Lack of Space is an obstacle for conducting HACCP; and 4. Negative experiences that relate to HACCP generally •Financial difficulties •HACCP as distortion •HACCP as a controversy

Significance: Assembling the HACCP team is the first step in the logical sequence for the application of the HACCP format recommended by the Codex Alimentarius Commission (CAC); therefore, this study tried best to discover the potentials required of HACCP team members and how that could reflect positively on HACCP implementation.

P1-97 Preventative Decontamination of an Aseptic Bottling Room Using Gaseous Chlorine Dioxide

MARK A. CZARNESKI and Kevin Lorcheim
ClorDiSys Solutions, Inc., Lebanon, NJ, USA

Introduction: The presentation will highlight the process and efficacy for the decontamination of aseptic manufacturing facilities with gaseous chlorine dioxide

Purpose: This presentation describes the effectiveness of using chlorine dioxide gas in order to decontaminate an aseptic manufacturing room during a scheduled maintenance

Methods: This presentation will start by discussing the need of the facility to decontaminate their aseptic bottling room during a scheduled shut down for maintenance. Background information concerning decontamination using chlorine dioxide gas will also be illustrated, discussing the process and consideration for its use. The setup and execution of the room decontamination will be explained and described as well. Finally, the methods for efficacy testing done afterward to confirm the success of the decontamination will be discussed along with the results. Pictures, figures, and graphs will be utilized where appropriate to enhance the presentation.

Results: Post-decontamination testing results will be shared, showing that all biological indicators were shown to exhibit no growth following post-exposure incubation following the decontamination. Benefits of decontamination with gaseous chlorine dioxide will also be discussed, including short downtime and no post-exposure residue

Significance: Preventative decontamination is becoming a more important aspect in the food industry. This presentation offers a case study of one company's use of chlorine dioxide gas for decontamination.

P1-98 Consumer Knowledge and Handling of Tree Nuts

LOUISE LEE, Christine M. Bruhn, Diane Metz and Maria Giovanni
University of California-Davis, Food Science and Technology, Davis, CA, USA

Introduction: Nuts contribute good taste as well as nutritional benefits to the American diet. Consumption of nuts has increased in the last decade. In 2007, 3.29 pounds (1.49 kg) of tree nuts were eaten per capita compared to 2.22 pounds (1.00 kg) in 1997 (1). While nut consumption can contribute to a healthy diet, recently nuts have been identified as a source of *Salmonella*.

Purpose: This study was designed to provide data on consumer awareness of nuts as a source of foodborne pathogens and to identify consumer purchase, storage, and usage of almonds, pecans, pistachios, and walnuts. This information can be used to guide consumer education and to assist in the development of risk assessment models should the nuts be contaminated.

Methods: An internet survey was designed to quantify the practices reported in the focus groups held in Northern California. Consumers who use nuts and were active in University of California Cooperative Extension programs such as 4H, gardening, or other activities as well as the general public were asked to volunteer either for the focus group or the internet survey.

Results: The 279 volunteers who took the survey in 2008 reported that raw nuts are most often eaten out of hand, baked in cookies, or tossed in salads. Other popular uses include adding to stir-fry dishes and to breakfast cereal. Typically consumers store almonds, pecans, and walnuts at room temperature or the refrigerator for as long as 6 months. Few consumers were aware that low-moisture foods such as nuts could on rare occasion be a source of foodborne illness. Only one-third believe that the health benefits of nuts are the same whether the nut is raw or pasteurized, or roasted. A majority of consumers reported that they would not change their family's use of nuts to prevent foodborne illness.

Significance: Raw nut are eaten out of hand and used in a variety of cooked and uncooked dishes. Consumer storage and usage can impact *Salmonella* survival. Data on consumer practices can be used to develop more accurate risk assessment models

P1-99 Multi-state Outbreak of *Escherichia coli* O157:H7 Infections Associated with Consumption of Mechanically-tenderized Steaks in Restaurants – United States, 2009

Wright A. Culpepper, Timothy Ihry, Carlotta Medus, Amanda Ingram, Diana Von Stein, Steven Stroika, Eija Hyytia-Trees, Scott Seys and MARK SOTIR
Centers for Disease Control and Prevention, Outbreak Response and Prevention Branch, Atlanta, GA, USA

Introduction: Escherichia coli O157:H7 is a major cause of bloody diarrhea and hemolytic uremic syndrome.

Purpose: In December 2009, several state health departments, CDC, and the USDA Food Safety Inspection Service (FSIS) investigated an increase in infections due to a specific strain of *E. coli* O157:H7.

Methods: We defined a case as illness in a patient from whom the outbreak strain of *E. coli* O157:H7, determined by pulsed-field gel electrophoresis, was isolated. We interviewed patients with a standard exposure questionnaire focusing on *E. coli* O157:H7 risk factors. Traceback investigations were conducted.

Results: We identified 25 cases in 17 states with onsets from October 3—January 31, 2010; 12 were hospitalized, 1 developed hemolytic uremic syndrome, and 1 died. Median age of patients was 30 years (range: 14-87 years); 42% were female. Of the 22 patients interviewed, 14 (64%) reported eating steak at a family-style restaurant; nine (41%) of the 22 ate at restaurant Chain X. Of the 14 who ate steak, 9 (64%) ate a 7 oz. sirloin, 1 (7%) reported eating sirloin tips, and 4 (29%) could not recall the cut of steak. All patients who ate steak ate them rare, medium-rare, or medium. Among the 8 who did not report eating steak, 7 (88%) ate ground beef. Traceback determined that seven Chain X customers consumed mechanically-tenderized steak from a single processor, Company Y. In late December, Company Y issued a voluntary recall of 248,000 pounds of beef. The recall included mechanically-tenderized steak and other products distributed to Chain X and several other restaurants.

Significance: This outbreak was linked to steaks served at restaurants in several states; steaks were mechanically-tenderized, traced to a single processer, and consumed rare, medium-rare, or medium. Commercial food establishments and consumers should be aware of the risks associated with undercooked mechanically-tenderized steaks.

P1-100 Detection and Enumeration of *Escherichia coli* O157:H7 and *Salmonella* Species in Raw Commingled Silo Milk

Emily E. Jackson, NEERAJ MADDI, Edibe S. Erten, Thomas E. Graham, John W. Larkin, Robert J. Blodgett, Joseph E. Schlesser and Ravinder M. Reddy
Illinois Institute of Technology, National Center for Food Safety and Technology, Summit-Argo, IL, USA

Introduction: Risk assessment and the Food Safety Objective (FSO) are increasingly being used to assure the safety of food technologies. Knowledge of initial microbial levels (H0) in raw milk intended for pasteurization will enable risk assessment of novel processes.

Purpose: A nationwide survey was conducted to determine initial microbial quality and levels of *Escherichia coli* O157:H7 and *Salmonella* species in raw silo milk intended for pasteurization.

Methods: Standard sampling procedures were used to collect 158 unique silo samples. Total Viable Count, Coliforms, *Enterbacteriaceae*, *E. coli* and *Staphylococcus aureus* counts were measured by using the TEMPO® Automated System (bioMérieux). Raw milk samples were initially screened by enriching 100 ml to detect *E. coli* O157:H7 and *Salmonella* species, with the mini VIDAS® (bioMérieux) ECPT or SLM assay, respectively, chromogenic and selective media, followed by confirmatory biochemical testing. Twenty-five milliliters of each positive sample was further analyzed using 10-fold dilutions in a 3-tube, 5-dilution Most Probable Number (MPN) technique. BAM methods were used to calculate the MPN using all dilutions, including the initial screening results.

Results: Generally, the samples were of good quality. *E. coli* O157:H7 was detected and enumerated in 18 of 198 samples (9.09%) at an average level of 0.0833 MPN/ml and *Salmonella* species were detected in 46 of 202 samples (22.77%) at an average level of 0.9909 MPN/ml. No correlation was observed between the general microbial quality and prevalence of *E. coli* O157:H7 or *Salmonella* species.

Significance: Although the prevalence rates observed were higher than those reported in the literature, the levels detected were low. The higher prevalence may be due to the use of a larger sample volume and more sensitive techniques and that the samples were taken from commingled silos, which contain milk, and, therefore contaminants, from multiple bulk tanks. The levels of pathogens measured can easily be eliminated by current milk pasteurization requirements.

P1-101 Cloning and Characterization of a Δ-*prfA Listeria monocytogenes* Strain Containing a Single Copy Genomic Artificial Internal Amplification Control for Use as Internal Sample Process Control

PETER ROSSMANITH, Karin Frühwirth, Sabine Fuchs, Patrick Mester and Martin Wagner
University of Veterinary Medicine Vienna, Vienna, Austria

Introduction: Real-time PCR for food pathogen detection is mostly used with internal amplification controls to monitor the enzymatic reaction and for determination of the efficiency of the reaction. Preliminary methodical steps such as sample preparation and DNA isolation and purification are not included in this kind of control and if at all, checked by external controls. This does not allow for control of single samples and thus negative results imply the possibility of false verification of the pathogen status of the investigated food samples. For this purpose there is need for an internal process control covering the whole detection process.

Purpose: Development and characterization of an internal process control based on a model organism as close related to the actual target pathogen as possible which does not influence the quantitative results of the underlying method for detection of the aimed pathogen.

Methods: A Δ-*prfA L. monocytogenes* EGDe strain was cloned with a phage insertion vector to result in a single copy inserted artificial real-time PCR target (IAC) amplified with the primers binding the *prfA* locus of *L. monocytogenes* resulting in a fluorescence signal not interfering with the respective signal of the *L. monocytogenes* wild type strain.

Results: The Δ-*prfA L. monocytogenes* EGDe strain was characterized as *L. monocytogenes* EGDe, the single copy status of the DNA insertion was demonstrated and the strain was used in context with matrix lysis sample preparation both with artificially and naturally contaminated food samples to demonstrate the use of the control. The resulting corrected values as obtained by the whole molecular detection protocol corresponded to the respective values of contamination as determined according to ISO 11920-2.

Significance: The internal sample process control based on IAC+, Δ-*prfA L. monocytogenes* EGDe enables in-sample control for real-time PCR detection of *L. monocytogenes* in food samples. The application of internal sample process controls is essential to cover all steps and integrated methods which are included in molecular biological pathogen detection to provide reliable results.

P1-102 Rapid and Specific Detection of the *invA* Gene in *Salmonella* spp. by iTPA

JEONG SOON KIM, Moonsang Jahng and Joon Kim
Samsung Everland Inc., Yongin-si, Republic of Korea

Introduction: Pathogenic *Salmonella* species are an important cause of infectious diseases throughout the world. *S. enterica* serovars Enteritidis and Typhimurium cause the majority of human gastroenteritis infections: a reported 40,000 cases of salmonellosis in the U.S. each year. Isothermal target and probe amplification (iPTA) is a novel technique that rapidly amplifies target DNA and FRET-based signal probes in isothermal environments.

Purpose: To propose a rapid and simple method through originally designed iTPA and fluorescence detector which doesn't need expensive DNA-analyzing instruments and electrophoresis technology. Here we report on the first study at developing a highly specific and sensitive iTPA assay for detecting *Salmonella* spp. in pre-enriched food samples.

Methods: This powerful amplification method employs the strand displacement activity of DNA polymerase and the RNA degrading activity of RNase H in the presence of DNA-RNA-DNA chimeric primers and probes. The detection of amplified FRET-based chimeric signal probes is achieved via a homogeneous solution without any post amplification handling. A set of four iTPA primers and one probe were designed based on the published *Salmonella* spp. and 40 other strains. The assay sensitivity was tested using serial dilution of *Salmonella* Typhimurium KCTC2515 culture ranging from 10,000,000 to 10 CFU/mL. The assay was evaluated in experimentally inoculated food samples.

Results: The *invA*-based iTPA assay was able to specifically detect all of 10 *Salmonella* spp. strains without amplification from 40 other strains. The detection limit was 40 ~ 50 CFU per assay.

Significance: The *invA*-based iTPA assay developed in this study was sensitive and specific, having great potential for future field screening of *Salmonella* spp. in the food industry.

P1-103 Selection and Characterization of Biotinylated DNA Aptamers for Capture and Detection of *Salmonella* Typhimurium Using Quantitative Real-time PCR

HARI PRAKASH DWIVEDI and Lee-Ann Jaykus
North Carolina State University, Raleigh, NC, USA

Introduction: Nucleic acid aptamers show advantages over traditional capture ligands such as antibodies, including reduced cost, ease of production and modification, and improved stability. Such ligands are being investigated for selective concentration and purification of pathogens from complex sample matrices such as foods.

Purpose: The purpose of this project was to identify DNA aptamers specific to *Salmonella* Typhimurium and apply them for the selective capture of *Salmonella* followed by detection by TaqMan™ quantitative real-time PCR (qPCR).

Methods: The whole cell-SELEX (Systematic Evolution of Ligands by EXponential enrichment) method was applied to a combinatorial library of biotin-labeled single stranded DNA molecules in an effort to identify DNA aptamers with binding specificity to *S.* Typhimurium. Aptamer specificity was enhanced using periodic counter-SELEX against select non-*Salmonella* genera. Aptamers with binding selectively to *Salmonella* were separated using flow cytometry, sequenced, and characterized for binding efficacy. An aptamer-magnetic bead based assay was developed to capture *Salmonella* followed by detection using qPCR targeting the *invA* gene.

Results: Out of 18 candidate aptamer sequences, two (S8-7 and S8-46) showed relatively high binding efficiency [13–14% of target cells (n = 200, 000) fluorescent with 300 pmoles of each aptamer]. Aptamer S8-7 was further characterized, demonstrating a dissociation constant (*Kd* value) of 1.73 ± 0.54 μM, and selectively binding 22% of target cells at a 6.94 μM concentration. Binding exclusivity analysis of S8-7 showed low apparent cross-reactivity with other foodborne bacteria including *E. coli* O157:H7 and *Citrobacter braakii*, and moderate cross-reactivity with *Bacillus cereus*. The lower limit of detection of the combined aptamer-magnetic bead capture assay followed by downstream detection using qPCR was 10^2–10^3 CFU of *S.* Typhimurium in a 290 μl sample volume. Capture efficiency ranged from 3–13%.

Significance: This study provides proof-of-concept that biotinylated aptamers targeting *S.* Typhimurium can be used for pathogen capture and subsequent detection. The candidate aptamers are currently being evaluated for their ability to capture *Salmonella* from complex sample matrices for subsequent detection by qPCR.

P1-104 Novel Phage Immuno-concentration Assay for Isolation of *Escherichia coli* O26, O103, O111, O145 and O157

Fanny Savoye, Marion Bouvier, JEAN-LOUIS PITTET, Christine Aguilhon and Delphine Thevenot
bioMérieux, RD Industry, Marcy L'Etoile, France

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) that cause hemorrhagic diarrhoeal disease and Haemolytic and Uremic Syndrome are associated with some predominant serotypes. Their high virulence status means that absence of the pathogenic *E. coli* strains must be guaranteed to ensure a safe release of foods.

Poster

Purpose: This study was designed to develop and optimize automated VIDAS immuno-concentration of *E. coli* O157, O26, O103, O111 and O145 (VIDAS ESPT) with the use of recombinant phage proteins for capture. The goal of the test is to allow sample preparation of enriched food products to facilitate further detection of pathogenic strains and reduce the number of false positive reactions.

Methods: Assay specificity was evaluated with 50 pure culture strains (*E. coli* O157, O26, O103, O111, O145, STEC and non-STEC). Assay sensitivity was determined using meat and dairy products enriched and then experimentally inoculated at different levels (10^1 to 10^7 CFU/ml). The test was then evaluated on food samples artificially contaminated with stressed cells and enriched.

Results: The inclusivity and exclusivity study clearly showed that the assay was highly specific for detection of the targeted strains. The limit of detection determined by serial dilution of O26, O103, O111, O145 and O157 cultures was found to be at least 10^4 cells/ml after enrichment. The test was shown to be sensitive enough for isolation of very low levels of stressed strains (1 to 5 CFU/25 g) in artificially contaminated foods.

Significance: This study has demonstrated that the VIDAS phage ESPT is a promising tool to isolate *E. coli* O157, O26, O103, O111 and O145 from food in a single assay, prior to establishing their pathogenic status with a PCR assay or to facilitate their identification on selective agar plates. This automated method will provide obvious technical and economic advantages to the food industry for routine testing.

P1-105 Isolation and Detection of Verotoxin-producing *Escherichia coli* from Foods

ALEXANDER GILL, Amalia Martinez-Perez and Burton Blais
Health Canada, Bureau of Microbial Hazards, Ottawa, ON, Canada

Introduction: Verotoxin (Shiga toxin) producing *Escherichia coli* (VTEC), including *E. coli* O157:H7 are significant foodborne pathogens. VTEC serotypes other than O157 account for 30% or more of VTEC illness in the USA and Canada. Though many methods are available for the detection and isolation of *E. coli* O157:H7, there is a shortage of methods to detect other VTEC.

Purpose: A protocol for the detection and isolation of five VTEC serogroups (O157, O26, O103, O111, and O145) from foods is described here.

Methods: Strains of five different VTEC (O157:H7, O26:H11, O103:H2, O111:NM, O145:NM) were inoculated individually into ground beef. 25 g samples of ground beef were prepared in triplicate and incubated in 225 ml of enrichment broth for 16–24 hours. The enrichment broths were screened by PCR for the genes of verotoxin 1 and verotoxin 2. Dilutions of the enrichment broths which tested positive for verotoxin genes were plated on agar medium and incubated for 24 hours. Colonies were screened to isolate presumptive VTEC. Presumptive VTEC isolates were confirmed by a cloth hybridization assay which simultaneously identifies the presence of the virulence factors verotoxin 1 and 2, intimin, enterohemolysin and identifies the serotypes O157, O26, O103, O111, O145. The same protocol was also applied to the isolation *E. coli* O157 from spinach, lettuce and cider.

Results: E. coli were detected and isolated from 25 g samples of ground beef, lettuce, spinach and cider when present at concentrations of 0.5 to 0.9 CFU/g. The other four VTEC were detected and isolated from ground beef at concentrations of 0.4 to 1.2 CFU/g.

Significance: These results demonstrate that this protocol is capable of reliably detecting and isolating *E. coli* O26, O103, O111, O145 and O157 from ground beef and *E. coli* O157:H7 from various food types, in the presence of normal background flora. The protocol can potentially be adapted to the analysis of clinical and environmental samples.

P1-106 Detection of *Escherichia coli* O157:H7 from Lettuce Using Treated Activated Charcoal and Real-time PCR

JUNG-LIM LEE and Robert E. Levin
University of Massachusetts, Dept. of Food Science, Amherst, MA, USA

Introduction: Vegetables, such as lettuce, can be contaminated during growth, harvesting, washing, packaging, shipping, and displaying. Several outbreaks of *E. coli* O157:H7 associated with lettuce consumption have been reported. Several types of detection methods have been developed for *E. coli* O157:H7, however, these methods are notably time consuming and labor intensive. Another critical problem is the number of cells needed in a sample to detect *E. coli* O157:H7.

Purpose: The purpose of this study was to develop a methodology that was fast and could detect relatively low numbers of *E. coli* O157:H7 in lettuce.

Methods: Romaine lettuce was purchased from a local retail source. The optimal sample preparation to detect *E. coli* O157:H7 from lettuce was determined by using various surfactants for detaching microbes and bentonite-coated activated charcoal for removal of PCR inhibitors. The real-time (Rti) PCR was used in this study for rapid detection. Primers that amplified the rfbE gene of *E. coli* O157:H7 were used.

Results: A SDS solution exhibited the greatest ability to detach cells from lettuce. When activated carbon coated with bentonite was mixed with target cell suspensions derived from lettuce, a high recovery of *E. coli* O157:H7 (93 ± 7.0%) was obtained. The Rti-PCR with the use of activated charcoal was able to detect below 1×10^3 CFU of *E. coli* O157:H7/g of lettuce. In contrast, the Rti-PCR without activated charcoal treatment failed to detect the same number of *E. coli* O157:H7. A linear range of DNA amplification was exhibited from 1.0×10^1 to 1.0×10^4 CFU/g by using the Rti-PCR.

Significance: These results indicate that the use of activated charcoal is an improved methodology for removal of PCR inhibitors and can be applied to detect *E. coli* O157:H7 from lettuce.

P1-107 The Use of DNA Aptamers for Capture of *Campylobacter jejuni* Prior to the Application of qPCR for Detection

SOO HWAN SUH, Hari P. Dwivedi and Lee-Ann Jaykus
North Carolina State University, Food, Bioprocessing and Nutrition Science, Raleigh, NC, USA

Introduction: Campylobacters are one of the most common causes of acute gastroenteritis worldwide. There is continuing need for improved methods to detect *Campylobacter jejuni* in foods and environmental samples, especially in the absence of reliable, commercial cultural or molecular protocols.

Purpose: The purpose of this study was to develop a rapid method to detect *C. jejuni* by combining a DNA aptamer-based capture-concentration step followed by quantitative real-time PCR (qPCR).

Methods: A previously identified biotinylated DNA aptamer (aptamer-229) with binding specificity for *C. jejuni* (A9a) was conjugated to streptavidin-coated magnetic particles for capture of *C. jejuni* of cells. The DNA from captured cells was extracted and amplified using a Taqman qPCR assay targeting the *C. jejuni glyA* gene. To confirm the binding specificity of aptamer-229, a cocktail of *C. jejuni* and non-*C. jejuni* [*Salmonella* enteric subsp. enteric (ATCC 13076), *Bacillus cereus* (9789), *Shigella flexneri* (12022) and *E. coli* O157:H7 (43895)] cells were processed for capture and detection. The sensitivity of the combined aptamer capture-qPCR assay was determined using a

Poster

serially diluted pure culture of *C. jejuni*; it was also compared to an immunomagnetic separation (IMS)-qPCR assay using anti-*Campylobacter* polyclonal antibodies.

Results: The qPCR standard curve demonstrated log linear detection in the range of 10 to 10^7 CFU *C. jejuni* cells per reaction, with a lower limit of detection of 50 CFU. When qPCR was preceded by the aptamer capture assay, the limit of detection of the combined method was 10^2 to 10^3 cells in 1 ml PBS buffer. The aptamer capture efficiency was 70 to 90% at when applied to 10^2 *C. jejuni* cells in 1 ml PBS buffer; 30% at 10^3 CFU/ml; and <5% at higher cell numbers. The capture efficiency of the IMS-qPCR assay was 4% at 10^3 CFU/ml and <1% at greater than 10^6 CFU/ml.

Significance: This study demonstrates that the aptamer-based capture assay could be applied in conjunction with qPRC for the detection of *C. jejuni*, and that this approach demonstrated higher binding efficiency (and better detection limits) than did an equivalent assay using antibody capture.

P1-108 A PCR Method for the Detection of *Listeria monocytogenes* Based on a Novel Target Sequence Identified by Comparative Genomic Analysis

Dandan Zhang, Lida Zhang, Dapeng Wang, Biao Suo and XIANMING SHI
Shanghai Jiao Tong University, Dept. of Food Science, School of Agriculture and Biology, Shanghai, China

Introduction: Listeria monocytogenes is an opportunistic foodborne pathogen that is a serious health hazard worldwide. The majority of published PCR detection assays for *L. monocytogenes* mainly utilized primers targeting genes of virulence factors or RNA subunit genes, which could yield false-positive or false-negative results. Using the whole genome sequences in public data bases, comparative genomic tools coupled with various algorithms could be utilized to mine novel targets for *L. monocytogenes*.

Purpose: The purpose of this study is to develop a PCR assay targeting a novel *L. monocytogenes*-specific diagnostic sequence combined with an internal amplification control (IAC).

Methods: The genome fragments (500-bp) of *L. monocytogenes* EGD-e were compared with the sequences of other *L. monocytogenes* strains and non-*L. monocytogenes* strains by BLASTN to mine specific sequences for *L. monocytogenes*. Using a primer set from one of the mined specific sequences, a PCR assay including an internal amplification control (IAC) was developed. The specificity and sensitivity of the PCR assay were evaluated and further tested in artificially contaminated milk samples.

Results: The PCR assay allowed amplificaiton of a 331-bp fragment only from the genomic DNA of *L. monocytogenes* strains and not from other *Listeria* species, as well as some non-*Listeria* species. The detection limit of the PCR assay was 55 copies/PCR with *L. monocytogenes* genomic DNA. Applying this PCR assay to artificially-contaminated milk samples, low levels of *L. monocytogenes* (1 to 10 CFU/ml of milk) were detected after 6-9 h incubation in selective culture enrichment (UVM1).

Significance: The novel species-specific target sequence identified by a comparative genomic approach offered a new molecular diagnostic marker for specific detection of *L. monocytogenes*, and this method could also provide a new tool in mining unique targets for other bacteria. The developed PCR assay with an IAC showed to be specific, sensitive, and applicable to the detection of *L. monocytogenes* from artificially-contaminated food.

P1-109 Mid-infrared Chemical Imaging for *Clostridium perfringens* Detection on DNA Microarray

BRIANNA L. SCHOEN, Sufian F. Al-Khaldi, Magdi M. Mossoba and Betsy J. Yakes
FDA, College Park, MD, USA

Introduction: Clostridium perfringens is one of the most common human pathogens. *C. perfringens* strains are divided into five toxin types on the basis of production of four major lethal toxins. The presence of these toxins in biological samples is often detected using PCR, restriction length fragment polymorphisms (RFLP), and pulsed field gel electrophoresis. However, these methods have limitations. There is a need for highly specific screening methods, such as DNA microarrays, to rapidly detect and identify foodborne pathogens.

Purpose: The purpose of this study was to use mid-infrared chemical imaging (IRCI) to detect *C. perfringens* toxin genes on DNA microarrays.

Methods: To build the DNA chip, each gene sequence was represented by one ~ 22-bp amino-modified oligonucleotide printed on glass slides prefunctionalized with succinimidyl ester groups. Both single and multiplex PCR was used to amplify DNA target regions of all five genes. Single- stranded DNA (ssDNA) was prepared from double-stranded amplicons using magnetic beads. Single-stranded DNA was biotinylated and bound to gold nanoparticles-streptavidin conjugates. Target hybridization was visualized by the silver enhancement of gold nanoparticles. The adlayer of silver, bound to hybridized microarray spots, formed the external reflective substrate that allowed confirmation and quantification of hybridization by infrared imaging.

Results: IRCI imaging of *C. perfringens* toxin genes was successful to provide a quantitative measure of spot intensity. The signal to noise ratio (SNR) values for the spots were significantly higher than those obtained by conventional fluorescence detection.

Significance: This data provides a new method for detection of foodborne pathogens using mid-infrared chemical imaging (IRCI) applied to microarrays.

P1-110 Development of a Scorpion™ Probe-based Real-time PCR Assay for *Cronobacter* spp. Detection

STEPHEN VARKEY, Daniel R. DeMarco and Roger Stephan
DuPont, Wilmington, DE, USA

Introduction: Enterobacter sakazakii is known to be an occasional contaminant of powdered infant formula (PIF). This opportunistic neonatal pathogen has recently been reclassified as a novel genus, *Cronobacter*. Within this genera exist six species, one of which, *C. genomospecies*, currently consists of only two known strains. The use of conventional and real-time PCR methods has been described in the literature; however, few, if any, commercial kits are currently available for *Cronobacter*.

Purpose: The purpose of this study was to evaluate the use of Scorpion® probe-based technology with existing primer sequences to develop a faster real-time PCR assay that is simultaneously 100% inclusive of the *Cronobacter* genus and exclusive of non-*Cronobacter* microorganisms. The use of probe detection allows for rapid cycling (< 1 h) and can provide additional specificity.

Methods: Studies evaluating the sensitivity of the new real-time assay were conducted using purified extracted DNA and select *Cronobacter* spp. Inclusivity and exclusivity studies were conducted using select *Cronobacter* spp. and closely related organisms.

Results: Sensitivity of the assay with purified extracted DNA was determined to be < 3 pg/reaction. With crude cell lysates of *C. genomospecies* (E797), the assay could detect as low as 10^4 CFU/ml. Inclusivity using a small panel of 47 *Cronobacter* spp. was 100%. Six strains of closely related *Enterobacter* species were not detected by the assay.

Poster

Significance: These results demonstrate the feasibility of developing a novel real-time PCR assay for *Cronobacter* spp. that allows for fast cycling with detection in less than one hour.

P1-111 Rapid and Sensitive Detection of *Vibrio parahaemolyticus* by a Loop-mediated Isothermal Amplification Assay

Jiro Nemoto, AKIRA OHSHIMA, Junji Arita, Tadashi Kojima, Masanari Ikedo, Akira Kamio, Hirotaka Konuma and Yukiko Hara-Kudo

Eiken Chemical Co. Ltd., Tokyo, Japan

Introduction: Thermostable direct hemolysin (TDH) or TDH-related hemolysin (TRH)-producing *Vibrio parahaemolyticus* is a pathogen which contaminates seafood. Total *V. parahaemolyticus*, including TDH or TRH- non-producing *V. parahaemolyticus*, is regarded as an indicator of seafood safety. In contrast with culture methods which are time consuming, loop-mediated isothermal amplification assays provide a rapid and sensitive method of detecting bacterial and virus pathogens.

Purpose: The purpose of this study was to develop rapid and sensitive detection methods for *V. parahaemolyticus* using the LAMP assay.

Methods: A set of LAMP primers comprised of four oligonucleotide primers targeting the *rpoD* gene were designed based on sequence data from the DNA Data Bank of Japan. The reaction mixture was incubated at 65°C for 60 min. Specificity and sensitivity were tested using 18 strains of *V. parahaemolyticus*, 22 strains from 12 *Vibrio* spp. other than *V. parahaemolyticus* and 26 strains from 11 bacterial species other than *Vibrio*. The LAMP assay developed in this study and a real-time PCR assay were evaluated in 14 seawater samples.

Results: All 18 strains of *V. parahaemolyticus* were positive and the other bacterial strains negative for the LAMP assay. Six CFU per reaction was the minimum level indicating a positive response in duplicate tests in each of the two *V. parahaemolyticus* strains, although three of four reactions had a level of 0.6 CFU per testing positive. Nine of 14 seawater samples were negative for *V. parahaemolyticus* by both real-time PCR and the LAMP assays. In three samples, *V. parahaemolyticus* was detected by both real-time PCR and the LAMP assays. In two samples, *V. parahaemolyticus* was detected by the LAMP assay, but not by the real-time PCR assay.

Significance: These data indicated that the LAMP assay targeting the *rpoD* gene rapidly and sensitively detected *V. parahaemolyticus*.

P1-112 Norovirus Recovery from Spiked Food Samples Using Histo-blood Group Antigens on Magnetic Beads

PENG TIAN, David Yang and Robert Mandrell

USDA, ARS, Produce Safety and Microbiology, Albany, CA, USA

Introduction: Noroviruses (NoV) cause an estimated 23 million annual cases of gastrointestinal disease in the United States, 50-67% of all foodborne illnesses, and of the NoV illnesses between 1973 and 2006 with a confirmed etiology, 58.3% were associated with leafy greens. Current methods to recover NoV from large wash volumes from complex samples like leafy greens are ineffective.

Purpose: Histo-blood group antigens (HBGA), which are recognized as receptors for human NoV, were conjugated to magnetic beads (HBGA-MB) and tested the ability to recover human NoV in food samples.

Methods: HBGA were conjugated to magnetic beads and added to 250 ml or larger volumes of wash samples of Romaine lettuce that had been spiked with a dilution of a human stool sample containing genotype II NoV. NoV were released from the surface of lettuce by a PBS wash with vigorous shaking, HBGA-MB were added and recovered using a recirculating immunomagnetic separation system. NoV recovery by a traditional PEG precipitation method was done also for comparison. NoV was measured by real time RT-PCR. Recovery of NoV by PEG and HBGA-MB, was 13% and 20%, respectively, resulting in 237- and 350-fold concentration. Recirculating immunomagnetic separation with HBGA-MB is a more rapid and simple method than conventional methods for recovery of NoV from large volume food samples.

Results: Recovery of NoV by PEG and HBGA-MB, was 13% and 20%, respectively, resulting in 237- and 350-fold concentration.

Significance: Recirculating immunomagnetic separation with HBGA-MB is a more rapid and simple method than conventional methods for recovery of NoV from large volume food samples.

P1-113 Immunomagnetic Concentration and Real-time RT-PCR Detection of Norovirus

SOPHIE ROY, Solange Ngazoa, Ismaël Fliss, André Darveau and Julie Jean

Université Laval, Dept. Food Science and Nutrition, Quebec, QC, Canada

Introduction: Norovirus (NoV) is the major agent of acute foodborne gastroenteritis in human worldwide. The concentration of viral particles found in food samples is very low but still sufficient to cause infections in human. Therefore, the concentration of viral particles prior to detection is necessary to achieve an accurate and sensitive monitoring of these viruses in clinical, food and environmental samples.

Purpose: The objective of this study was to develop and evaluate the efficacy of an immunomagnetic concentration method combined to real-time RT-PCR for the sensitive and specific detection of NoV.

Methods: Polyclonal antibodies have been produced against a synthetic peptide corresponding to specific region of the viral capsid protein. The antibodies obtained were purified by affinity and covalently immobilized to magnetic beads. The viral particles were captured by the magnetic beads and were lysed before being submitted to amplification by TaqMan RT-PCR.

Results: The approach using synthetic peptide was shown to be simple and very efficient for generating specific antibodies against NoV. The detection limit of the concentration/detection approach was 0.001 RT-PCR units using clinical NoV samples. The concentration/detection approach has also been applied to lettuce and strawberries spiked samples and NoV was detected at a concentration as low as 10 RT-PCR units.

Significance: The development and application of this strategy may contribute to a better concentration and detection of NoV in different samples including foods.

P1-114 Norovirus Recovery from Foods and Food Contact Surfaces is Improved with Alternative Elution Buffers and Cationically-charged Magnetic Beads

SUSAN J. DOWNER, Amy N. Mann and Jennifer L. Cannon

University of Georgia, Center for Food Safety, Griffin, GA, USA

Introduction: An estimated 9 million illnesses occur annually in the USA due to foodborne norovirus (NoV). To assist in outbreak investigations and monitor the safety of foods, improved methods for detecting NoV in foods are needed.

Purpose: Here, alternative buffers for virus elution and a novel concentration method using positively-charged magnetic beads were investigated for enhanced recovery of human NoV and murine norovirus (MNV), a NoV surrogate.

Methods: Foods (carrots, lettuce and berries) and food contact surfaces (stainless steel knives, vegetable brushes, and latex and nitrile gloves) were inoculated with diminishing amounts of partially purified human NoV or MNV (1 to 1,000 viral particles) prior to elution

with various elution buffers (modifications in composition, pH, and ionic strengths) and physical disruption techniques (vortex, massage, sonication, glass beads). Percent recoveries were determined via realtime RT-PCR or plaque assay following PEG precipitation or recovery using cationic magnetic beads.

Results: While viral recovery varied among the items (~20–50%), the highest and most consistent viral recovery for both foods and food contact surfaces was achieved using a 0.1 m PBS buffer containing 1 M NaCl and 0.05% Tween-20, resulting in a lower detection limit of 10 to 500 viruses for food and food contact surfaces. Using cationic beads for concentration, inoculums of 100 to 2,000 viruses could be recovered from food items.

Significance: Results indicate that the use of a high ionic strength buffer containing a non-ionic detergent is a promising means for eluting NoV from foods and food contact surfaces and positively charged magnetic beads can improve virus recovery and detection.

P1-115 Combined Concentration and Extraction Method for Bacteria and Viruses from Contaminated Water and Leafy Vegetables

Julie Brassard, Évelyne Guévremont, MARIE-JOSÉE GAGNÉ and Lisyanne Lamoureux
Agriculture and Agri-Food Canada, Saint-Hyacinthe, QC, Canada

Introduction: Water and leafy vegetables eaten fresh are more and more reported as being involved in foodborne illness cases. The pathogenic agents responsible for these infections are mainly bacteria and viruses and are present in very small quantities on the contaminated food matrices. Laboratory techniques used to isolate or detect the contaminating agent differ enormously according to the type of microorganisms, generating time and economical losses.

Purpose: The purpose of this study was to optimize a unique method which allows in the same step the recovery and concentration of these two main types of pathogenic organisms.

Methods: Water and spinach samples were artificially contaminated with the feline calicivirus (FCV), rotavirus, hepatitis A virus (HAV), *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Salmonella* Typhimurium. The principle of the recovery technique was based on the use of a positively charged membrane which allows the adsorption of both viruses and bacteria present in the water or in the rinse from the vegetables. After the filter elution, bacteria were isolated on selective media and the viruses underwent a supplementary concentration step on Amicon column.

Results: All viruses and four bacterial species used in this study were recovered and detected from the two types of samples. Using conventional microbiology, this filtration technique allowed a detection level superior to 10^2 CFU/g for *S.* Typhimurium, *E. coli*, and *L. monocytogenes*, *C. jejuni* and to 10^2 PFU/g for FCV, HAV and rotavirus in classical RT-PCR.

Significance: This combined method can be applied to other bacterial and viral species and will possibly allow a costs reduction as well as lower the number of laboratory manipulations and time associated to the search and identification of the responsible agent for foodborne illnesses, whether it is of viral or bacterial origin.

P1-116 Coliphage in Produce Flume Water Detected in Less Than Eight Hours Provide Same Day Fecal Risk Indicator

ROBERT S. SALTER, Ernestine Conklin and Gregory Durbin
Charm Sciences Inc., Lawrence, MA, USA

Introduction: Produce contaminated by fecal pathogens is a public health concern. Rapid and simple methods are needed to predict fecal contaminated fruits and vegetables and to assess in-process treatment effectiveness. Water, used to process and rinse produce, contains a representative sample of the production-batch microbial flora. Coliphage are viruses to fecal bacteria that can be detected in water in 5.8—8 h and that could serve as a same day contamination indicator. They are prevalent in fecal material and they are more robust to log reduction treatment steps than coliform and *E. coli*, their fecal-bacteria- indicator counterparts.

Purpose: Evaluate 5.5–8 h presence/absence coliphage method with flume water spiked with waste water effluent and compare results to EPA Method 1601. Evaluate MPN versions of the method in comparison to plaque quantitation methods.

Methods: Fast Phage™ Test Kits, Charm Sciences Inc., provide 5.5–8 h fluorescent indication of somatic or male specific coliphage in 100 ml equivalent to EPA Method 1601. Flume water from spinach and lettuce processing was obtained by overnight shipping. Chlorine residuals 1–4 ppm were thiosulfate neutralized. Primary waste water, 0.45 um filtered was spiked at 1.3–1.5 plaque forming unit/100 ml (PFU/100 ml) for n = 10 presence absence evaluation, and 10—50 PFU/ml for 5.5–6 h MPN evaluation. MPN devices used were 4 ml-TEMPO Card™ (bioMérieux) and 100 ml-Quantitray™ (IDEXX). Coliphage plaques were quantitated by double layer agar (DAL) technique and EPA Method 1602.

Results: Somatic and male specific coliphage in spiked flume water produced at least n = 5 positives of n = 10 replicates similar to presence/absence results with spiked DI-RO water. Both somatic MPN methods yielded results within 0.5 log of DAL and 1602 plaque quantitation.

Significance: Coliphage in flume water can be detected in 5.5—8 h. This could provide a working day indicator of fecal contamination to the produce industry. Correlative work is needed to relate coliphage presence and enumeration to pathogen occurrence.

P1-117 DNA PROfiling for Detection and Characterization of *Salmonella* in Peanut Butter

BRITTANY E. PORTER, Hyun Jung Kim, Ho-ming Pang, Angie Oppedahl, Gary W. Procop and Byron F. Brehm-Stecher
Iowa State University, Food Science and Human Nutrition, Ames, IA, USA

Introduction: The polymerase chain reaction (PCR) is a rapid and sensitive tool for detecting pathogens in foods. However, as typically applied, PCR does not take full advantage of the informational content of target nucleic acids. Next-generation sequencing tools are available, but are still too costly for routine use in food labs. New approaches able to bridge the gap between traditional PCR and next-generation sequencing could enhance food safety efforts by providing both rapid presence/absence testing and detailed genetic characterization of isolates.

Purpose: The purpose of this study was to evaluate a novel capillary electrophoresis-based approach for obtaining sequence data directly from PCR amplicons and to use this method for rapid detection and characterization of *Salmonella* in peanut butter.

Methods: Creamy-style peanut butter was artificially spiked (2 CFU/g) using either a laboratory strain (*S.* Typhimurium ATCC 13311) or two strains (*S.* Tennessee and *S.* Typhimurium) isolated during the last two national peanut butter outbreaks. Samples were incubated in Buffered Peptone Water at 37°C for 18 h, homogenized by stomaching, and 1 ml aliquots were removed and centrifuged briefly to sediment solids. DNA was extracted from the clear aqueous supernatant using the PrepMan Ultra reagent and fluorescent, *Salmonella*-specific PCR amplicons were generated using primers targeting the 16S-23S intergenic spacer region. Amplicons were chemically digested with a reagent cleaving specifically at A and G residues, and the resulting populations of labeled fragments were analyzed via high-throughput capillary electrophoresis (HT-CE).

Results: Amplicons were generated from all *Salmonella* strains tested, using both standard (2.5 h) and abbreviated (90 min) PCR protocols. Combined PCR and HT-CE required only ~3 h. Analysis of chemically restricted, labeled amplicons via HT-CE (DNA PROFiling)

Poster

resulted in clear, superimposable DNA profiles for all strains. Identical results were obtained using both the standard and abbreviated PCR protocols, either from pure culture or from spiked peanut butter, with excellent reproducibility between separate experiments.

Significance: Our data highlight the promise of DNA PROFiling for providing rapid sequence-based characterization of *Salmonella* in complex foods. This approach for combined detection and genetic characterization of foodborne pathogens provides the food industry with a valuable new tool for advanced food safety.

P1-118 Evaluation of the Discriminatory Abilities of Different Subtyping Methods for *Escherichia* Species

SARITA RAENGPRADUB WHEELER, Jacob K. Cannon, Preciaus D. Heard and Mark W. Carter
Silliker, Inc., Food Science Center, South Holland, IL, USA

Introduction: Bacterial subtyping methods allow for characterization of isolates beyond the species and subspecies level. DNA-based or molecular subtyping methods include band-based (e.g., repetitive extragenic palindromic sequence-based PCR or rep-PCR) and sequence-based (e.g., multilocus sequence typing or MLST) methods that have emerged as approaches to potentially discriminate strains more effectively or more rapidly than phenotype-based methods such as serology.

Purpose: The objectives were: (1) to assess the utility of a semi-automated rep-PCR-based typing system (DiversiLab), to effectively differentiate *Escherichia*, including several strains of Shiga toxin-producing *E. coli* (STEC) and (2) to compare the discriminatory abilities of rep-PCR typing and MLST for *E. coli*.

Methods: Ninety-eight *Escherichia* strains, including 69 strains obtained from the STEC Center at Michigan State University, were used in this study. DNA extraction, rep-PCR, and PCR product separation and detection were carried out according to DiversiLab protocols. DiversiLab software was used to analyze fingerprints using the Pearson Correlation to calculate similarity. Allele profiles, as defined by a seven loci-MLST (st7), for 35 strains were available through the STEC Center isolate database.

Results: Ten strains, representing four different *E.* non-*coli* species, displayed unique rep-PCR fingerprints and showed low similarity (average 66%) to nearly all *E. coli*. All STEC O157:H7 and O157:non-motile strains clustered together with the majority displaying one characteristic pattern; other H variants showed low similarity to this cluster and to one another. For a select subset of 35 *E. coli* strains, 21 st7 MLST types were represented. One particular allele profile, ST 106, represented seven different serotypes and seven unique rep-PCR patterns, suggesting that MLST may not be as discriminatory for *E. coli* as serotyping or rep-PCR typing.

Significance: Rep-PCR is an effective subtyping method for differentiation of *Escherichia* strains that is comparable to serotyping as unique fingerprints were generated for the majority of isolates representing different serotypes. With further expansion of the DiversiLab *E. coli* Reference Library, the DiversiLab System™ can be used to rapidly confirm *Escherichia* serotypes and can be a useful tool for surveillance.

P1-119 PCR-based Method for Rapid Detection of Enterococci from Meat Products

Musarrat Jahan, Richard A. Holley, Denis O. Krause and KAVITHA PALANIAPPAN
University of Manitoba, Food Science, Winnipeg, MB, Canada

Introduction: Enterococci (considered members of the lactic acid bacteria) now include species of vegetable plant as well as animal intestinal origin. Although generally non-pathogenic, their presence in large numbers in foods may indicate a lapse in sanitation. Their ability to serve as a genetic reservoir of transferable antibiotic resistance is of concern.

Purpose: Conventional culture methods for identification of enterococci from food sometimes give false positive results, as some species lack typical characteristics. Moreover, these methods require at least 3 d to yield conclusive results. This work reports the development of a PCR-based assay to detect enterococci at the genus level within 24 h in meat and processed meat products by targeting the 16S rRNA sequence.

Methods: Published 16S rRNA sequences were aligned and used to design genus specific primers (EntF and EntR). The primers were able to amplify a target region of 678 bp from *E. faecalis* ATCC 7080. Subsequently, the complete nucleotide sequence of this amplicon was determined. After primary identification by PCR the *Enterococcus* positive samples were plated on two selective media (KF-*Streptococcus* agar and Brain Heart Infusion agar with 6.5% NaCl). Enterococci were then isolated by colony patch techniques and identities confirmed by API 20 Strep strips.

Results: The primers were able to amplify genomic DNAs from 18 species of enterococci, but there was no amplification with 32 species from closely-related genera (*Pediococcus*, *Lactobacillus*, *Streptococcus* and *Listeria*) and species of *E. coli* and *Salmonella*. Meat samples, including fermented sausage, ham, and fresh sausage batter, were tested for the presence of enterococci and so far two strains of *E. faecium* (fresh sausage batter) and *E. avium* (ham) have been found.

Significance: Results show that this combined method can be used to rapidly detect enterococci in meat samples and subsequently isolate and identify the strains.

P1-120 Development of a Rapid Method for Identification of Heat-resistant Fungi *Byssochlamys*, *Talaromyces*, *Neosartorya* and *Hamigera*

KOICHI HOSOYA, Motokazu Nakayama, Tetsuhiro Matsuzawa, Yusuke Hiro, Yumi Imanishi, Hajime Tokuda and Takashi Yaguchi
Kao Corporation, R&D-Safety Science, Ichikai-Machi, Haga-Gun, Japan

Introduction: Heat-resistant fungi, genera *Byssochlamys, Talaromyces, Neosartorya* and *Hamigera*, contribute significantly to the spoilage of heat-processed foods due to the formation of heat-resistant ascospores. The method of identifying these genera is based on morphologic characteristics. These methods take a long times to cultivate fungi suitable for identification and are required to discriminate the morphologic specialty. We attempted to develop the new rapid and convenient method of identifying heat-resistant fungi using PCR-specific amplification.

Purpose: The purpose of this study was to develop a rapid and convenient method for genus level identification of heat-resistant fungi.

Methods: The partial ßtubulin gene and 28SrDNA D1/D2 domain of the heat-resistant fungi were sequenced directly from PCR products using primers Bt2a/ Bt2b and NL1/NL4. Based on these data, genus-specific primers were designed from a particularly highly conserved site at the 3' terminal in the target genus that had no conservation or similarity with other genera. To estimate identifying potency of these primers, PCR reactions using extracted genomic fungi DNA and these primers were performed as follows: 30 to 35 cycles of denaturation at 95 to 98°C for 10 s, annealing at 55 to 61°C for 1 min, and extension at 72°C for 1 min.

Results: The relation between designed primers and PCR products was shown as follows: In PCR using B1F/B1R, PCR products were detected for homothallic species of genus *Byssochlamys, B. fulva, B. nivea, B. lagunculariae* and *B. zollerniae*. In PCR using the H2F/2R primers, PCR products detected for genus *Hamigera*. In PCR using T11F/T2R, T12F/T12R and Te1F/Te1R, PCR products detected for genera

Poster

Talaromyces mainly species. In PCR using N2F/N2R, PCR products detected for genera *Neosartorya* and *Aspergillus fumigatus*, and using the Af1/Af1R, PCR products detected *A. fumigatus* only. In PCR using these primer sets, the other strains involved in food spoilage and environmental contamination were not detected.

Significance: These data suggest that PCR reaction using these primers can identify heat-resistant fungi and shorten identification period from 14 days to a day.

P1-121 Discrimination of Sound and Fusarium-infected Wheat Using an Electronic Nose

ODEAN M. LUKOW and Kathy Adams
Agriculture and Agri-Food Canada, Cereal Research Centre, Winnipeg, MB, Canada

Introduction: The electronic nose has the potential of measuring both the quantity and quality of odor from all types of wheat, whether sound or disease infected. The FOX 3000 Electronic Nose, Alpha M.O.S., France, was used in our laboratory to distinguish wheat varieties grown at different locations using samples of grain and different flour fractions.

Purpose: The purpose of this study was to investigate: (1) wheat variety distinguishability using an electronic nose and (2) distinguishability of grain with infected with Fusarium head blight and producing distinct odors related to varying levels of infection.

Methods: For the electronic nose analyses, a standardized methodology was used to obtain readings for at least five of the twelve metal oxide sensors in the range of 0.7 to 0.9. Differences in six varieties were investigated with outliers removed prior to final analyses. One variety, Snowbird, was used to investigate the differences in Fusarium head blight infection. Electronic nose data were examined by principal component analysis (PCA).

Results: PCA analyses produced measurable discrimination for wheat varieties within environments with indexes ranging from 61 to 83. Variety differences between environments had discrimination indexes ranging from 59 to 97. Varying levels of infection by Fusarium head blight (0%, 2.2%, 4.1%) in the variety Snowbird could be discriminated with a PCA discrimination index of 94.

Significance: Electronic nose technology shows promise in wheat variety discrimination both for the food industry and for the agricultural community. This technology also has the ability to identify the presence of Fusarium disease in wheat and therefore can be an asset to the food industry in identifying food components harmful to human health.

P1-122 Method to Concentrate and Detect Pathogens in Produce Wash: Automated Concentration System, Lateral Flow Immunoassays and MSD PR2 1500

SONIA MAGANA, Sarah M. Schlemmer and Daniel V. Lim
University of South Florida, CMM, Tampa, FL, USA

Introduction: Current methods for detection of pathogens in dirty matrices require enrichment steps to isolate microorganisms and increase their number to detectable levels. However, enrichment can take days to provide confirmation of positive contamination.

Purpose: One of our laboratory's goals is to develop comparable but faster methods for pathogen detection in dirty matrices. These protocols include methods for the food industry for detection of low levels of foodborne pathogens in large lots of produce.

Methods: Produce (lettuce and spinach: 1.3 to 4.6 kg) was washed in 50 L of dechlorinated tap water for 10 min to generate produce wash (PW). PW spiked with *Escherichia coli* O157:H7 at 10^2 to 10^5 CFU/ml was concentrated to a 0.4 L sample (retentate) using a patent-pending Automated Concentration System (ACS) developed in our laboratory. The retentates were analyzed for *E. coli* O157:H7 ATCC 35150 on two rapid detection platforms; a lateral flow immunoassay (LFI) (EHEC VIP Gold™, BioControl) and an electrochemiluminescence assay-based sensor (SECTOR® PR2 Model 1500, Meso Scale Diagnostics).

Results: Concentration of PW resulted in 1.2 to 2 log increases in total CFU/ml and total MPN/100 ml and 2 log increases in *E. coli* O157:H7 CFU/ml (spiked samples). Unspiked retentates generated 33% false positives on LFIs and 0% false positives on the PR2 1500. Retentates containing 10^4 to 10^7 CFU/ml of *E. coli* O157:H7 were detected with the PR2 1500 whereas LFIs detected *E. coli* O157:H7 at levels of 10^6 to 10^7 CFU/ml.

Significance: Concentrating produce wash with the ACS and identifying *E. coli* O157:H7 with rapid detection platforms was successful and could prove useful to the food industry for rapid testing of produce prior to delivery to the consumer.

P1-123 Performance Testing of Selective Enrichment Media for *Listeria monocytogenes* Using Single Bacterial Cell Manipulation

DAGMAR SCHODER, Barbara Röder, Martin Wagner and Peter Rossmanith
University of Veterinary Medicine, Vienna, Austria

Introduction: In the present study, a recently developed method for single bacterial cell manipulation (SBCM) was applied to validation of enrichment media.

Purpose: The purpose of this study was performance testing of selective and unselective enrichment media in the range of < 10 cells avoiding stochastic effects caused by dilution.

Methods: The performance of Oxoid One-broth-*Listeria* and Half-Fraser broth was compared to tryptone soy broth with 6% yeast extract (TSB-Y). The growth of both stressed and unstressed cells was investigated. *Listeria monocytogenes* cells were manipulated by SBCM as previously published. Inocula of 1, 2, 3 and >3 cells were added to the respective media and the samples were analyzed after 24 and 48 h of incubation by determination of the optical density of the offspring cultures. Additionally enrichment broths/samples were streaked on selective and unselective solid media by the semi-quantitative three-loop technique and real-time PCR targeting the *prfA* gene were performed for determination of cell counts of the offspring cultures.

Results: A significant difference in the performance of selective media compared to unselective TSB-Y was identified for unstressed *L. monocytogenes* cells. Chilling stress resulted in no offspring cultures of *L. monocytogenes* in Half-Fraser broth as well as in Oxoid One-broth-*Listeria* when 1 cell was inoculated compared to 70% offspring in TSB-Y. A coherence of increasing cell numbers in the inocula to the number of positive offspring cultures was determined.

Significance: The use of SBCM generating single cell inocula independent from stochastic effects caused by dilution is a novel and successful tool in performance testing of enrichment media supporting conventional validation procedures. This approach avoids the influence of Poisson distribution and enables the direct evaluation of bacterial cell growth in the range of < 10 initial cells. This provides additional information for more precise determination of the performance of selective and unselective media.

P1-124 Stability of Calibration Function (Standards) in Nucleic Acid-based Food-pathogen Detection

Barbara Roeder, Martin Wagner and PETER ROSSMANITH
University of Veterinary Medicine Vienna, Christian Doppler Laboratory for Molecular Food Analytics, Vienna, Austria

Introduction: Results of real-time PCR depend on a calibration function when using the Ct-method for quantification and the accuracy of this standard is all-important. Long-term storage of standards is saving cost and time, avoiding laborious production on a daily basis in routine application. Nevertheless, aqueous solutions of DNA are prone to degradation during storage.

Purpose: The aim of this study was the determination of the causative parameter of DNA-standard degradation and the underlying mechanism impairing the amplification reaction during real-time PCR.

Methods: Real-time PCR assays targeting the *prfA* gene of *Listeria* and the *fimA* gene of *Salmonella* have been used to investigate the influence of long-term storage (>100 d), the GC-value, shear forces, DNA target length, chemical reactions within the storage buffer, glycerolstorage, subsequent thaw and freeze cycles, and the influence of remaining cellular enzymes after isolation, at -20°C ± 0°C, and 4°C. Tests were performed for initial low and high DNA target numbers.

Results: The stability of DNA-standards is influenced by shearing of long DNA fragments (bacterial genomes) if the standard is frozen. Short fragments (~100 bp) are not influenced by shearing during long-term storage or by subsequent thaw and freeze cycles. Depurination of the DNA and following mismatches on the primer attachment sites are biasing real-time PCR results if DNA is stored at 4°C or with glycerol. This effect is increased by primary amines such as Tris or by Mg-ions as included in the PCR buffer. By finally testing the resulting benchmark treatment for DNA-standard storage a deviation of Δ0.2 Ct-values was obtained by real-time PCR after 100 days storage in H_2O containing 50% glycerol.

Significance: Preservation of DNA-standards in 50% glycerol in ddH_2O enables long-term storage for real-time PCR. Depurination and shearing of the DNA are avoided, thus providing reliable results using the Ct-method for quantification.

P1-125 *Micrococcus roseus* and *Serratia marcescens* as Colored Bacterial Indicators: A Simple Strategy during Design and Development of a New Method for Sample Pre-treatment

PETER ROSSMANITH, Karin Fruewirth, Beate Suess, Erich Schopf and Martin Wagner
University of Veterinary Medicine Vienna, Christian Doppler Laboratory for Molecular Food Analytics, Vienna, Austria

Introduction: In the present study, chromogenic (red) bacteria were used to simulate actual target bacteria during setup and optimization of an isolation process of bacteria, designed for food samples.

Purpose: Isolation of bacteria from food in the context of molecular biological detection of food pathogens is a multistep process. Development of such a separation method requires continuous monitoring of the location of the presumable targets in the sample tubes. Therefore, red colored pigmented bacteria were used as substitutes for the actual target bacteria, during the establishment of a new sample preparation technique.

Methods: Visibility of the pigmented bacteria within the complex sample matrices served to allocate bacterial content during the various steps necessary for finalization of the method protocol. Prior to application, the chromogenic bacteria *Micrococcus roseus* and *Serratia marcescens* were confirmed to withstand the physical (e.g., centrifugal forces) and chemical (e.g., lysis buffer composition) conditions required during establishment of the new technique.

Results: The suitability of these model bacteria to substitute for the actual target pathogens (*Salmonella enterica* subsp. *enterica serovar* Typhimurium and *Listeria monocytogenes*) was assured by testing the physical properties of the model bacteria with respect to the proposed separation methods.

Significance: The use of these pigmented bacteria as substitutes for actual colorless target bacteria during design and development of a bacterial isolation method is a simple and inexpensive application. The presumptive bacterial targets can be allocated simply by visualization of their bright red color silhouetted against the background sample matrix. Application of colored bacterial indicators saves a huge amount of time and resources, as the proof of principle of new methods is possible in rapid succession.

P1-126 Statistical Data Analysis of Real-time PCR Results Derived from Single Copy Amplification

PETER ROSSMANITH and Martin Wagner
University of Veterinary Medicine Vienna, Christian Doppler Laboratory for Molecular Food Analytics, Vienna, Austria

Introduction: The validation of real-time PCR systems and above all the proof of the detection limit of this method is a frequently and intensively discussed topic. We present a statistical method for the accurate determination of DNA amounts < 10 target molecules using real-time PCR. The implication of this method is the possibility of distinct validation of real-time PCR assays and the generation of absolute DNA standards needed for quantification with this enzymatic method in routine diagnostics.

Purpose: The purpose of this study was to evaluate a novel validation tool for real-time PCR assays based on the theoretical possibility of the amplification of one single DNA target. The ability to detect such low DNA target concentrations reliable by real-time PCR should be clearly demonstrated. Consequently, a validation method based on this pre-requisites should be established which allows the absolute evaluation of real-time PCR assays.

Methods: Real-time PCR was carried out by targeting a 274 bp fragment of the *prfA* gene of *L. monocytogenes*. Fit of the empirical data to the theoretical predictions was tested using the Kolomogorow - Smirnov (K-S) test using the SPSS 14.0 statistical software package.

Results: The ability of the *prfA* real-time PCR assay to detect reliable one target molecule could be clearly demonstrated ($p_{avg.}$=0.52). The coherence of the results of samples containing < 10 target molecules and samples containing DNA amounts within the range of fluorescent measurement could be clearly demonstrated. The evidence for the accuracy of the newly developed validation-method was shown both statistically and with direct demonstration. The explicit determination of assays with a detection limit of one copy and assays with such a limit of three copies is exemplary demonstrated. We also demonstrate that real-time PCR at best starts from the first cycle with certain efficiency and proceeds with this efficiency until saturation of the reaction.

Significance: The results show that an absolute validation of real-time PCR assays is possible. The Ct - values of certain initial target amounts are fixed in dependence of the efficiency of the reaction. An absolute determination of DNA amounts is possible independent of conventional measurement methods. The validation tool also allows on-line monitoring of real-time PCR results in routine diagnosis.

P1-127 A Quantitative and Highly Sensitive Method for Detection of Ricin in Complex Food Matrices

XIAOHUA HE and David Brandon
USDA-ARS-WRRC, Albany, CA, USA

Introduction: The potential use of ricin as a bioweapon in food highlights the necessity for developing food-specific detection methods.

Purpose: The purpose of this study is to develop a sensitive assay for ricin in foods.

Methods: An immuno-PCR (IPCR) assay for the detection of ricin in three economically important food matrices is described.

Results: The direct adsorption of ricin onto the wells of a microtitration plate was compared to indirect immobilization via a capture antibody (sandwich IPCR). The latter procedure provided much greater sensitivity. We also compared a protocol with the immunoassay and PCR conducted in a single plate to a two-step procedure in which the PCR was conducted in a second plate, following release and transfer of the DNA marker. The two-step procedure proved 1000-fold more sensitive for ricin detection, so this format was used to detect ricin in spiked samples of ground beef, chicken egg, and milk, and the results were compared to those obtained from ELISA. The limit of detection (LOD) for ricin reaches as low as 10 fg/mL in PBS buffer, 10 pg/mL in chicken egg and milk samples, and 100 pg/mL in ground beef extracts. Comparable ELISA results were in the 1 to 10 ng/mL range. Further optimization of the sandwich IPCR was performed by comparing various antibody combinations. Among the four formats investigated, the pAb/pAb combination yielded the lowest LOD.

Significance: The IPCR developed in this study is useful in countering intentional biothreats. It provides regulatory agencies and the food processing industry with analytical tool for enhanced food security.

P1-128 Validation of Fluorescence Polarization Immunoassay for Ochratoxin A

DONG MIN KIM, Hyang Sook Chun, Sung-Wook Choi, Sergei A. Eremin, Eun Hye Choi and Hyun-Joo Chang
Korea Food Research Institute, Sungnam, Republic of Korea

Introduction: A fluorescence polarization immunoassay (FPIA) is the immunoassay using a fluorescent labeled antigen as tracer in competitive reaction to detect analytes. This method is a very short period to produce test results, user-friendly format and relatively inexpensive to make. Ochratoxin A (OTA), a mycotoxin produced by *Aspergillus* and *Penicillium* species, can remain in food and feed for a long time in spite of processing procedures and induce health problems for human and animal that intake OTA contaminated food.

Purpose: The purpose of this study was to validate FPIA established previously using a new tracer for detection of OTA in food, and to make it a rapid and applicable technique for a screening test in the field.

Methods: Newly synthesized 4′-(aminomethyl) fluorescein (4-AMF) OTA as a tracer and OTA monoclonal antibody were used. After the extraction condition to minimize matrix effect was determined with different solvent, validation studies to confirm sensitivity, accuracy, precision, trueness and specificity of FPIA for detection of OTA were carried out. For this study, OTA free sample and several concentrations of OTA spiking sample were used and all experiments were done in triplicate.

Results: When optimizing analytical condition considering salt concentration, pH and solvent, dynamic range of this assay was 0.3 - 3000 ng/mL. The FPIA for OTA exhibited comparable and acceptable recoveries from intra- and inter-day assays. Cross reactivity test showed that the monoclonal antibody had great activity for OTA than for Ochratoxin B, but no activity for L-pheylalanine and coumarin even at the highest concentration (3000 ng/mL). In the natural OTA contaminated sample, this assay was well correlated with HPLC and ELISA.

Significance: These results indicate that established FPIA could be useful to detect OTA in food and/or feed rapidly and accurately.

P1-129 Detection of Mustard Residue Contamination in Food Commodities by an Immunoassay-based Test

MOHAMED M. ABOUZIED, Michael E. Sarznski and Stephen L. Taylor
Neogen Corporation, Research and Development, Lansing, MI, USA

Introduction: Allergic reactions to mustard, including severe anaphylaxis, have been reported. Mustard is one of the listed allergens in EU labeling directives and the food industry is required to test for its presence in final products.

Purpose: Develop an immunoassay-based test that can be used in the food industry to monitor mustard contamination in incoming ingredients and in the finished product.

Methods: A 30-min Mustard Allergen Test for quantitative analysis of mustard contamination in finished food products and incoming raw ingredients was developed. The test is a Sandwich Enzyme-Linked Immunosorbent assay (S-ELISA). Polyclonal antibodies against specific mustard protein markers were used as the capture and detector antibodies. For quantitative analyses, a standard curve of mustard flour ranging from 0 to 25 PPM (0 to 1 µg/ml) was used. Samples were extracted by shaking 5 g of ground samples with 125 ml of extraction buffer (Tris/EDTA) in a hot water bath. Extracts were filtered and filtrates were used directly for ELISA analyses. Extracts were added to antibody-coated wells in which mustard proteins bind to the capture antibody during a 10-min incubation period. Any unbound protein was washed away and the anti-mustard horseradish peroxidase-labeled antibody (detector antibody) was added. The detector antibody bound to the mustard protein during a 10-min incubation period. Unbound enzyme-labeled antibody was washed away and a substrate was added. Color developed as a result of the presence of bound-labeled antibody during a final 10-min incubation period. Absorbance readings of samples were compared with those of the standards and the concentrations in parts per million (PPM) were calculated.

Results: Mean recovery of mustard from various spiked samples was found to be 88%. Limit of detection was found to be less than 2.5 PPM (0.1 µg/ml) determined as mustard flour. The test is capable of detecting residual contamination of mustard in ingredients, finished products, and rinses and on environmental surfaces. No cross-reactivity was observed with any other spices, plant, or animal proteins.

Significance: The test can be used to detect inadvertent mustard contamination in ingredients, finished products such as flours, tomato products, spices, salad dressing, meat products, and bakery products.

P1-130 Establishment of an HPLC Method for the Detection of Malachite Green and Leucomalachite Green in Aquaculture Food Products of Animal Origin

Kyeongyeol Kim, QING LIU, Neema Kassim, Jeong-Eon J. Song, Won-Bo W. Shim and Duck-Hwa D. Chung
Gyeongsang National University, Division of Applied Life Science, Jinju, Republic of Korea

Introduction: Malachite green (MG), a triphenylmethane dye used as fungicide in fish farming industry, has been reported to be toxic and carcinogenic to humans. MG is mainly metabolized into leucomalachite green (LMG) in fish and LMG also has carcinogenic activity in human and animals.

Purpose: This study established a detection method for MG and LMG in fish by high-performance liquid chromatography (HPLC) equipped with photodiode array detector.

Methods: Samples such as flatfish, armorclad rockfish, flathead mullet and konosirus punctatus were extracted with ammonium acetate solution followed by acetonitrile and isolated by partitioning into dichloromethane which was then evaporated to dryness. The redissolved residues were cleaned up through alumina and propylsulfonic acid phase prior to analysis by HPLC system (mobile phase: 5 mM ammonium acetate : acetonitrile = 4:6, wavelength: 618 nm). For validation, specificity, accuracy, precision, detection and quantification limits (LOD, LOQ) were determined from samples spiked with each of MG and LMG at 5, 10 and 20 ng/g.

Results: As a result, linearity (R2) for MG and LMG (1 – 200 ng/g) were 0.999 and 0.999, respectively. Precision (relative standard deviation) and accuracy (recovery) were in acceptable range of 0.15–1.17% and 73.63–87.07% for MG, and 0.08–1.76% and 73.50–91.48% for LMG. LOD and LOQ were 2 and 3 ng/g respectively, for both MG and LMG.

Significance: These results indicated that the established method was satisfactory to analysis MG and LMG in aquaculture food products of animal origin.

P1-131 Use of Flow Cytometry to Determine Minimum Inhibitory Concentration of Thymol

JULIE MCKINNEY, Renee R. Boyer and Joseph Marcy
Virginia Tech, Food Science and Technology, Blacksburg, VA, USA

Introduction: Conventional methods for determining the antimicrobial effectiveness of a substance can be time consuming. Methods that rely on visual interpretation have a high margin of error due to the subjective nature of visual observations.

Purpose: In this study, a flow cytometry (FC) method was evaluated to determine if it was more sensitive than visual turbidity evaluation to determine the Minimum Inhibiroty Concentration of Tymol of an antimicrobial. To assess this, thymol was screened for its ability to inhibit the growth of *Saccharomyces cerevisiae* in a 10% apple juice medium for up to 8 weeks.

Methods: S. cerevisiae was tagged with SYTO® BC dye for visualization of the yeast in the flow cytometer. Fifty microliters of each thymol concentration to be tested was added to AbGene micro-titer test tubes in triplicate, followed by 950 µl of AJM and 4 µl of yeast inoculum. The final yeast concentration in each tube was approximately 2 log CFU/ml. Tubes were incubated at 30°C. Prior to being analyzed by FC, tubes were visually inspected for turbidity at days 0, 2, and weeks 1, 2, 4, 6, and 8.

Results: The MIC for thymol on day 56 was 1,000 ppm using both procedures. There was no significant difference in MIC's determined at each inspection time between the two methods.

Significance: Results obtained by FC and visual observation provided similar MIC values. However, FC was able to provide information beyond growth or no growth. By observing the concentration of yeast at each sampling period, growth can more precisely be equated to inhibition by a particular concentration when compared to the growth at other concentrations. FC may also allow detection of initial yeast growth earlier than visual inspection, as long as appropriate observation times are included.

P1-132 Development of Immunochromatography for Rapid Detection of 6-Chloronicotinic Acid

Kyeongyeol Kim, SHIFEI ZHAO, Neema Kassim, Won-Bo W. Shim and Duck-Hwa D. Chung
Gyeongsang National University, Division of Applied Life Science, Jinju, Republic of Korea

Introduction: 6-Chloronicotinic acid (6-CNA) is an intermediate of agrochemicals. It can contaminate drinking water and agricultural products presenting an important risk to human health.

Purpose: The aims of this study were to develop a rapid immunochromatography (ICG) assay for the detection of 6-CNA in food and environmental samples, and to confirm the results of the ICG by high performance liquid chromatography (HPLC).

Methods: The immunochromatography method based on a polyclonal antibody was developed and optimized in this study. For validation of the ICG, soil and apple samples spiked with 6-CNA at 3, 5, 7, 10 µg/g were extracted with PBST, and water samples spiked with 10, 20, 60 µg/g of 6-CNA was extracted with PBST. All extracts were applied to ICG strip. Additionally, the spiked samples were analyzed by HPLC and the results were compared with those of the ICG.

Results: The visual detection limit of the ICG for the 6-CNA was 5 µg/mL, and the results were obtained within 15 min after sample loading. This method showed a cross-reaction to 5-amino-2-chloropyridine at 10 µg/mL, but no cross-reaction to other related compounds (Atrazine, 2,4-Dichloro, 2,4,5-T, 2,4-methyl, 4-(2,4-Dichloro), 3,4-D, 2-(2,4-Dichloro)) was observed. The results of soil, water and apple samples spiked with 6-CNA by ICG and HPLC were in good agreement.

Significance: The ICG test-strip was sufficiently sensitive, simple, accurate and convenient for rapid screening of 6-CNA in agricultural and environmental samples.

P1-133 Utility of Microsatellite DNA Fingerprinting Analysis for Source Tracking in Beef Trim and Ground Beef Products

SONGHAI SHEN and Mansour Samadpour
IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA

Introduction: Beef trim and ground beef products are usually prepared from a mix or combination of meat pieces coming from several individual carcasses. These individual carcasses or animals may be differentiated using microsatellite DNA fingerprinting analysis.

Purpose: The purpose of this study was to assess the extent of meat combinations in ground beef patties and beef trim using microsatellite DNA fingerprinting.

Methods: Five sets of beef trim excision samples collected using the IEH N60 sampler for *E. coli* O157 analysis, and ground beef patties produced by five different meat packers were analyzed. DNA was extracted from several sub-samples coming from each set and tested using microsatellite DNA fingerprinting procedure.

Results: Of the sub-samples obtained from the 5 beef trim samples, at least 27 DNA genotypic patterns were observed. In addition, a few sub-samples from each sample of the 5 beef trim samples showed common genotypic patterns, e.g., sub-samples from sample #1 and #3 shared a common pattern, sub-samples from sample #2 and #4 shared the same pattern, and #2, #4 and # 5 exhibited a common genotypic pattern. For ground beef samples, meat from all five packages exhibited 8-11 different genotypic patterns. No common pattern was observed among the ground beef samples from the five different meat packers, although a few sub-samples within the each of the 5 samples showed a common pattern.

Significance: Results from this study indicated that the beef trim excision samples and the ground beef products contained meat from several individual animal carcasses. This poses a higher risk of cross-contamination, hence, a more robust sampling procedure needs to be in place to ensure true sample representation for pathogen screening.

P1-134 Differentiation of Tuna and Bonito Species in Frozen and Canned Products by PCR Method

Zora Hubalkova and EVA RENCOVA
Veterinary Research Institute, Toxicology and Pharmacology, Brno, Czech Republic

Introduction: Tuna fish belong among the most economically important fishery resources because they are typically used to manufacture canned products, the main format for marketing of these species. The Council Regulation (EEC) No. 1536/92 laid down common marketing standards for preserved tuna and bonito to improve the profitability of tuna production in the Community in order to keep products of unsatisfactory commercial quality off the market. The legislation defines the standard governing-states-specific rules for the tuna marketing.

Purpose: The purpose of this study was to develop the PCR method for the differentiation of tuna (*Thunnus thynnus*, *T. albacare*, *T. alalunga*, *T. obesus*, *T. tonggol*, *T. maccoyii* and *Katsuwonus pelamis*) and *bonito* (*Sarda sarda*).

Methods: The ATPase gene based on searching in GenBank database was selected. Specific primer pair amplifying partial sequence of ATPase gene (100 bp) in tuna species was designed.

Results: The targeted 100 bp PCR products were detected only in tuna species. The amplicon was identified even in canned fish.

Significance: The developed one-step PCR method represents simple and fast control tool for the differentiation of tuna and bonito fish in fish products. Financially supported by grant MZe 00027 16202.

P1-135 Screening of Natural Antimicrobials for Inhibition of *Escherichia coli* O157:H7 in a Solidified Apple Juice Medium

TAKIYAH ABDULMALIK, Renee R. Boyer, Joshua B. Gurtler and Julie McKinney
Virginia Tech, Blacksburg, VA, USA

Introduction: Naturally occurring antimicrobials such as plant extracts and essential oils have been used in the food industry for years. Due to increased consumer demand for minimally processed juices there has been increased interest in the use of novel antimicrobial compounds isolated from natural substances.

Purpose: The purpose of this study was to evaluate ten naturally occurring compounds for their antimicrobial effect against *Escherichia coli* O157:H7 in a solidified apple juice medium.

Methods: Spiral Gradient Endpoint software was used to determine stock concentrations for all compounds. Each of the ten antimicrobial compounds was spiral plated onto separate 15 cm plates containing apple juice agar (AJA) to create a concentration gradient on the plate (AJA = plate count agar supplemented with 10% of Motts apple juice, glucose, 4.68% w/v; sucrose, 0.18%; and fructose 5.94%). An 8-log CFU/ml culture of *E. coli* O157:H7 CIDER (clinical isolate from apple cider outbreak) was then vertically streaked across the gradient and evaluated after incubation (37°C for 24 h) to determine the minimum inhibitory concentration (MIC) of each compound.

Results: The compounds exhibiting the most significant inhibition of *E. coli* O157:H7 were sorbic acid (MIC = 55.91 mg/L) and cinnamic acid (MIC = 59.96 mg/L) ($P < 0.05$). Methyl paraben, ethyl paraben, butyl paraben, propyl paraben and eugenol had significantly lower antimicrobial effectiveness than sorbic acid and cinnamic acid. The least effective compound was diacetyl (781.78 mg/L), requiring an ending concentration nearly 3 times greater than any other compound.

Significance: Two of the ten compounds evaluated had significant antimicrobial activity at low levels against *E. coli* O157:H7 and have potential to be added to a juice beverage to control pathogen growth. Future studies may address the additive or synergistic inhibitory effects of these antimicrobials.

P1-136 Withdrawn

P1-137 Effect of Pulsed Electric Field on Surface Charge and Membrane Damage to *Escherichia coli* O157:H7 Cells in Apple Juice

DIKE UKUKU, Hyun-Gyun Yuk and Howard Zhang
ARS-USDA, FSIT-ERRC, Wyndmoor, PA, USA

Introduction: Bacterial cell surfaces have net negative electrostatic charge due to the presence of ionized phosphoryl and carboxylate moieties on the outer envelope exposed to the extracellular environment. It has been reported that antibiotics can reduce the negative charge while certain metals and high pH have the propensity to reverse the charge from negative to positive. Pulsed Electric Field (PEF) treatments, a non-thermal process, have been reported to injure and inactivate bacteria in liquid foods. However, the effect of this treatment on bacterial cell surface charge and hydrophobicity has not been investigated.

Purpose: The purpose of this study was to evaluate bacterial cell surface charge and hydrophobicity of *E. coli* cells before and after PEF treatment in relation to injury and death.

Methods: Apple juice (AJ, pH 3.8) purchased from a wholesale distributor was inoculated with *Escherichia coli* O157:H7 at 6.8 log CFU/ml, processed with a PEF at 32.2 kv/cm, 18.4 A with pulse width of 2.6 μs and a flow rate of 120 ml/min. Treatment condition was periodically adjusted to achieve outlet temperatures of 47.5 and 57°C. Treated samples were plated (0.1 ml) on Sorbitol MacConkey Agar (SMAC) and Trypticase Soy Agar (TSA) plates to determine injured populations and viability loss. Bacterial cell surface charge and hydrophobicity of untreated and PEF-treated *E. coli* O157:H7 was determined using hydrophobic and electrostatic interaction chromatography.

Results: Immediately after treatment at 47.5°C, the average cell populations determined on TSA plates and SMAC plates was 4.9 log and 4.7 log, respectively. At 57°C treatment, *E. coli* cell populations determined averaged 3.9 and 2.4 log on the TSA and SMAC plates, respectively. The relative *E. coli* cell surface charge and hydrophobicity before PEF treatment averaged 0.462 ± 0.112 and 32.10 ± 8.12, respectively. PEF treatment at 47.5°C and 57°C decreased the above values to 0.335 ± 0.121 and 12.85 ± 3.30 and 0.337 ± 0.114 and 6.94 ± 3.85, respectively. There was no leakage of intracellular UV-intracellular materials.

Significance: The results of this study indicate that damage of PEF treatment to *E. coli* bacteria occurs mostly at the outer cell envelope exposed to the extracellular environment as evidenced by the reduction of cell surface charge after the PEF treatment.

P1-138 Decontamination of Mycotoxin Patulin by Alkalization in Apple Cider Processing

TING ZHOU and Rong Tsao
Agriculture and Agri-Food Canada, Guelph Food Research Center, Guelph, ON, Canada

Introduction: Patulin is a mycotoxin produced primarily by *Penicillium expansum*, a fungus causing blue mould rot in apples and other fruits. Contamination of patulin is one of the most important food safety concerns for apple cider/juice. Since pasteurization will not remove patulin because it is heat stable, the industry needs innovative approaches to reduce patulin in products.

Purpose: The purpose was to test the effect of various alkalines on the degradation of patulin in rotten apples and to determine their potential application in apple cider processing.

Methods: Patulin contaminated apples, obtained by inoculation of apples with *P. expansum*, were soaked in water or water solutions of alkalines at various concentrations and incubation periods before being used to make apple ciders, which were tested for patulin levels using a HPLC method. In the pilot experiment, NaOH was added into the washing tank. Patulin levels were determined for the washing water samples and their corresponding apple cider samples, which were taken at 10 min intervals before and after the treatment.

Results: The soaking treatment with NaOH and Na_2CO_3 at the concentrations of 0.1–0.5 M for > 10 min significantly reduced the patulin levels of the resulted cider samples as compared to those soaking in only water. The treatments with NaOH at 0.25 and 0.5 M reduced the patulin to undetectable levels within 30 and 10 min, respectively. In the pilot experiment, the patulin level of the washing water reached to 1400 ppb when NaOH was added to a final concentration of 0.25 M. The NaOH rapidly lowered the patulin level of to below 50 ppb within 30 min and also significantly reduced the patulin levels in the apple cider samples.

Significance: The data indicate that alkalization can be an effective approach to reduce patulin levels in apple products.

P1-139 UV-light to Improve Toxicological Safety of Apple Products

FAN WU, Ting Zhou, Keith Warriner and Tatiana Koutchma
Ontario Agriculture College, Dept. of Food Science, Guelph, ON, Canada

Introduction: Patulin [4-hydroxy-4H-furo (3, 2-c)-pyran-2-(6H)-one], a mycotoxin produced by several species of *Aspergillus*, *Penicillium* and *Byssochlamys* is a secondary metabolite produced by a wide range of fungi including *Penicillium expansum*, which is a common contaminant of apples. Therefore, the level of patulin becomes the important toxicological safety indicator in apples and apple-based products such as fresh apple cider.

Based on the research that UV light is capable of achieving a 5-log or greater reduction of human pathogens in apple juice exposed to a dose level of 14.32 mJ/cm^2, I hypothesized that UV light will be effect to patulin degradation since its peak absorbance is 276 nm, which is close to the germicidal UV wavelength of 254 nm.

Purpose: In order to degrade the patulin and decontaminate the toxin-producing fungi and foodborne pathogen effectively, treatment conditions should be optimized with considering of UV intensity. The objective was to evaluate effects of UV light on patulin reduction in model buffer using collimated beam UV apparatus, and quantify effects of pH, absorbance and vitamin C on degradation kinetics of patulin.

Methods: Clear model cider buffers (simulated buffer) with patulin concentration of 1000 ppb, 500 ppb, and 100 ppb were exposed to UV light from 10 to 120 min. The samples were placed at the end of the collimator tube at UV fluent rate of 280 mW/cm^2. The concentration of patulin before and after UV treatment was measured using High Pressure Liquid Chromatography (HPLC), which is assembly consisted of a pump (Model W600), an auto sampler (Model 717P) and a PDA detector (Model 1998). A Primesep-D (150 × 4.6 mm) reverse phase column was used for patulin detection with a mobile phase consisting of 95% water (containing 1% formic acid) and 5% acetonitrile.

Results: It was found that patulin in clear apple buffer was reduced 70%, 60%, 55% in 1000 ppb, 500 ppb, 100 ppb, respectively after 120 min with 280 mW/cm^2, that is the dose of level is 223 mJ/cm^2.

Significance: This method proved that the degradation is independent of incident intensity (at same dose level) and initial concentration of patulin. The study showed that the patulin degradation is heavily influenced by ascorbic acid, suspended particles owing to their preferential absorption and scattering of UV light. It is recommended an approach to reduce the risk of patulin in apple cider buffer, which also helps the manufactures build new system of keeping the products more safe.

P1-140 Comparison of Dehydratable Film and Various Conventional Methods for Evaluation of Drinking Water

CRISTINA F. ABREU, Adriana R. Tassinari, Ana Claudia N. Barboza, Heloisa C. Ruppert, DeAnn L. Benesh, Romeu C. Neto, Neusely Silva, Edson A. Nour and Jose L. Pereira
University of Campinas, Microbiology, Campinas, Brazil

Introduction: The use of 3M™ Petrifilm™ Plates is not listed as an US Environmental Protection Agency [EPA] recommended methodology for drinking water assessment and is not published in the Standard Methods for the Examination of Water and Wastewater.

Purpose: The aim of the study was to compare 3M Petrifilm Aerobic Count [AC] Plate and 3M Petrifilm Coliform and *E. coli* Count [EC] with Plate Count Agar [PCA] M-Endo Agar Less [Endo], respectively, following EPA 821-B-03-004 procedures.

Methods: Samples were obtained from two rivers that are suppliers of drinking water for São Paulo state/Brazil (prior to supplier station), two wells and two fountains. River water was used to spike drinking water, after chlorination stress [0.1 mg/L – 5 minutes]. Wells and fountains were naturally contaminated. Physical-chemical parameters were evaluated. Samples were divided into two aliquots for testing by both methods. For coliform analysis, each aliquot was divided in 10 samples of 100 mL and filtered with 0.45 um membrane. Membranes were transferred to Endo and EC (35°C for 24 h and 48 h, respectively). From each of the 10 replicates per method, 2 plates were randomly chosen and 10 colonies of each were picked and inoculated in PCA and 320 colonies were confirmed with Enteric/Nonfermenter Kit. For heterotrophic counts, 10 replicates was made, 1 mL of each sample, was plated onto AC and PCA (35°C for 48 h).

Results: For coliform analysis, samples were analyzed by matrix. 50 results from spiked drinking water were transformed to log base. Recovery: Median was EC = 1.9085 and Endo = 1.8603. The result from Mann-Whitney test W = 2786.5 shows we can not reject the equality between methods since W is > 2525.0. Petrifilm shows different variability with *P*-value = 0.014, with lower SD to EC = 0.1585 than Endo SD = 0,2908. For the untreated water matrix, 40 coliform results were evaluated and EC results were statistically different and lower than the reference method. For heterotrophic counts, matrices were analyzed together and 90 results were obtained. The recovery is not significantly different for 3M since W = 8455.5 greater than > 8145.0 with medians for Petrifilm AC = 2.0294 and PCA = 2.0212. With a p-value of 0.253, Petrifilm AC results were not statistically different for the PCA results.

Significance: The statistical analysis indicates a strong correlation between traditional method and 3M Petrifilm Plates results. The recommendation would be additional study to confirm the claim of 3M Petrifilm Plates for drinking water testing. Acknowledgment: Bruno Constantino, Enelton Fagnani, Fernando Pena Candello Ligia Maria Domingues, Norma Miya and Interlab S.A.

P1-141 Characterization of Low-pH Tolerant *Bacillus* Species and Their Behaviors in Bottled Vegetable/Fruit Juice

SHINYA MIYAMOTO, Ryuji Yamamoto, Yoshihiro Ido, Etsuko Shibutani and Hisato Ikemoto
SUNTORY BEVERAGE and FOOD LIMITED, Beverage Development and Design Dept., Kawasaki, Japan

Introduction: Bacillus subtilis and *B. coagulans* have been recognized as aerobic spore-forming bacterial species which grow well under pH conditions below 4.5. However, there have been a series of new description on *Bacillus* species from acidic environments since 2000. As they appeared growing well at pH below 4.0, their spoilage risks were strongly suggested in pasteurized vegetable and fruit juice products.

Purpose: In this study, effects of acidification and oxygen availability were examined on the germination and growth of *B .acidicola* (described in 2005), *B. ginsengihumi* (described in 2008) and *B. shackletonii* (described in 2004) to recognize their impact on the quality of juice products.

Methods: Type strains were obtained from culture collections, and wild strains were further isolated from juice and soil samples as well. Their phylogenetic analysis was carried out based on 16SrRNA gene sequences to show their close positionings.

Results: All type strains and wild isolates were mesophiles and shown to grow at acidic pH as low as 3.2. *B. shackletonii* exhibited the highest heat resistance (D(95°C) = 48 min),which exceeded *Alicyclobacillus acidoterrestris*, but not *B. amyloliquefaciens*. The optimum pH

for germination was found to be 5.0—5.5. *B. acidicola* was the most acidotorelant, but the rate of germination reduced to 10% at pH 4.0 and less than 0.1% at pH 3.5. Some of the strains appered strictly aerobic. When *B.acidicola* was challenged in vegetable/fruit juice (pH 4.1) at the initial population of 10—100 CFU/ml, it exceeded 100,000 CFU/ml in a test tube with a sufficient head space volume while it did not reach 1,000 CFU/ml in a hot-filled PET bottle.

Significance: This study indicated the existence of a low-pH tolerant *Bacillus* species described only lately. They grow at acidic pH conditions as low as 3.2, suggesting their potential spoilage risks in vegetable and fruit juice products distributed under ambient conditions. It seems that the limitation of oxygen availability in the package works effectively against their growth in current commercial beverages; however, we would need a close observation on newly described sporeformers.

P1-142 *Salmonella* spp. Survival on Lemon Slices and in Transfer to Beverages

RACHEL MCEGAN and Michelle D. Danyluk
University of Florida, CREC, Lake Alfred, FL, USA

Introduction: Lemon slices are commonly added to beverages in the food service industry. Little is known about microbial risks associated with this practice.

Purpose: The objectives of this study were to determine survival of *Salmonella* on lemon slices stored on ice or at room temperature (RT) and transfer of *Salmonella* from lemon slices into beverages.

Methods: A five-strain cocktail of *Salmonella* (5 log CFU/slice) was spot-inoculated onto the flesh, peel or albedo of sliced lemons and held on ice or at RT for up to 24 h. *Salmonella* were enumerated by plating on selective agar during the 24 h of storage. To determine transfer from lemon slices to beverages, inoculated lemon slices (peel or albedo) were dried for 1 h then added to 575 ml water or iced tea (at 4°C) and stirred; a 5-tube MPN was immediately performed.

Results: Salmonella inoculated onto peel and albedo decreased 2 log CFU/slice in 24 h when stored on ice. When inoculated onto flesh, *Salmonella* decreased 1 log at time 0 h, fell below the limit of detection (1.9 log CFU/slice) from 1 to 8 h, and were again detectable at 24 h. When stored at RT *Salmonella* inoculated onto the peel and albedo decreased by 0.1 and 1.2 log CFU/slice, respectively, over 24 h. *Salmonella* on the flesh of lemons at RT decreased to below the limit of detection in 2 h, remaining undetectable through the remainder of the experiment. The addition of a peel-inoculated lemon slice resulted in 10.2 and 8.933 MPN/ml for water and iced tea, respectively. The addition of an albedo inoculated lemon slice resulted in 0.673 and 0.246 MPN/ml of water and iced tea, respectively.

Significance: The demonstrated ability of *Salmonella* to survive on lemon slices, and transfer into beverages, suggests a potential food safety risk associated with improperly handled sliced lemons.

P1-143 Effect of Milk Fat Content on the Efficacy of Pulsed Electric Fields for the Inactivation of Native Bacteria in Milk

OSCAR RODRIGUEZ GONZALEZ, Markus Walkling-Ribeiro, Shesha H. Jayaram and Mansel W. Griffiths
University of Guelph, Canadian Research Institute for Food Safety, Guelph, ON, Canada

Introduction: Milk is a complex food system in which microorganisms can grow easily and inactivation of the latter is a challenge. Several major microbial groups can be differentiated by culture methods including coliforms that indicate unsanitary conditions, and psychrotrophs that grow at refrigeration temperature.

Purpose: This study was conducted to evaluate the effect of the fat content on the inactivation of natural microflora using pulsed electric fields (PEF), to compare its effectiveness with thermal pasteurization, and to provide solutions for incorporating PEF in milk processing.

Methods: Prior to inactivation treatments, raw milk was incubated with 1/10 of milk incubated at 18°C for 24 h, then separated, standardized to four fat levels (1% to 4%), and homogenized; the skim milk was also micro-filtered. Three combinations of voltage, frequency, and residence time were applied, obtaining energy inputs of 832, 1478, and 1663 kJ/L with outlet temperatures below 60°C.
Total mesophiles, coliforms, and psychrotrophs were analyzed following standard methods.

Results: No differences were found in the reduction of coliforms in PEF treated milk between the four fat or energy levels ($P \geq 0.05$), but within energy levels at 3.8% fat. The reduction at 1663 kJ/L ranged from 2.7 to >3.7 log CFU/mL. For psychrotrophs PEF applied at 832 and 1478 kJ/L caused a lower reduction ($P < 0.05$) in 3.8% fat-milk (2.3, and 2.1 log CFU/mL) compared with milk of 1.0% fat content (>3.9 log CFU/mL). The combination of microfiltration with PEF resulted in >3.7 log CFU/mL reduction for all the microbial groups analyzed after PEF treatment using 832 kJ/L.

Significance: The results of this study suggest that the inactivation trends, in relation to milk processing and PEF parameters, depend on the microbial population analyzed. The present findings could aid in identifying factors which have to be considered for the incorporation of a PEF system in a milk processing line.

P1-144 Effect of Pulsed Electric Field on Microbial Quality and Protein Profiles of Milk and Milk Concentrates

SIEH NG, Heather Craven, Jason Wan, Kerryn King, Roderick Williams and Piotr Swiergon
CSIRO Food and Nutritional Sciences, Innovative Food Processing, Werribee, VIC, Australia

Introduction: Pulsed Electric Field (PEF) is a potential alternative to traditional thermal processing for microbial inactivation at reduced heat requirements, with minimum adverse impact on functional properties of foods.

Purpose: To evaluate the advantages of PEF in conjunction with mild heat for the inactivation of milk microflora, extension of shelf life and preservation of milk proteins.

Methods: Raw milk and milk concentrates (18% total solids) were pasteurized (72°C 15 s) or treated by PEF at 36 to 49 kV/cm for 2 to 20 µsec with outlet temperatures of 40 to 72°C (depending on the application), using a laboratory scale system. Microbial populations were monitored by viable plate count before and after processing and during storage at 4°C. Bacteria were identified with Microgen *Bacillus* ID. Size exclusion chromatography was used to characterize the major whey proteins and the size distribution of micelles.

Results: For raw milk and milk protein concentrates, PEF reduced the total and thermoduric count by 2 and 0.5 log respectively. The total count of raw milk was reduced significantly more by PEF than by pasteurization ($P < 0.05$). During the six weeks of storage at 4°C, no microbial growth was detected in the PEF treated milk, compared with an increase in count of 6 log in the pasteurized milk due to growth of *Bacillus brevis*. The whey proteins and the casein micelle size appeared to be unaffected by PEF treatment under the conditions used.

Significance: These data suggest that PEF combined with mild thermal treatment can provide additional quality attributes for milk that thermal pasteurization alone cannot achieve.

P1-145 Ultraviolet Light Inactivation of Microorganisms in Soy Milk Using Dean Flow Technology

SRINIVASARAO BANDLA, Ruplal Choudhary and John Haddock
Southern Illinois University-Carbondale, Plant, Soil and Agricultural Systems, Carbondale, IL, USA

Introduction: Nonthermal methods are promising technologies for processing of different foods to meet the consumer demands of safety, taste, healthy, organic and freshness. These technologies have advantages over traditional thermal preservation methods such as ability to minimize the loss of flavors and nutrients and improve energy efficiency. Ultraviolet (UV-C) light treatment has been established as a nonthermal germicidal process for water purification and fresh juices with laminar, turbulent and dean flow patterns. UV-C treatment at 254 nm can be used for the disinfection of microorganisms in liquid food and retain quality without affecting functional, structural and sensory characteristics.

Purpose: The objective of the study is to design a novel UV reactor for microbial disinfection of soymilk and evaluate its efficacy in reduction of *Escherichia coli* W1485.

Methods: The designed reactor consists of a perfluoroalkoxy (PFA) tubing (1/16" ID and 1/8" OD) wrapped in the form of a coil around the UV ballast, having UV intensity of 347 mW/cm^2. The coiled tube generates secondary turbulence in fluid, called dean flow. UV light efficacy was tested at different flow rates of soy milk: 25, 50 and 75 ml/min. *Escherichia coli* W 1485 was inoculated at 1.37 x 10^7 CFU/ml of soy milk. After treatment, the number of surviving cells was determined using the plate count technique.

Results: The designed dean flow UV reactor inactivated *Escherichia coli* W 1485 to non-detectable levels at 25, 50 and 75 ml/min, which corresponds to residence times of 34.1, 17.1 and 11.4 s and UV dose of 11,840, 5,920 and 3,945 mJ/cm^2.

Significance: Dean flow UV reactor effectively reduced the microorganisms to non-detectable levels. A scale-up study of the dean flow UV reactor for soy milk will help in successful transmittal of this technology to the soy milk industry.

P1-146 Development and Evaluation of a Novel Real-time PCR with Internal Amplification Control for the Detection of *Listeria monocytogenes* in Milk

NISHANT KUMAR and Yi Chen
Food and Drug Administration, Center for Food Safety and Applied Nutrition, Division of Microbiology, Microbial Methods Development Branch, College Park, MD, USA

Introduction: Listeria monocytogenes is a gram-positive intracellular foodborne pathogen that can cause the sometimes fatal disease listeriosis among high-risk populations such as pregnant women and immunocompromised individuals.

Purpose: The purpose of this study was to develop, optimize, and evaluate a TaqMan real-time PCR scheme for the detection of *L. monocytogenes* in foods.

Methods: Two sets of primers with corresponding TaqMan probes were designed to target *L. monocytogenes* and an internal control. The target of the primer pair was invasion-associated protein (iap). An artificially synthesized internal amplification control was simultaneously co-amplified for each PCR reaction. Standard curves were generated by plotting the threshold cycle value against the bacterial number (log CFU per mL) via linear regression. The PCR efficiency and detection limits were subsequently calculated. The developed PCR was then applied to test artificially contaminated whole milk. Variable sample enrichment time and different DNA extraction methodologies were further evaluated.

Results: The assay was both specific and sensitive as it amplified all 55 *L. monocytogenes* strains and did not amplify any of the 32 non-*L. monocytogenes* strains. PCR efficiency ranged from 85 to 101%, depending on the serotype of *L. monocytogenes* tested. The detection limit was 30 copies of DNA per reaction in pure culture. Without enrichment of milk inoculation, the detection limits were: 10^6 CFU/ml with PrepMan® Ultra; 10^5 CFU/ml with Roche Applied Science High Pure PCR Template Preparation Kit, 10^5 CFU/ml with Qiagen DNeasy Blood and Tissue Kit and 10^3 CFU/ml with Promega Wizard® Genomic DNA Purification Kit. With 24-h enrichment of milk, all kits achieved a detection limit of 1 CFU/ml with the developed PCR scheme.

Significance: The TaqMan real-time PCR scheme developed in this study proved to be an effective method for the rapid and reliable detection of *L. monocytogenes* in whole milk and potentially other food matrices.

P1-147 Impact of Disinfectants and Surface Materials on *Listeria monocytogenes* in Mixed Species Biofilms

Geoff Knight, Cathy McAuley and HEATHER CRAVEN
CSIRO Food and Nutritional Sciences, Werribee, VIC, Australia

Introduction: The presence of *Listeria monocytogenes* within processing environments is a significant risk to the safety of dairy products and must be effectively managed to prevent product contamination.

Purpose: To determine the efficacy of various disinfectants and surfaces for the control of *L. monocytogenes* in biofilms using a model system that simulates fouling and cleaning events of a dairy factory.

Methods: Mixed species biofilms of pseudomonads, coliforms, *Kocuria* and *L. monocytogenes* (previously isolated from dairy factory environments) were established on factory floor tiles (Group 1A, slip resistance R12/B) and subjected to a fouling and cleaning (FC) cycle over a 24 h period. During the cleaning step, biofilms were exposed to a chlorinated alkaline cleaning product for 5 min and rinsed with water. Disinfectants including acid anionic (AA), hypochlorite (H), peroxyacetic acid (POAA) and quaternary ammonium compound (QAC) products were applied after cleaning at recommended concentrations for 10 min. Survival of *L. monocytogenes* in mixed species biofilms on a range of surface materials (wall tiles, polymer surface coatings and stainless steel) was also evaluated after one FC cycle. Test surfaces were swabbed using Whirlpak sponges and total and *Listeria* counts determined using plate count agar and a thin agar layer (TALOX) technique respectively.

Results: The POAA- and QAC-based products were the most effective for inactivating *L. monocytogenes* within a mixed species biofilm, although POAA was more effective than QAC in the inactivation of the other bacteria present. After one FC cycle, significantly ($P < 0.05$) higher levels of *L. monocytogenes* ($\log_{10}$ CFU cm^{-2}) were found on factory floor tiles (4.5) compared to an epoxy-based coating (3.0), stainless steel (2.2), glazed tiles (1.1) and a polyurethane-based coating (0.1). Cleaning without disinfection was relatively ineffective for the removal of *L. monocytogenes*.

Significance: Choice of surface materials and disinfectants has a significant impact on the survival of *L. monocytogenes* in mixed species biofilms. Thorough and regular application of POAA or QAC following cleaning will improve control of *L. monocytogenes* in dairy factory environments.

P1-148 Inhibitory Effects of Exopolysaccharide Produced by *Lactobacillus fermentum* E432L on the Attachment and Invasion of *Listeria monocytogenes* on HT-29 Cell

HYUN SUN YUN, Jin Lee, Seung Hye Choi, Sejong Oh and Sae Hun Kim
Korea University, Division of Food Bioscience and Technology, Seoul, Republic of Korea

Introduction: *Listeria monocytogenes* is a facultative intracellular foodborne pathogen that causes commonly called listeriosis with a mortality rate of up to 30%. Recently, many studies reported that adhesion of *L. monocytogenes* and entry into epithelial cells of the small intestine involve several bacterial cell surface proteins. One of these proteins, internalin A, promotes entry into human cells by interacting with the host receptor E-cadherin. This study suggests that EPS was shown to mediate expression of specific proteins related to biofilms on the biotic surfaces, such as HT-29 cell.

Purpose: The EPS in this study was examined with regard to their ability to inhibit the attachment and invasion of *L. monocytogenes* Scott A to HT-29 cell.

Methods: Biofilm formation by *L. monocytogenes* Scott A strains was assessed on biotic surfaces using HT-29 cell. Then, the mRNA levels of virulence-associated genes of *L. monocytogenes*, such as iap, inlA, inlB, actA, plcA, plcB, and hlyA were observed in the presence of 1.0 mg/ml EPS by semi-quantitative RT-PCR analysis. Related to this, *Caenorhabditis elegans* killing assays were performed as initially described by Tenor et al. (2004) with modification.

Results: EPS caused about log 1 reduction of adhesion of *L. monocytogenes* Scott A on the HT-29 cell at the concentration of 10^6 CFU/ml (MOI 1:1). However, the inhibitory effects of EPS at the concentration of 10^8 CFU/ml (MOI 100:1) did not show significant differences. Therefore, as the MOI increased, the inhibitory effects of added material were not shown in HT-29 cell. Therefore, all tested EPS at the concentration of 10^6 CFU/ml significantly reduced the attachment and invasion of *L. monocytogenes* Scott A to the HT-29 cell. In addition, these results suggest that EPS prevented adhesion and invasion of *L. monocytogenes* Scott A on HT-29 cell at the lower MOI. EPS caused the repression of virulence-associated genes (inlA, plcB, hlyA, and iap) and showed the prolonged survival ability on the *L. monocytogenes* infected host model.

Significance: Until present, the molecular basis of the effects evoked by probiotic bacteria has not been well characterized. Probiotic bacteria including *L. fermentum* are known to have a wide range of effects on the antimicrobial activity as GRAS (Generally Recognized As Safe). Further understanding of the virulence-associated genes of the relationship between probiotic bacteria and the host should clarify the contribution of these microorganisms to host physiology and allow enhanced application of these approaches as prevention of *L. monocytogenes*-associated diseases.

P1-149 Bactericidal Activity of GRAS Lauric Arginate against *Listeria monocytogenes* in Milk and Queso Fresco Cheese

Kamlesh Soni, RAMAKRISHNA NANNAPANENI, Wes Schilling and Viodelda Jackson
Mississippi State University, Mississippi State, MS, USA

Introduction: Recently in 2009 and 2010, consumers in the state of New York were warned not to eat a certain variety of Mexican style cheeses including Queso fresco cheese (QFC) due to the possibility of *Listeria monocytogenes* contamination. Studies show that QFC is an excellent substrate for the growth of *L. monocytogenes* and spoilage bacteria due to high moisture (45–55%), low salt content and near neutral pH

Purpose: In this study, we have evaluated the effectiveness of GRAS lauric arginate (LAE) on *L. monocytogenes* reduction in QFC as a function of: (i) substrate (broth, milk and cheese), (ii) LAE concentration, and (iii) storage time at 4°C. We also determined if the addition of 200 ppm LAE affected the sensory properties of QFC.

Methods: The long term bactericidal effect of LAE against *L. monocytogenes* was evaluated in skim milk and whole milk for up to 15 days and in QFC for up to 28 days at 4°C at 200 ppm to 800 ppm concentrations. *L. monocytogenes* was enumerated in control and LAE treated samples after 0, 1, 7, 14, 21 or 28 days of storage at 4°C.

Results: When a 4 $\log_{10}$ CFU/ml of *L. monocytogenes* was inoculated in physiological saline or tryptic soy broth, it was reduced to a non-detectable level within 30 min with 200 ppm LAE. By contrast, when a 4 $\log_{10}$ CFU/ml of *L. monocytogenes* was inoculated in whole milk or skim milk, the reduction of *L. monocytogenes* was approximately 1 $\log_{10}$ CFU/ml within 24 h with 200 ppm LAE. When 800 ppm LAE was added to whole or skim milk, the initial 4 $\log_{10}$ CFU/ml of *L. monocytogenes* was non-detectable within 24 h and no growth of *L. monocytogenes* was observed for 15 days at 4°C. Subsequently, by the surface application of 200 ppm or 800 ppm LAE on vacuum packaged QFC, the reductions of *L. monocytogenes* within 24 h at 4°C were 1.2 and 3.0 $\log_{10}$ CFU/g, respectively. The overall growth of *L. monocytogenes* in QFC was also decreased by 0.3—2.6 and 2.3–5.0 $\log_{10}$ CFU/g with 200 ppm and 800 ppm LAE, respectively, compared to untreated controls over 28 days storage at 4°C. Sensory tests revealed that consumers could not determine a difference ($P > 0.05$) between queso fresco cheese that was treated with 0 and 200 ppm LAE. In addition, no differences ($P > 0.05$) existed between treatments with respect to the flavor, texture and overall acceptability of the cheese.

Significance: LAE shows the potential for use in dairy products since it is bactericidal against *L. monocytogenes* and does not impact sensory quality. However, LAE would need to be used at higher concentrations than the current regulations allow or may need to be combined with other antimicrobials to maximize its effectiveness against *L. monocytogenes* in QFC.

P1-150 Detection and Enumeration of *Listeria monocytogenes* and *Bacillus cereus* in Raw Commingled Silo Milk

EMILY E. JACKSON, Edibe S. Erten, Neeraj Maddi, Thomas E. Graham, John W. Larkin, Robert J. Blodgett, Joseph E. Schlesser and Ravinder M. Reddy
Food and Drug Administration, National Center for Food Safety and Technology, Summit-Argo, IL, USA

Introduction: Risk assessment and the Food Safety Objective are increasingly being used to assure the safety of food technologies. Knowledge of initial microbial levels (H_0) in raw milk intended for pasteurization will enable risk assessment of novel processes.

Purpose: A nationwide survey was conducted to determine initial microbial quality and levels of *Listeria monocytogenes* (LM) and *Bacillus cereus* in raw silo milk intended for pasteurization.

Methods: Standard sample collection procedures were used to collect 202 unique silo samples. Total Viable Count, Coliforms, *Enterobacteriaceae*, *E. coli* and *Staphylococcus aureus* counts were measured by using the TEMPO® Automated System (bioMérieux). Aerobic spore and presumptive *B. cereus* counts were determined by modified standard procedures. Raw milk samples were initially screened by enriching 100 ml to detect *Listeria* species, including LM, with the mini VIDAS® (bioMérieux) LIS assay, chromogenic and selective media, followed by confirmatory biochemical testing. Twenty-five milliliters of each positive sample were further analyzed using 10-fold dilutions in a 3-tube, 5-dilution Most Probable Number (MPN) technique. BAM methods were used to calculate the MPN using all dilutions, including the initial screening results.

Poster

Results: Generally, the samples were of good quality and contained low levels of both aerobic spores and *B. cereus*. *Listeria* species were detected and enumerated in 115 of 199 samples (57.79%) using the VIDAS assay. The levels detected ranged from < 0.0055 to 9.2465 MPN/ml, depending on the assay used. LM was detected in 99 of 202 samples (49.01%) at an average level of 0.3937 MPN/ml. No correlation was observed between the general microbial quality and prevalence of *Listeria* species and LM.

Significance: Although the prevalence rates observed were higher than those reported in the literature, the levels detected were low. The higher prevalence may be due to the use of more sensitive techniques and samples from commingled silos, which contain milk, and, therefore contaminants, from multiple bulk tanks. The levels of pathogens measured can easily be eliminated by current milk pasteurization requirements.

P1-151 Characterization of *Staphylococcus aureus* Strains Isolated from Raw Milk Intended for Artisan Cheesemaking

DENNIS J. D'AMICO and Catherine W. Donnelly
University of Vermont, Burlington, VT, USA

Introduction: *Staphylococcus aureus* is an important cause of bacterial mastitis in milking animals characterized by chronic infections that are often difficult to cure. Several outbreaks of human illness related to milk and cheese have been reported worldwide as the result of the ingestion of enterotoxins produced by some strains of *S. aureus*.

Purpose: The purpose of this study was to examine the genetic and phenotypic diversity, the enterotoxigenicity and the antimicrobial resistance of *S. aureus* strains isolated from raw milk used for the production of artisan cheese in the State of Vermont.

Methods: Ninety isolates from cow, goat and sheep's milk collected during routine surveillance over a three year period were examined. Additional isolates collected from whey, brine, curd and human nare samples were analyzed as well.

Results: Cross tabulations revealed that the 17 different ribotypes identified among the 94 food isolates examined were typically associated with a specific animal species and that more than half were unique to individual farms. In general, specific ribotypes were also associated with phenotypic variation in hemolysis patterns, lipase expression and the presence or absence of bound coagulase. Limited antimicrobial resistance was observed among the isolates with resistance to ampicillin (15%) or penicillin (12%) as the most common. Two isolates of the same ribotype obtained from the same farm were resistant to oxacillin with 2% NaCl. In general, staphylococcal enterotoxin (SE) production, or the lack thereof, was also linked to specific ribotypes and more than half (56%) of isolates produced toxin. Overall, 34 of the 38 isolates tested produced toxin type C (SEC) alone.

Significance: The recurrence of individual ribotypes on specific farms over time further illustrates the chronic nature of infection. Although these data demonstrate that strains found in raw milk intended for artisan cheese manufacture are capable of enterotoxin production, SEC is not typically linked to foodborne illness. The unexpected finding of limited antimicrobial resistance is an area that requires further investigation.

P1-152 Behavior of *Escherichia coli* O157:H7 during the Manufacture and Aging of Raw Milk Gouda and Stirred-curd Cheddar Cheeses

DENNIS J. D'AMICO, Marc J. Druart and Catherine W. Donnelly
University of Vermont, Nutrition and Food Sciences, Burlington, VT, USA

Introduction: Pathogenic *Escherichia coli* can readily contaminate raw milk on the farm as dairy cattle are a known reservoir of *E. coli* including enterohemorrhagic strains such as serotype O157:H7.

Purpose: The purpose of this study was to compare the fate of *E. coli* O157:H7 during the manufacture and aging of Gouda and stirred-curd Cheddar cheeses made from raw milk.

Methods: Cheeses were manufactured in a lab scale cheese vat from unpasteurized milk experimentally contaminated with one of 3 strains of *E. coli* O157:H7 at an approximate concentration of 20 CFU/ml. Samples of milk, whey and curd were collected for enumeration throughout the manufacturing process. Finished cheeses were vacuum sealed and aged at 9 ± 1°C. Cheese samples were removed for detection and enumeration at set intervals during the aging period until *E. coli* O157:H7 was no longer detected following selective enrichment.

Results: Overall, counts in both cheese types increased almost 10-fold from initial milk inoculation levels to an approximate concentration of 145 CFU/g observed in cheeses on day 1. From this point, counts dropped significantly over 60 days to mean concentrations of 25 and 5 CFU/g of Cheddar and Gouda, respectively. Levels of *E. coli* O157:H7 fell and stayed below the cultural detection limit of ≥ 5 CFU/g after an average of 94 and 108 days in Gouda and Cheddar, respectively, yet remained detectable following selective enrichment for more than 270 days in both cheese types. Changes in pathogen levels observed throughout manufacture and aging did not significantly differ by cheese type.

Significance: In agreement with previous studies our results suggest that the 60-day aging requirement alone is insufficient to completely eliminate viable levels of *E. coli* O157:H7 in Gouda or stirred-curd Cheddar cheese when manufactured from raw milk contaminated with low initial levels of this pathogen.

P1-153 Food Safety and Quality Criteria of Dairy Farms: A Case Study of Turkey

Nevin Demirbas, DUYGU TOSUN, Figen Cukur, Ozlem Yildiz and Evren Golge
Ege University, Agricultural Economics, Izmir, Turkey

Introduction: Today, besides meeting the need in the quantity of food requirement, food safety and production according to quality criteria are the main problems of providing food. As in the other food products, "food safety" in milk and dairy products appear to be the most important quality criteria. The food safety process for milk and dairy products starts at the dairy farms which is the first step in supply chain.

Purpose: In this study, food safety and quality criteria of dairy farms were determined in the province of Izmir, one of the most important production centers in Turkey, according to the number of dairy farms and milk production. The knowledge level of dairy farmers on the quality of milk, food safety and quality system practices was studied. Also, related problems and solutions were examined in this paper.

Methods: The number of surveys applied was 103; five counties and 20 villages connected to these five counties were involved in the surveys. The knowledge of the farmers on the standards to be followed during milk production were evaluated according to the three-point Likert Scale.

Results: Only seven of the farms which were studied had milk cooling tanks. Also equipment necessary to measure quality of milk were inadequate. On the other hand, animal health and hygiene criteria were mostly applied on farms before and after the production. The knowledge level of farmers on food safety and quality of milk was determined to be inadequate.

Significance: The studied farms are faced with various technical and economical problems which make it difficult for farms to adapt to food safety systems. In terms of public health, besides the technical inadequacy, the level of knowledge of the farmers on food safety and quality systems applications should be increased.

P1-154 Comparison of Growth and Recovery of *Alicyclobacillus acidoterrestris* from Five Different Juices with a Four-day Procedure Utilizing a Method Based on CO_2 Production, a Six-day Procedure Based on a Modified Plating Method, and a Traditional Ten-day Plating Method

PATRICIA RULE, Stan Bailey, Jacquelyn Miles and Tony Dworetsky
bioMérieux, Hazelwood, MO, USA

Introduction: *Alicyclobacillus acidoterrestris* is a spore forming heat tolerant acid-loving organism known to cause spoilage in the fruit and juice beverage industries by the production of an off flavour known as guaiacol.

Purpose: Since first isolated in 1984 from spoiled apple juice, the reproducible growth and recovery of *Alicyclobacillus acidoterrestris* remains a difficult challenge requiring a 5 day pre-incubation period as well as an additional 5-day recovery on plates in very specialized and complicated media. A reduced pre-incubation of 3 days in a simple YSG medium was reviewed with the standard plating method as well as an alternate 24-h screening method based on CO_2 production using the BacT/ALERT® (BTA) Microbial Detection System.

Methods: *A. acidoterrestris* spores were inoculated at multiple levels into 50 gram samples of apple, orange, pear, white grape and concord juice products in three separate trials over a 6-month period. Samples were plated 1 mL each onto K Agar and YSG Agar and 10 mL into BTA culture bottles following a reduced 3 day pre-incubation time frame and compared to the traditional method of 5 days pre-incubation and 5 days plate recovery. Triplicates of the CO_2 detection method were evaluated for reproducibility of time to detection.

Results: The overall agreement between the 24-h CO_2 screening method to the traditional plating method was 92.5% in Trial 1, 100% in Trial 2 and 90% in Trial 3. The CO_2 screening method resulted in the shortest time to detection (4–9 h) with apple, white grape and pear juice, while the longest time to detection (19–21 h) was observed with orange juice. There was one false negative with the BTA screening method in a single replicate of the initial trial due to weak CO_2 production. An overall total of 4 false positives were observed by the CO_2 screening method, with 1 in the initial trial and 3 in the third trial. The three additional positives observed by the CO_2 screening method in the third trial were due the presence of a non- guaiacol naturally occurring *Alicyclobacillus* species in the orange and pear juices that were not observed in the 10-day traditional method with K Agar but were observed on the YSG Agar plated after 3 days pre-incubation.

Significance: The less complex YSG broth supported the growth and recovery of *A. acidoterrestris* in a shortend 3-day preincubation time frame compared to the traditional 5 day preincubation method. *A. acidoterrestris* could be observed as early as 3 days on the YSG agar plate and in < 24 h in the CO_2 screening method. Both plating and CO_2 screening methods required additional guaiacol confirmation as non-guaiacol producing *Alicyclobacillus* species could also grow.

Poster

P2-01 Evaluation of an Automated Immunoassay for *Salmonella* Detection in Pet Foods and Environmental Surfaces

DENISE HUGHES, Jennifer Chen and Selina Begum
DH MICRO Consulting, Greenacre, NSW, Australia

Introduction: The VIDAS *Salmonella* Easy (Easy SLM test, AOAC Method 020901) has previously been validated for all foods using lactose broth and other FDA BAM enrichment media.

Purpose: The objective of the study was to extend the application of the Easy SLM test to pet food and environmental surfaces.

Methods: In the extension study, samples were pre-enriched in buffered peptone water. An aliquot was then transferred to a secondary enrichment broth, *Salmonella* Xpress 2 (SX2) broth,which was incubated overnight before testing in the VIDAS instrument. In the Easy SLM protocol, a single selective broth replaces two broths in traditional methods and eliminates the need for transfer to M broth prior to the VIDAS assay. This study included method comparison according to AOAC guidelines for 2 foods (moist and dry pet food) and five surfaces (stainless steel, plastic, rubber, ceramic and concrete).

Results: There were no significant differences between the Easy SLM test and the FDA BAM reference method using an unpaired Chi-square test at the 5% level. However, the Easy SLM detected 5 additional confirmed positive samples for moist pet food, which had a high microbial load. There were no false positive results using the Easy SLM method and the use of chromogenic media (ChromID *Salmonella* agar) for confirmation gave equivalent results to traditional selective agars.

Significance: The Easy SLM method provides significant savings in media, tubes, pipettes and time spent in transferring cultures and results are available on the second day after sample set up. This makes the Easy SLM method an attractive option for routine *Salmonella* testing of food and environmental samples.

P2-02 Rapid Detection of *Salmonella* in Pet Food: Design and Evaluation of an Under 12-hour Workflow Using Real-time PCR

PRIYA BALACHANDRAN, Robert Tebbs, Maxim Brevnov, Manohar Furtado and Olga Petrauskene
Life Technologies, Foster City, CA, USA

Introduction: According to the 2009/2010 National Pet Owners Survey, 62% of U.S. households own a pet, and estimated sales within the U.S. market for pet food alone is $17.4 billion. Consequently, PCR-based rapid and robust testing of pet food for foodborne pathogens is gaining importance. Food samples create unique challenges, particularly with respect to sample preparation, for detection by PCR.

Purpose: To create a simplified workflow, including sample preparation for detection of 1—3 CFU of *Salmonella* in dry pet food in under 12 hours using real-time PCR.

Methods: 25 g of dry pet food was spiked with 1—3 CFU of *Salmonella*. 225 ml of pre-warmed Brain Heart Infusion Broth was added, samples were homogenized and enriched at 37°C, 225 rpm for 9 hours. DNA from enriched samples was extracted using the PrepSEQ™ Nucleic Acid Extraction Kit automated on MagMAX™ Express with some modifications. The enriched samples were passed through a filter-clarification column by low-speed centrifugation, followed by Proteinase K digestion and nucleic acid extraction. Samples were analyzed by real-time PCR using the MicroSEQ® *Salmonella* spp. detection kit run on the Applied Biosystems 7500 Fast Real-Time instrument. All enriched samples were also confirmed by culture by plating on CHROMagar™ *Salmonella* plates.

Results: Dry pet food is one of the more difficult matrices to process using the PrepSEQ™ Nucleic Acid Extraction Kit because of incomplete removal of food debris during sample preparation, and carry-over into the final eluted samples. This poses a challenge with respect to ease-of-use for the end user due to potential transfer of PCR inhibitory components to the assay reaction. We found that pre-digestion of samples with Proteinase K greatly reduced the food debris carryover in the eluted sample, while robustly detecting the target. The food debris in the elution plate was further reduced using a pre-clarification/ filtration tray, which eliminated large particles of food from entering the sample prep. Overall, these modifications improved detection by up to 2 Cts which allowed for reducing the enrichment time. Using the PrepSEQ™ Nucleic Acid Extraction Kit automated on MagMAX™ Express-96, a 100% correlation was observed between real-time PCR results and culture confirmation.

Significance: In this study, we have optimized a complete workflow for the detection of 1—3 CFU of *Salmonella* in dry pet food in under 12 hours. Sample preparation, a critical step for quality PCR detection, was optimized using multiple approaches. In conclusion, we demonstrate a rapid pathogen detection system for monitoring presence of *Salmonella* in pet food.

P2-03 Evaluation of a Real-time PCR Assay and Novel Sample Collection and Processing Protocol for Genus *Salmonella* Detection and Monitoring in Environmental Sampling Programs

DANIEL DEMARCO and Stephen Varkey
DuPont Qualicon, Wilmington, DE, USA

Introduction: The use of PCR-based methods for *Salmonella* spp. detection and monitoring in environmental sampling programs has shown tremendous growth in recent years. DuPont Qualicon recently developed a Scorpion® probe-based real-time PCR assay for genus *Salmonella* detection. This assay has previously demonstrated excellent performance characteristics in terms of inclusivity, exclusivity and sensitivity in studies with pure culture and purified DNA. The assay has also been developed as an alternative approach to environmental sampling compared to traditional methods. The protocol involves the use of the real-time PCR assay in conjunction with a novel sample collection device, cell resuscitation step and fast enrichment protocol.

Purpose: The purpose of this study was to evaluate the use of a Scorpion® probe-based real-time PCR assay and sample collection procedure for environmental *Salmonella* monitoring. The real-time PCR method was compared with a commercially available alternate PCR method as well as a traditional culture method.

Methods: Spike studies on stainless steel surfaces with select strains of *Salmonella* spp. were conducted. Spike concentration was determined based on achieving fractional positive results. Studies were conducted comparing the new real-time assay with traditional culture methods and an alternate commercially available PCR method.

Results: Results indicated that the real-time PCR method with a cell resuscitation step detected all true positive samples in as little as 16 h (n = 10). In contrast, the traditional culture method and alternative PCR protocol detected statistically significantly fewer true positives (X^2 = 6.84) and required a minimum of 24 h to complete. Additional studies have shown promising results with for the real-time PCR method with enrichment times as short as 11 h, and the data suggests that even faster times may be possible.

Significance: These results demonstrate the feasibility using a real-time PCR assay for *Salmonella* spp. in conjunction with a novel approach for sample collection and preparation from environmental surfaces. The assay offers a significant improvement in total time to results and improved performance versus culture methods or alternative PCR technologies that use traditional sample collection and processing techniques.

Poster

P2-04 Development and Validation of a Rapid Lateral Flow Test Strip Method for Environmental Monitoring of *Salmonella* Enteritidis in Poultry Houses

MARK MULDOON, Adam Leaphart, Vera Gonzalez, Meredith Sutzko and James Stave
Strategic Diagnostics Inc., Newark, DE, USA

Introduction: Each year, over 100,000 cases of human illness are caused by the consumption of *Salmonella* Enteritidis (SE, *Salmonella* serogroup D1)-contaminated shell eggs. In order to reduce this incidence, the US FDA has recently put in place regulations that require egg producers to monitor poultry houses for SE and, if found, to test shell eggs for the presence of SE and divert contaminated eggs from the table market. This regulation is likely to increase production costs as a result of increased testing and possible holding of product.

Purpose: The purpose of this study was to develop and validate a rapid, lateral flow test strip method for the detection of SE in environmental drag swabs used for monitoring SE in egg producing poultry houses.

Methods: A monoclonal antibody was developed against *Salmonella* serogroup D1 serovars including SE. The antibody was configured in a lateral flow test strip device and tested against a panel of 351 *Salmonella* strains representing 20 serogroups including 141 from serogroup D1. The test strip was coupled to a 24-h enrichment protocol using SDI RapidChek Select media for the analysis of both artificially- and naturally-contaminated environmental drag swab samples taken from a commercial poultry house. The results were compared to the results obtained using the 96-h US FDA cultural reference method for environmental drag swabs.

Results: In the inclusivity study, the lateral flow test strip method showed 100% sensitivity and 93% specificity for *Salmonella* serogroup D1 strains. For the method comparison using artificially contaminated drag swab samples, the test strip method gave 16 positives whereas the reference method gave 14 positives. There were no false positives or false negatives found. The Mantel-Haenszel Chi-square analysis showed no significant difference between the methods (0.52). For the analysis of naturally-contaminated drag swabs (n = 25) the rapid test strip method gave two (2) *Salmonella* serogroup D1 positives with no other *Salmonella* positives found whereas the cultural reference method gave no *Salmonella* serogroup D1 positive samples and one (1) non-serogroup D1 *Salmonella* positive sample.

Significance: The rapid, 24-h test strip method was highly specific for *Salmonella* serogroup D1 strains including SE. Furthermore, it showed equivalent performance as the 96-h cultural reference method. This new method should be a valuable tool for environmental monitoring and control of SE in poultry houses by reducing testing costs and ensuring safe product.

P2-05 Evaluation of a Sampling Technique for Recovery of *Salmonella* spp. from Soil

Estefany López-Murillo, Ofelia Rodríguez-García, Cristina Martínez-Cárdenas and MAYRA MÁRQUEZ-GONZÁLEZ
Universidad de Guadalajara, Farmacobiología, Guadalajara, Mexico

Introduction: Salmonella spp. is a ubiquitous organism. The presence of this pathogen in farm environments increases the risk of contamination for the product. Crop production farms have large areas. Traditional soil sampling by weight involves composed samples from different points at the farm. Using drag swab sampling can increase the surface sampled at the farm. Drag swab methods for recovery of *Salmonella* spp. have been tested on poultry environments. However, the environmental conditions found in produce farms may difficult the recovery of the pathogen from soil samples.

Purpose: The purpose of this study was to evaluate swabbing vs. traditional sampling method for the recovery of *Salmonella* spp. from produce farm soil.

Methods: Salmonella spp. isolates from environmental samples (*S.* Montevideo, *S.* Derby, *S.* Poona, *S.* Newport, *S.* Typhimurium and *S.* Infantis) were used to inoculate soil samples at high and low levels. Uninoculated samples were used as control. For both inoculated and uninoculated soil, twelve replicates were sampled by weighting 25 g and using a drag swab moistened with skim milk. All samples were analyzed by using traditional culture method.

Results: All of the 12 uninoculated samples were negative for both methods. There were no false positive results. For the low level inocula (0.11 MPN/g) there were 10 confirmed positive results for the weigh sampling, and 12 confirmed positives for the drag swab method. All 12 samples were positive for the high level inocula (0.34 MPN/g). There was not significant difference between the sampling methods at the 5% level using McNemar's test for paired data.

Significance: Drag swabbing moistened with skim milk is a reliable method for the recovery of *Salmonella* spp. cells from farm environments.

P2-06 A *Salmonella* Test System for the Detection of the Pathogen in Food, Feed and Environmental Samples

MEREDITH SUTZKO and Francois Le Nestour
Strategic Diagnostics, Newark, DE, USA

Introduction: Salmonella has been implicated as a major cause of human foodborne illnesses worldwide. A variety of food types have been identified as vehicles of infection for salmonellosis, including raw meat and poultry, fresh produce, processed meats, dairy products and fruit juices. Several control measures have been implemented along food production lines to reduce the levels of *Salmonella* in these matrices; however, microbiological testing remains a key role in preventing foodborne salmonellosis Thus, sensitive, fast, and reliable detection methods are needed to monitor foods for *Salmonella* spp. and ensure that safe food is being supplied to the consumer.

Purpose: The aim of the study is to evaluate the performance of the RapidChek® SELECT™ *Salmonella* test system against the ISO reference method (NF EN ISO 6579:2002) for the detection of *Salmonella* spp. in food and animal feeding stuffs.

Methods: A method comparison study was conducted to determine accuracy, specificity, sensitivity, and relative detection level. Three hundred eighty (380) samples from six food categories were analyzed by both methods. Selectivity was evaluated by testing fifty (50) target microorganisms and thirty (30) non-target microorganisms.

Results: The test system demonstrated 92% relative accuracy. The relative sensitivity of the method was 93%. The relative specificity was 97%. The relative level of detection of the test method varies from 0.7 to 1.3 CFU/25 g depending on food type while the limit of detection of the reference method varies from 0.6 to 0.9 CFU/25 g depending on food type. The selectivity of the method was satisfactory.

Significance: The target pathogen can be detected at very low levels of contamination in as few as 24 hours with the test system. Confirmation of a positive result can be accomplished in as little as 3 days with the test method versus 4 to 5 days with the reference method.

P2-07 Preliminary Evaluation of a New Next-day Method for Detection of *Salmonella* in Food Products

Laure Puthod, Peggy Noel, Peggy Nomade, Jean-Louis R. Pittet and CELINE DOMINGOS
bioMérieux, R&D, Marcy L'Etoile, France

Introduction: Although a few rapid next-day methods for detection of *Salmonella* in food have been described recently, there are generally drawbacks to their adoption for routine industry use including their high cost and practical limitations.

Purpose: The goal of this study was the preliminary internal evaluation of a new, automated next-day method for the detection of *Salmonella* in food products, the VIDAS UP *Salmonella* method.

Methods: 25 g of food samples, 1/10 diluted in buffered peptone water with 1ml of a specific additive, were enriched for 22–24 hours at 41.5°C prior to immunoassay detection. The immunoassay is a sandwich, 2 step assay using a cocktail of monoclonal antibodies and recombinant phage proteins. They target both somatic and flagellar antigens allowing detection of both motile and non-motile strains.

Results: Sensitivity study: the new method was compared to the AOAC and ISO 16140 validated, VIDAS Easy *Salmonella* method. 51 food products, 10 replicates per product, were artificially contaminated with 30 stressed cells of *Salmonella* at a level comprise between less than 1 and 9 cells/25 g of products. 372 positive results were obtained with the new method and 378 with the VIDAS Easy *Salmonella* method. There were no significant differences between the two methods using an unpaired chi-square test at 5% level. Specificity study: 168 food products were tested in parallel by the new method and with the reference method. A specificity of 99.4% was obtained with the VIDAS method.

Significance: The VIDAS SPT method showed satisfactory results when tested on artificially contaminated and uninoculated food samples. The very simple protocol makes it a promising tool for the next-day detection of *Salmonella* in food products.

P2-08 Single-step Enrichment Protocol for Use with a Lateral Flow Immunoassay for Detection of *Salmonella* spp. in Raw Meats, Raw Shrimp and Chicken Carcass Rinse

Rebecca Hoerner, Jill Feldpausch and MARK MOZOLA
Neogen Corp., Lansing, MI, USA

Introduction: Rapid detection of *Salmonella* spp. in raw meats requires a sample enrichment method in which the antagonistic demands of *Salmonella* recovery and suppression of growth of competing microflora are balanced. In this study, single-step enrichment in Rappaport-Vassiliadis (RV) broth was compared to traditional two-stage enrichment (buffered peptone water (BPW) followed by selective enrichment in RV broth and tetrathionate brilliant green (TT) broth) for detection of *Salmonella* spp. in raw meats, raw shrimp, and chicken carcass rinse using the Reveal *Salmonella* lateral flow immunoassay.

Purpose: The purpose of the study was to compare the performance of the Reveal *Salmonella* assay using single-step RV broth enrichment with that of reference culture procedures for detection of *Salmonella* spp. in a variety of sample types.

Methods: Raw ground turkey, ground beef (fresh and frozen),and shrimp were inoculated with *Salmonella* spp. and held for 48–72 hours at 2–8°C before analysis (-20°C for frozen ground beef). Chicken carcass rinse samples were inoculated on the day of analysis. 25-g test samples were enriched in RV broth for 20–24 hours at 42°C and tested in the Reveal assay. A parallel set of 25-g samples were tested by the USDA-FSIS reference culture method (US FDA method for shrimp) and required 48 hours of enrichment before plating to selective/differential agars. For chicken carcass rinse samples, 30 mL of rinse was added to 30 mL of double-strength RV broth or to BPW for the Reveal and USDA-FSIS analyses, respectively. All positive Reveal results were confirmed by plating from the RV broth enrichments. A total of 9 trials were performed. Performance of the methods was compared by Chi-square analysis.

Results: A total of 228 samples were tested. There were 85 Reveal assay positive samples and all were confirmed by plating from the RV broth enrichments. Specificity of the Reveal assay was therefore 100% in these trials. There were a total of 95 positive samples by the reference culture methods. There were no statistically significant differences in the number of positives obtained by the two methods in individual trials, except in one trial with raw shrimp where the culture method detected more positives.

Significance: In these trials, the Reveal *Salmonella* method with single-step enrichment in RV broth showed comparable sensitivity to that of the reference culture methods for detection of *Salmonella* spp. in a variety of raw meats and chicken carcass rinse. This procedure offers the dual advantages of providing results within 20–24 hours and single-step enrichment, eliminating the need to make transfers during the enrichment process.

P2-09 Novel Automated Workflow Reducing Time to Result for Detection of *Salmonella* in Food Matrices

MAXIM BREVNOV, Priya Balachandran, Jack Regan, Craig Cummings, Robert Tebbs, Nikolay Sergeev, Manohar Furtado and Olga Petrauskene
Life Technologies, Applied Biosystems, Foster City, CA, USA

Introduction: Detection of low levels of pathogenic microorganisms in food by molecular methods is often difficult due to the limited amount of target DNA molecules, complexity of food matrices and presence of PCR inhibitors. We have developed fast and easy workflow, including an automated sample preparation method, for extracting total nucleic acids and the highly sensitive detection of food pathogens using real-time PCR and Reverse Transcriptase real-time PCR.

Purpose: To design a novel automated sample preparation solution to detect 1 CFU of *Salmonella enterica* following enrichment in various food matrices using our highly sensitive, pathogen-specific real-time PCR systems.

Methods: A highly efficient magnetic-bead based total DNA capture method was developed for sample preparation and evaluated using Applied Biosystems 7500 Real-time PCR system. The developed method enables the isolation of total Nucleic Acids (NA) from food samples that are free of PCR inhibitors and ready for downstream applications like RT real-time PCR. The method employs a proprietary multi-component surface chemistry to isolate effectively total NA. The reagents are packaged for automated processing of individual samples and achieving high efficiency of total NA extraction. The protocols are optimized for automated extraction of total NA from a variety of food type samples. The sample preparation workflow was validated on milk samples spiked with *Salmonella*. Briefly, 25ml of milk samples were spiked with 1–3 CFU of *Salmonella* and enriched in 225 ml Buffered Peptone Water, according to FDA-BAM standard procedures. The extracted nucleic acids were evaluated by real-time PCR and reverse transcriptase real-time PCR. Enriched samples were confirmed by plating on *Salmonella* CHROMagar plates.

Results: 100% correlation was obtained between real-time PCR results and plate confirmation. The sample preparation method was highly efficient in removal of PCR inhibitors, as demonstrated by robust detection of the internal positive control included in the assay. Both DNA and RNA were efficiently extracted as demonstrated by positive signal obtained by real-time and reverse transcriptase real-time PCR assays.

Poster

Inclusion of the reverse transcriptase step improved the real-time PCR signal by 4–7Ct, enabling shorter time-to-results. Sample preparation and real-time PCR could be completed in under 2 hours, following enrichment.

Significance: The ease-of-use, rapidity and efficiency of our novel sample preparation method in combination with real-time PCR or RTreal-time PCR is ideal for detection of low amounts of food pathogens in difficult food matrices in a reduced time frame.

P2-10 Comparison of Different Sample Preparations for the Recovery of *Salmonella* from Internally-contaminated Whole Tomatoes

HUA WANG, Mindi Russell, Vikas Gill, Kari Belin, Steven Monday, Eric Brown and Thomas Hammack
U.S. FDA, College Park, MD, USA

Introduction: During the past decade, the consumption of fresh and fresh-cut tomatoes has been linked to 12 *Salmonella* outbreaks in the U.S. *Salmonella* has become a persistent environmental contaminant for tomatoes in the field and post processing.

Purpose: To determine the relative efficacies of whole soak (current BAM *Salmonella* method), quartering, stomaching and blending methods for detecting *Salmonella* from internally contaminated whole tomatoes.

Methods: Three *Salmonella* serovars were used as inocula. Red round tomatoes were immersed in an inoculum bath that was ca. 20°C cooler than the tomatoes for 15 min and air dried overnight. Tomatoes were swabbed with 95% ethanol and air dried, and stored under refrigeration for 3 days before culture analysis. Quartered, stomached, blended and whole tomatoes were pre-enriched in Universal Preenrichment broth and incubated for 24 h at 35°C. The BAM *Salmonella* culture method was followed thereafter. The internalization using GFP+ *S.* Typhimurium was viewed after a 15-min soak bath. Multiplex PCR analysis was performed on 24 h incubated preenrichment broths. This assay was designed to preliminarily identify *Salmonella* through three genes *gapA* (internal control), *invA* and *apeE* (*Salmonella*-specific genes). The assay has been validated for its sensitivity (inclusivity testing, 147 serotypes) and specificity (exclusivity testing, 30 non-*Salmonella*) from pure culture.

Results: The results show the stomaching (87) is more effective than whole soaks (74) or quartering (74) for the recovery of *Salmonella*. A blend method is under evaluation. Multiplex PCR assay has inclusivity rate of 99.3% and an exclusivity rate of 100%.
The PCR and culture method results were closely aligned with a 97.7% agreement rate. Bath inoculation shows positive internalization at tip of stem scar.

Significance: This study addressed a need to develop an improved BAM *Salmonella* sample preparation method for the detection of *Salmonella* from internally contaminated tomatoes. Multiplex PCR assay can be used as a rapid screening method.

P2-11 Evaluation of Rapid Screening Techniques for Detection of *Salmonella* spp. from Artificially Contaminated Produce Samples after Pre-enrichment According to FDA BAM and a Short Secondary Enrichment

Ken J. Yoshitomi, Karen C. Jinneman, Palmer A. Orlandi, Stephen D. Weagant, Ruben Zapata, Cecelia M. Garcia, Betty A. Strietelmeier and WILLIS M. FEDIO
New Mexico State University, Las Cruces, NM, USA

Introduction: Outbreaks of salmonellosis have been associated with fresh fruits and vegetables. Detection of *Salmonella* is based on conventional enrichment and isolation on selective media which is both time consuming and labor intensive.

Purpose: The objective of this study is to evaluate the utility of an accelerated plating procedure and rapid screening technique for *Salmonella* detection.

Methods: Food matrices tested included Romaine lettuce, cilantro, jalapeno peppers, tomatoes and cantaloupes. Produce was inoculated with *Salmonella* at ~ 2.5 CFU/sample, 7.5 CFU/sample and 25 CFU/sample. Six replicates at each inoculum level were tested per food matrix, as well as six uninoculated controls. After 24-h pre-enrichment, subcultures were made into TT and RV broths. After 7-h incubation at 42°C, plates were streaked for isolation of *Salmonella* and the selective enrichments were reincubated for a total of 24 h and used for conventional BAM cultural isolation and the VIDAS-SLM assay. At both 7-h and 24-h incubation, portions of the TT broths were used for Neogen Reveal and RapidChek *Salmonella* tests. RV broths were also tested at both 7 h and 24 h but only on the Neogen devices.

Results: Performance of the procedures varied by commodity and by *Salmonella* strain used for the evaluation. The seven hour accelerated plating procedure worked well for 4/6 to 6/6 of all produce samples inoculated at the lowest level. Both the RapidChek and Neogen Reveal tests worked as well as the VIDAS-SLM after 24-h enrichment for most strains tested but failed to detect the pathogen after 7-h selective enrichment in nearly all of the artificially contaminated samples. The Neogen Reveal test can be used as an alternative to the VIDAS-SLM for detection of *Salmonella* from both 24-h TT and RV BAM selective enrichments. The RapidChek could be used with 24-h TT BAM enrichments. Strains that do not grow well in TT could be missed by the RapidChek.

Significance: The seven hour selective enrichment procedure worked well to accelerate the isolation of *Salmonella* from contaminated samples.

P2-12 Comparison of 3M™ Petrifilm™ Aerobic Count Plates with Pour Plates for Determination of Aerobic Plate Counts in Fermented Chile Mash

JORGE A. BEALL, Ruben Zapata, Cecelia M. Garcia, Elizabeth A. Strietelmeier, Nancy C. Flores and Willis M. Fedio
New Mexico State University, Food Safety Laboratory, Las Cruces, NM, USA

Introduction: Fermented chile pepper mash (*Capsicum annuum* cv. Mesilla Cayenne) is a major food product in New Mexico. There are few reports on the fermentation process or on methods to monitor it.

Purpose: In the current study we examined a pour plate procedure with an overlay using plate count agar and Petrifilm Aerobic Count Plates for enumeration of total bacteria during the fermentation of chile mash.

Methods: Chile mash was obtained from a local processor. A laboratory simulation of the chile mash fermentation was conducted in plastic pails. Samples were stored at room temperature and over a 3 month period, 56 samples were examined. Additionally, 50 chile mash samples were obtained directly from commercial fermentation vats and examined within 2 h of collection. Serial dilutions of the chile mash were prepared in Butterfield's Phosphate Buffer. One mL portions of the diluted samples were aliquoted in duplicate onto the AC Plates and into empty Petri dishes. Pour plates were prepared with melted and tempered plate count agar (PCA). Once the plates had solidified, they were overlayed with about 10 mL of PCA to minimize spreaders. Plates were incubated at 30°C for 48 h and enumerated. Paired difference tests (two tailed t-tests) were conducted on log transformed data to compare the results of the two plating procedures.

Results: For the laboratory chile mash samples, many of the AC Plates became difficult to read as the gelling agent was being liquefied by the organisms growing on the plates, particularly towards the end of the fermentation. However, no significant differences ($\alpha = 0.05$) were seen between the counts obtained between the pour plates and the AC plates. Plate counts were within one log cycle in 51/56 (91.4%) of the

Poster

samples. For the commercial chile mash fermentation, no significant differences were seen between the AC Plate counts and the pour plate counts (α = 0.05). Plate counts were within one log cycle for 46/50 (92%) of the samples.

Significance: 3M™ Petrifilm™ AC plates are a good alternative to pour plates for the determination of the total aerobic counts in chile mashes. They are easier and more convenient to use than pour plates and suitable for food processors with limited laboratory capacity.

P2-13 Development of Methods for the Detection and Isolation of *Salmonella* from Cloves

VIKAS GILL
Food and Drug Administration, College Park, MD, USA

Introduction: During the past decade the annual per capita consumption of spices in the United States increased by 60%. During this time there has been an increase in spice related *Salmonella* outbreaks.

Purpose: To develop more sensitive methods for detection and isolation of *Salmonella* from various spices, including cloves, using an immunomagnetic capture system (Pathatrix), multiplex PCR, and sonication methods. These methods were compared with the current BAM *Salmonella* culture method.

Methods: Two *Salmonella* serovars were used as inocula. Four varieties (Madagascar, Brazil, Ceylon, and Spice Island aka Zanzibar) of cloves were used. The sample size for cloves was 10 g and 25 g. The cloves were pre-enriched in tryptic soy broth and incubated for 24 h at 35°C. One set of samples was treated with the Pathatrix method and the other with BAM *Salmonella* culture method. Pure culture samples are also evaluated using the BAM *Salmonella* culture method, which served as positive control.

Results: The Pathatrix showed a 4 to 5 log increase in sensitivity over the BAM for 10-g samples of Madagascar cloves. There was no difference for the 25 g samples. The Pathatrix showed up to 2 log increased sensitivity for 10-g and 25-g samples of Brazil and Ceylon cloves. PCR and sonication methods are under development.

Significance: The current BAM *Salmonella* culture method recommends a sample size of 1 g/1 litre of broth for cloves. This method increases the sample broth ratio of 1:1000 to 1:40, a 40-fold increase.

P2-14 Comparison of Different Pre-enrichment Broth for the Recovery of *Shigella* Species in Fresh Produce

INSOOK SON, Guodong Zhang and Keith Lampel
CFSAN, U.S. FDA, College Park, MD, USA

Introduction: *Shigella* are difficult to isolate from food matrices since they are easily outgrown by competitive indigenous microorganisms in foods. Another disadvantage is that *Shigella* are usually present in low numbers, and in some foods, are dwarfed in comparison to the resident microbial population, a major challenge for successful isolation.

Purpose: The purpose of this study is to evaluate four pre-enrichment media for the recovery of *Shigella* from four fresh produce commodities.

Methods: Cilantro, lettuce, potato salad, and shrimp were inoculated with *S. boydii*, *S. dysenteriae*, *S. flexneri*, and *S. sonnei* and stored at 4°C for 7 days. Four pre-enrichment broths, tryptic soy broth with streptomycin (TSBS), acid enrichment (AE), *Shigella* broth (SB), and *Shigella* broth with streptomycin (SBS) were added to each food and incubated for 6 and 24 h at 37°C. Each culture was streaked onto BioLog *Shigella/Aeromonas* rainbow agar on 0, 3, and 7 sampling days. For confirmation, a latex agglutination test was used. Each experiment was performed in triplicate.

Results: Overall, 6 h of enrichment was less effective in regard to the isolation of *Shigella* than 24 h, regardless of the food matrix and pre-enrichment broth. TSBS, SBS and SB were suitable broth in most cases, but results varied depending on the strain used and food matrix. For example, with *S. boydii*, TSBS performed better with lettuce and potato salad, whereas SBS and SB did better with cilantro and shrimp, respectively. As for *S. sonnei*, TSBS was more effective in lettuce and shrimp, but SB had better results with cilantro and potato salad. TSBS did well with *S. flexneri* in all produce tested.

Significance: Comparison of pre-enrichment media has shown that streptomycin added to broth may be more effective means to isolate *Shigella* in fresh produce.

P2-15 Comparison of Two Chromogenic Agars with MacConkey and Xylose Lysine Deoxycholate Agars for Isolation and Detection of *Shigella* from Foods

GUODONG ZHANG and Keith A. Lampel
Food and Drug Administration, DM-ORS-CFSAN, College Park, MD, USA

Introduction: *Shigella* outbreaks are widely reported worldwide, in both developed and developing countries. It remains a challenge to isolate *Shigella* spp. from foods using conventional microbiological media.

Purpose: The main objective of this study was to evaluate the effectiveness of newly developed chromogenic media, Rainbow™ agar *Shigella/Aeromonas* (RA agar) and CHROMagar *Shigella* for the isolation and detection of *Shigella* spp. in foods.

Methods: All four *Shigella* species, *S. sonnei, S. flexneri, S. dysenteriae* and *S. boydii*, were studied. CHROMagar *Shigella* and RA agar were compared with xylose lysine deoxycholate agar (XLD) and MacConkey agar (MAC) for their effectiveness to isolate *Shigella* spp. in artificially-contaminated foods (4.85 $\log_{10}$ CFU/g food), including lettuce, parsley, cilantro, spinach, potato salad, and shrimps.

Results: In most cases, CHROMagar *Shigella*, Rainbow™ agar and MAC were significantly ($P < 0.05$) more effective than XLD in detecting *Shigella* spp. in these foods. The overall average *Shigella* populations detected by CHROMagar *Shigella*, RA agar, MAC and XLD were 4.51, 4.41, 4.27 and 3.90 log CFU/g produce, respectively. Cilantro, parsley and spinach showed a moderately or very high background microflora on all four media. For potato salad and shrimp, which had low background microflora on all media studied, CHROMagar *Shigella*, RA agar and MAC were significantly ($P < 0.05$) superior in recovering *Shigella* spp. than XLD, except for the case of *S. flexneri* in shrimps, where MAC was significantly ($P < 0.05$) better than CHROMagar *Shigella*, RA agar and XLD.

Significance: In conclusion, CHROMagar *Shigella*, RA agar and MAC were superior to XLD for the isolation and detection of *Shigella* spp. from foods, primarily due to the mauve or black colors of *Shigella* colonies on these media compared with the colorless colonies on XLD.

P2-16 Validation of a Real-time PCR Assay for Screening *Escherichia coli* O157:H7 in Foods

MORGAN WALLACE, Frank R. Burns, Bridget Andaloro, Lois Fleck, George Tice, Jeff Rohrbeck, Eugene Davis, Dawn Fallon, Bill Centrella, Wendy Warren, Ryan Welsh and Deana DiCosimo
DuPont Qualicon, Wilmington, DE, USA

Introduction: *E. coli* O157:H7 is a foodborne pathogen sometimes found in raw beef and produce that can cause serious, and sometimes fatal, illness at a very low infectious dose (as few as 10 organisms). Since culture-based methods can be difficult and time-consuming, and

Poster

since the organism is difficult to isolate when in the presence of an excess of competing flora, well-validated rapid methods for the detection of this pathogen are needed.

Purpose: This study evaluated the inclusivity, exclusivity, and effectiveness of the BAX® System Real-Time PCR assay for screening *E. coli* O157:H7. Artificially contaminated beef trim, ground beef, lettuce and spinach were tested and results compared with the appropriate USDA or FDA reference method.

Methods: Inclusivity testing was performed at ~10^5 CFU/mL, while exclusivity testing was performed at 10^8 CFU/mL. In eight studies of the above referenced matrices, samples were inoculated with *E. coli* O157:H7 at levels expected to yield fractional positive results, using spike levels of 0.7—2.0 CFU per analytical portion (375 g beef trim, 65-g ground beef, and 25-g produce).

Results: For inclusivity testing, all 62 target strains (including O157:H non-motile and rough strains) were found to be reactive, while all 72 non-target strains were non-reactive to the assay. For effectiveness testing, which included 160 spiked and 40 unspiked samples for test and reference methods, 100/160 spiked test method samples were positive and 72/160 spiked reference method samples were positive, with no false positive and two false negative results by the test method at a short incubation time (one from ground beef and one from lettuce) and no false negatives at extended incubation times.

Significance: This data indicates that this PCR method for the detection of *E. coli* O157:H7 is as effective as culture-based methods while providing significant time and labor savings.

P2-17 Evaluation of New Technology to Detect *Escherichia coli* O157:H7 in Ground Beef and Spinach Using a Novel Food Security System

Jenkyn E. Powell, Kristine Klemens, TRACI HAYES, Dorn L. Clark, Jefferey J. Koziczkowski, Roy P. Radcliff, Haleigh Millward, Stephanie Thatcher, Kelly Winterberg and Abby Bird
Idaho Technology, Salt Lake City, UT, USA

Introduction: The *E. coli* O157:H7 LT Food Security System (FSS) is a PCR-based detection method that rapidly and specifically identifies *E. coli* O157:H7 in food. The entire procedure takes under 10 h to return positive or negative results with the PCR portion taking less than an hour. The method includes: a single 8-to-9 h enrichment of samples, lysis of bacteria to release DNA, amplification and melting of target DNA in Idaho Technology's R.A.P.I.D. LT instrument, internal amplification controls, and automatic interpretation of results by the system software. Samples may be tested individually, or pooled after enrichment and tested.

Purpose: The *E. coli* O157:H7 LT FSS was evaluated for sensitivity, specificity, ruggedness and reagent stability in an AOAC Performance Tested Methods study. The system was compared to the reference methods for both raw ground beef and uncooked spinach.

Methods: Samples of ground beef and spinach (25 g) were inoculated at levels to provide recovery of ~1 CFU of *E. coli* O157:H7 per 25-g sample after equilibration. All enrichments were performed in Buffered Peptone Water (BPW) at 42°C and were incubated and tested after 8 h for individual samples and 9 h for pooled samples (50 mL from 1 inoculated to 50 mL from 4 uninoculated samples). Results were compared statistically to unpaired sample sets that were tested with the applicable reference method. Specificity testing used an inclusivity panel of 60 *E. coli* O157:H7 isolates and an exclusivity panel of 45 non-*E. coli* O157:H7 strains including *E. coli* O157:non-H7 isolates. The ruggedness portion of the study evaluated the system's ability to withstand minor user variations in set-up. The stability evaluation looked at shelf life and lot-to-lot variation.

Results: The *E. coli* O157:H7 LT FSS is equivalent or better than the reference methods for ground beef and spinach. The system detected all 60 *E. coli* O157:H7 isolates and did not detect 45 closely related strains. The system is robust and reproducible as demonstrated by ruggedness and stability studies.

Significance: The *E. coli* O157:H7 LT FSS takes less than 10 h to obtain a result where the reference method can take 3 to 4 days.

P2-18 Development and Preliminary Validation of a New Lateral Flow Immunoassay for Detection of *Escherichia coli* O157:H7 and O157:NM Strains in Foods

PAUL LEWIS, Barry Neiditch, Edan Hosking, Jill Feldpausch, Rebecca Hoerner, Tim Goldy, Paul Norton, Frank Klein, Mark Mozola, Michael Wendorf and Jennifer Rice
Neogen Corp., Lansing, MI, USA

Introduction: A new lateral flow immunoassay (Reveal 2.0) has been developed for specific detection of *E. coli* O157:H7 and O157:NM in foods. Compared with the current Reveal device, the 2.0 test uses a new antibody configuration; a highly inclusive polyclonal antibody is used on the capture line, and a monoclonal antibody specific for O157:H7 and O157:NM strains is used in the colloidal gold conjugate. The device architecture has also been changed and a chemical treatment of the enriched test sample replaces the boiling step. Additionally, a common enrichment medium may be used for both single-shift and overnight determinations, allowing results to be obtained in a time frame ranging from 8 to 24 hours.

Purpose: The purpose of this study was to evaluate the inclusivity and exclusivity characteristics of the new test and to assess the performance of the test in detecting *E. coli* O157:H7 in inoculated beef samples.

Methods: For inclusivity testing, *E. coli* O157:H7 and O157:NM strains were grown in Reveal *E. coli* 2.0 Medium for 8 or 20 hours at 42°C. Tests were performed on 1:10 and 1:100 dilutions. For exclusivity testing, non-O157:H7/NM *E. coli* and other *Enterobacteriaceae* were grown in tryptic soy broth with yeast extract for 8 and 20 hours at 42°C. Tests were performed on undiluted cultures and on 1:10 dilutions. For testing of inoculated beef trim and ground beef samples, 25-g samples were added to 225 ml pre-warmed Reveal 2.0 Medium and incubated at 42°C for 8 or 20 hours and then tested with the Reveal device. A parallel set of samples was enriched and tested according to the procedures of the USDA-FSIS reference culture method. Performance of the Reveal and reference methods was compared by Chi-square analysis.

Results: In inclusivity testing, all O157:H7 and O157:NM strains tested positive. All non-O157:H7/NM *E. coli* strains and other Enterobacteriaceae tested negative. In 4 trials with beef samples, the Reveal and USDA reference methods detected a total of 46 and 52 positives, respectively. The number of positive results by the two methods was statistically equivalent in all 4 trials.

Significance: Results of preliminary validation studies show that the Reveal 2.0 and USDA reference methods have comparable sensitivity in detecting *E. coli* O157:H7 in inoculated beef samples. The Reveal 2.0 method offers the significant advantage of improved specificity. Based on results generated to date, the test does not react with *E. coli* O157:non-H7 strains or with other bacteria that may contain the O157 antigen, e.g., certain strains of *Citrobacter*. Strains of these types are a common cause of false-positive results in some screening methods.

P2-19 ATP-Bioluminescence Immunoassay Detection of *Escherichia coli* O157:H7 in Concentrated Produce Wash

DAWN M. HUNTER, Sonia Magaña, Sarah M. Schlemmer and Daniel V. Lim
University of South Florida, Cell Biology, Microbiology, and Molecular Biology, Tampa, FL, USA

Introduction: *Escherichia coli* O157:H7 is one of the leading causes of foodborne disease outbreaks associated with fresh produce. The infectious dose is low, and the concentration in natural samples is often below the detection limits of most rapid methods. Consumer exposure to this pathogen could be reduced by improved detection techniques.

Purpose: The purpose of this study was to develop a rapid, specific method to detect *E. coli* O157:H7 in fresh produce and discriminate between live and dead cells.

Methods: Lettuce and spinach (1–5 kg) were washed in 50 L of dechlorinated tap water, sieved, and concentrated using an Automated Concentration System to produce retentate. Samples were unspiked or spiked with *E. coli* O157:H7 (10^2–10^5 CFU/ml) prior to concentration. Retentates were secondarily concentrated by syringe filtration and backflushed using sodium phosphate buffer with sodium polyphosphate to recover the secondary retentate (SR). SR were analyzed using an ATP-bioluminescence immunoassay.

Results: Concentration of the produce wash yielded 2 $\log_{10}$ increases in the amount of *E. coli* O157:H7 in spiked samples and 1-2 $\log_{10}$ increases in total CFU/L. Detection of *E. coli* O157:H7 occurred at 10^4 CFU/L post-concentration in all SR samples. Signal-to-noise ratios were on the order of 10^1 RLU for positive samples. Detection was not affected by the presence of fecal coliforms or non-O157:H7 *E. coli* in the retentate. Total assay time was less than 4 h.

Significance: Produce wash concentration increases the total CFU/L to levels within detectable limits of an immunoassay-based system. Detection using an ATP-bioluminescence immunoassay was successful, and is a rapid way to detect live *E. coli* O157:H7 in produce.

P2-20 Limit of Detection Study for the Detection of *Escherichia coli* O157:H7 in Refrigerated Cookie Dough and Its Ingredients Using a Commercial PCR Detection System

CYNTHIA M. STEWART, Michelle Smoot, Brian Kupski, Jeanette Jones-Franklin, Kenya Williams, John Marugg, Sophie Butot and Stephanie Campbell
Silliker, Inc., Food Science Center, South Holland, IL, USA

Introduction: In 2009, refrigerated cookie dough was related with the possible presence of pathogenic *Escherichia coli* O157:H7. In order to incorporate additional microbial testing into the over all food safety plan, validation of methodology for testing of the raw dough and its ingredients were necessary.

Purpose: The objective of this study was to evaluate the limit of detection (LOD) of the BAX® system *E. coli* O157:H7 MP assay to detect *E. coli* O157:H7 in cookie dough and its ingredients. The FDA BAM method served as the reference method.

Methods: *E. coli* O157:H7 was inoculated into cookie dough and its ingredients and was stored to allow for adaptation of the microorganism. Portions (25 g) of the inoculated product were transferred to pre-warmed enrichment media and homogenized (5 replicates) to achieve ca. 10^3 CFU/g. From this homogenized sample, 25 ml was transferred to 200 ml pre-warmed enrichment broth + 25 g uninoculated sample and homogenized (ca. 10^2 CFU/g). This was repeated in series 5 additional times. The PCR test method was executed according to manufacturer's instructions and separate sets of samples were prepared and tested according to the FDA BAM method; both methods were followed through confirmation.

Results: A 5-tube Most Probable Number calculation was performed and the results were used to compare the LOD between the PCR test method and the FDA BAM method. For the cookie dough, margarine, egg powder, sugar, molasses and pecans, oats, cocoa powder, wheat flour, coconut, and peanut butter, there was no difference in the sensitivity of the PCR test method as compared to the FDA BAM reference method; the PCR method appeared to be slightly more sensitive with raisins, rice flour and chocolate morsels.

Significance: The BAX® *E. coli* O157:H7 MP test method is comparable in its limit of detection of *E. coli* O157:H7 to the FDA BAM method for the items included in this study, and therefore is suitable for use for the detection of *E. coli* O157:H7 in refrigerated cookie dough and its ingredients.

P2-21 Comparison of *Escherichia coli* O157:H7 Recovery Using Standard and Experimental Enrichment Procedures

MICHAEL A. GRANT
U.S. Food and Drug Administration, Bothell, WA, USA

Introduction: Monitoring of food samples for *E. coli* O157:H7 is challenging because even small numbers of viable organisms have the potential to cause illness. Foods with high background counts of non-pathogenic microorganisms are particularly difficult because these populations may interfere with detection procedures.

Purpose: The purpose of this study was to compare three standard methods and two experimental methods for their ability to selectively enrich small numbers of *E. coli* O157:H7 in food samples with high natural background counts.

Methods: Three strains of *E. coli* O157:H7 were introduced into vegetable rinsates or hamburger so that the initial ratio of target to nontarget cells was at least 1:10,000. Three standard enrichment procedures were used – International Organization for Standardization (ISO), U.S. Department of Agriculture (USDA) and U.S. Food and Drug Administration (FDA). Two versions of an experimental Acid Enrichment procedure were also used. After the specified incubation period, growth of target cells was assessed by viable counts on Rainbow Agar plus tellurite and novobiocin (RTN) and Sorbitol MacConkey Agar plus tellurite and cefixime (TCSMAC), as well as by qPCR detection of stx_1 and *stx*2 genes.

Results: In four of five inoculated foods, either the experimental Acid Enrichment procedure, or a modified version incorporating 2 µmol nitrite yielded the largest viable counts on both RTN and TCSMAC agars. Recovery after the experimental enrichments was typically one to two log units higher than the other methods and ranged from $\log_{10}$ 8.48 to 9.13. Similarly, PCR cycle threshold values ranged from 17.7 to 21.4 for *stx*1 and 16.3 to 19.9 for *stx*2, corresponding to approximately 5×10^8 gene copies/ml to 1.1×10^9 gene copies/ml.

Significance: These data suggest that the experimental enrichment procedures may be useful in facilitating detection of *E. coli* O157:H7 in certain food types.

P2-22 Evaluation of Growth Media for Resuscitation of Stressed Enterohemorrhagic *Escherichia coli*

DANIEL ADAMS, Peixuan Zhu, Daniel R. Shelton, Steingrimur Stefansson, Shuhong Li and Cha-Mei Tang
Creatv MicroTech, Inc., Potomac, MD, USA

Introduction: Enterohemorrhagic *E. coli* (EHEC) contamination on fresh produce is a major cause of foodborne illness outbreaks. Detecting EHECs on fresh produce requires enrichment, and this can be impacted by the enrichment medium used, growing and storage conditions that may have stressed the bacterial cells, and the EHEC strain involved.

Poster

Purpose: To identify the optimal enrichment medium for rapid resuscitation of stressed EHEC cells for the major EHEC strains.

Methods: We compared 24 enrichment media. Six EHEC strains were used: four O157:H7, one O26, and one O111. Control experiments were conducted using 200 microliter of medium and 2 microliter of conditioned *E. coli* strain culture. Plates were incubated at 42°C, with OD at 620 nm measured hourly for eight hours. Cells were stressed by subjecting to low pH (pH = 3.0 for 30 min), desiccation (cells dried for 30 min), and nutrient depletion (removing nutrients for 24 hours). The doubling time was based on the increase from OD 0.010 (~10^6 CFU/ml) to 0.100 (~10^7 CFU/ml). Resuscitation time was determined as the time required for stressed cells to reach OD 0.100.

Results: Resuscitation and doubling times varied greatly among the media. Twelve media had doubling times of 18 – 30 min for all six strains under the control conditions. Cells stressed by low pH and nutrient depletion recovered in an average of 90 minutes, while desiccated cells averaged 4 hours. Not all of the media were able to resuscitate desiccated cells within an 8 hour period, suggesting that desiccation renders many cells nonviable. Four media provided the most consistent and rapid resuscitation, doubling times for all stains tested and stressed conditions: LB broth, Creatv's proprietary Media B, NZCYM, and SOC Media.

Significance: Media were identified that are suitable for enrichment of a variety of stressed EHEC strains for produce safety testing.

P2-23 Influence of Liquid or Solid Media Amended with Magnesium and Calcium Ions on Resuscitation of Pulsed Electric Field-injured *Escherichia coli* O157:H7 Cells

DIKE UKUKU
USDA-FSIT-ERRC-ARS, Wyndmoor, PA, USA

Introduction: Consumers are demanding juices that receive no heat or minimal heat treatment for health reasons and convenient for use. Pulse Electric Field (PEF) treatments, a non-thermal process have been reported to injure and inactivate bacteria in liquid foods. However, information on conditions required for resuscitation of PEF injured *E. coli* cells is limited.

Purpose: The purpose of this study was to evaluate the behavior of PEF-injured *E. coli* cells in apple juice and plate count agar amended with Ca^{2+}, Mg^{2+} and 0.1% pyruvate or Catalase.

Methods: Apple juice (AJ, pH 3.8) inoculated with *Escherichia coli* K-12 at 7.8 log CFU/ml was treated with PEF at 32.2 kv/cm, 18.4 A with pulse width of 2.6 µs at 55°C and a flow rate of 120 ml/min. Treated samples were plated (0.1 ml) on Sorbitol MacConkey Agar (SMAC) and Trypticase Soy Agar (TSA) plates to determine percent injury and viability loss. Also, samples were analyzed for leakage of intracellular UV-materials as a function of membrane damage. The PEF treated juice was amended with 0, 1.5 and 3.0 mM calcium and magnesium and 0.1% pyruvate or Catalase, stored at 5 and 23°C for 24 h. Periodically (0, 2, 4, 6, 8, 10, 12 and 14 h), the ability of damaged cells to recover in these media were monitored by plating 0.1 ml on SMAC and TSA plates containing 0, 1.5 and 3.0 mM calcium and magnesium and 0.1% pyruvate or Catalase.

Results: The initial surviving cell populations determined after PEF treatment on TSA plates amended with 0 mM calcium and magnesium and 0.1% pyruvate or Catalase averaged 3.9 log CFU/ml, and the population determined on plates with 1.5 and 3.0 mM calcium and magnesium averaged 4.2 log CFU/ml. Cell populations in all apple juice without Ca and Mg cation decreased from 3.9 log to an average of 1.8 log CFU/ml while the populations in apple juice containing these cations decreased to only 2.7 log during storage at 5 and 23°C for 24 h.

Significance: The results of this study indicate that Ca and Mg cation arrested further inactivation of damaged cells by aiding cellular repair of the PEF damaged surface structure of *E. coli* cells. However, this effect was found to be dependent on the type of cation species and the media used.

P2-24 Optimizing *Escherichia coli* O157:H7 Detection on Lateral Flow Immunoassays Using Concentration and Immunomagnetic Separation

SARAH M. SCHLEMMER, Kelly M. Leach, Sonia Magana and Daniel V. Lim
University of South Florida, Cell Biology, Microbiology and Molecular Biology, Tampa, FL, USA

Introduction: Lateral flow immunoassays (LFIs) are routinely used for detection of bacterial pathogens in the food industry because of their small size and rapid results after sample application. However, sample pre-enrichment of at least 8 hours is required. This enrichment is required because: 1) bacterial pathogens, if present, typically occur at very low concentrations in samples although these levels are sufficient to cause human disease, and 2) LFI limits of detection (LOD) are 10^5 CFU/ml or greater.

Purpose: The purpose of this study was to reduce total LFI time without the use of enrichment, while also optimizing the LFI specificity.

Methods: Produce wash samples, spiked with different concentrations of *E. coli* O157:H7, were processed through a patent-pending Automated Concentration System (ACS) to increase the level of *E. coli* within the samples (retentate). A fraction of each retentate was subjected to immunomagnetic separation (IMS) with beads (Dynal) labeled with anti-*E. coli* O157:H7 antibody (KPL) to further concentrate the target organisms while reducing non-targets. The final sample was tested on the VIP Gold for EHEC LFI (BioControl).

Results: The combined ACS-IMS procedure was completed in 4 h with an LOD of 10^2 CFU/ml in the initial spiked sample. Specificity of the LFIs was initially tested without IMS on samples of the following non-target bacteria at 10^8 CFU/ml PBS: *Aeromonas hydrophila, A. caviae, Citrobacter freundii, Enterobacter aerogenes, E. cloacae, Enterococcus faecium, E. faecalis, E. coli* (non-O157 strains), *Listeria monocytogenes, Proteus mirabilis, Salmonella enterica* subsp. *enterica* serovars Choleraesuis and Typhimurium, *Serratia marcescens, Shigella flexneri, S. sonnei* and *Staphylococcus aureus*. A false positive result was generated by *E. aerogenes*; however, this false positive was resolved by the addition of IMS. The combined ACS-IMS procedure increased the overall target cell concentration by 1000-fold, eliminated the requirement for lengthy enrichment and reduced non-specific binding on the LFIs.

Significance: This procedure could prove useful to the food industry for rapid produce screening prior to product delivery to consumers.

P2-25 Design of a Real-time PCR Assay for Detection of *Listeria* Species in Food and Environmental Surfaces

ROBERT S. TEBBS, Paolo Vatta, Craig A. Cummings, Patrick Zoder, Priya Balachandran, Lily Wong, Arlene Nunez, Pius Brzoska, Manohar R. Furtado and Olga V. Petrauskene
Applied Biosystems, Foster City, CA, USA

Introduction: The genus *Listeria* is composed of eight species; *L. monocytogenes, L. innocua, L. welshimeri, L. seeligeri, L. ivanovii, L. grayi* and two recently discovered new species, given the provisional names *L. marthii* and *L. rocourtiae*. *L. monocytogenes* accounts for nearly all listeriosis cases in humans. *Listeria* spp. testing is an indicator test for the potential growth of *L. monocytogenes*.

Purpose: To create a real-time PCR assay capable of detecting all *Listeria* species in a single reaction mix for use in food and environmental testing.

Methods: Seven *Listeria* strains representing five species were sequenced by the SOLiDTM system. The new sequences, together with publicly available sequences were used for assay development. The assay targets the conserved rnpB gene in *L. monocytogenes, L. innocua, L. ivanovii, L. welshimerii, L. marthii* and *L. seeligeri* as well as the more divergent *L. grayi*, in a single reaction, and includes an internal positive control. Food samples (25 g) were spiked with *Listeria* spp. to generate fractional positive recovery and enriched in Buffered *Listeria* Enrichment Broth for 24 h. Enriched samples were prepared using PrepSEQ™ sample preparation and Real-time PCR was run on the 7500Fast instrument and results analyzed using Rapid Finder Express software (Applied Biosystems).

Results: The assay showed 100% detection of all *Listeria* strains tested (evaluated all *Listeria* species except *L. rocourtiae*), and no detection of a panel of non-*Listeria* species at ~10^8/ml. The PCR efficiency was 100 ± 10% with a limit of detection at 10 genomic copies. Detection of *Listeria* spp. from food matrices (smoked salmon, roast beef, hot dogs, infant formula and milk) showed 100% correlation between real-time PCR results and plate confirmation.

Significance: Real-time PCR allows for detection of *Listeria* spp. in less than 27 h compared to 3 to 4 days for presumptive positive detection of *Listeria* using traditional culture techniques.

P2-26 Evaluation of Quantitative Methods for *Listeria monocytogenes* Using Statistical Process Control Charting

LINDA M. SMOOT, Wendy McMahon and Stefano Colombo
Silliker, Corporate Quality, Columbus, OH, USA

Introduction: *Listeria monocytogenes* is a foodborne pathogen most reportedly associated with ready-to-eat (RTE) foods that can support the growth of this organism. RTE foods containing <100 CFU/g that do not support the growth of this organism are considered low risk. Method uncertainty associated with *L. monocytogenes* enumeration protocols must also be considered when interpreting microbiological results.

Purpose: The objective of this study was to use Statistical Process Control (SPC) Charting to compare enumeration methods for *L. monocytogenes* using a quantified reference material in ultra high temperature (UHT) milk.

Methods: Quantified lyophilized pellets of *L. monocytogenes* were used to inoculate 10 ml of UHT milk. The inoculated samples were enumerated using the ISO 11290-2: 2005 protocol and Rapid *L. monocytogenes* BioRad method as outlined in the manufacturer's instructions. Colony forming units per gram were determined after 24 h of incubation at 37°C. Trials were conducted at seven different laboratories using three different lots of reference material.

Results: Mean values of CFU/g recovered for each of the three lots were determined for both the ISO 11290-2:2005 and Rapid Lm BioRad chromogenic plating methods. The ISO method consistently recovered higher levels of *L. monocytogenes* after 24 h of incubation. Differences in recovery were 0.29, 0.28 and 0.50 $\log_{10}$ CFU/g for lots 1, 2 and 3, respectively. Variation associated with each method was also determined for each of the three lots as defined by the difference between the upper and lower control limits. The ISO method demonstrated equal to or less variation when compared to the Rapid Lm BioRad chromogenic method. Differences in the variation of the two methods were 0.00, 0.16 and 0.31 $\log_{10}$ CFU/g for lots 1, 2 and 3, respectively.

Significance: Differences in overall recovery and variation of *L. monocytogenes* enumeration methods are not well defined. SPC charting can be used to determine method uncertainty and provide information to better interpret results given the low target level of <100 CFU/g in certain RTE foods.

P2-27 Evaluation of a *Listeria* Species Xpress Method for the Detection of *Listeria* Species in Foods: AOAC Official Method Collaborative Study

ROBERT JECHOREK, Ronald Johnson and Jean-Louis Pittet
rtech laboratories, St. Paul, MN, USA

Introduction: Rapid screening for *Listeria* is extremely important in food safety because of the potential for serious disease.

Purpose: The objective of this study was to demonstrate in a multi-laboratory study, the effectiveness of the alternative method for rapid screening of *Listeria* species in a variety of food products when compared to traditional reference methods.

Methods: A collaborative study was conducted to compare the VIDAS® LSX method and the standard cultural methods for the detection of *Listeria* species in foods. Included in the comparative analysis was the optional application of the Heat and Go system and chromID™ Ottaviani Agosti (OAA) chromogenic confirmation media. Six food types were tested: Vanilla ice cream, cheddar cheese, raw ground beef, frozen green beans, deli turkey and cooked shrimp. Each food, inoculated with a different *Listeria* strain at 2 levels and uninoculated test portions, were analyzed by each method. A total of 15 laboratories representing government and industry participated.

Results: In this study 1,890 tests were analyzed in the statistical analysis. There were 483 positive by the VIDAS LSX method using the sample boiling step, 476 positive by the LSX method using the Heat and Go system and 437 positive by the standard culture methods. The OAA chromogenic plates were comparable to the reference method plates in confirming presumptive LSX results. The resulting Chi-square analysis of 0.25 and 0.11 respectively, indicates that overall, there were no statistical differences between the LSX method using a test sample boiling step and the LSX method with the Heat and Go system when compared to the standard methods at the 5% probability level.

Significance: The alternative method provides the detection of *Listeria* species in foods with negative or presumptive positive results in less than 30 h compared to at least 5 days for the cultural methods.

P2-28 Comparison of CASA®, a New Chromogenic Medium and mCCDA, for the Enumeration of *Campylobacter* spp. in Poultry

JÉRÔME THEPAUT and Véronique Boeffard
AES CHEMUNEX, Bruz, France

Introduction: In the European Union *Campylobacter* is the most commonly reported gastrointestinal bacterial pathogen in human. Campylobacteriosis is caused by thermotolerant *Campylobacter* spp. mainly represented by both species *C. coli* and *C. jejuni*. Enumeration and identification of *Campylobacter* species on traditional media are often difficult due to a lack of selectivity and an absence of specific colonies characterization.

Purpose: The aim of this study was to evaluate the performances of CASA®, a new selective chromogenic medium, versus the mCCDA (modified Cefoperazone Charcoal Desoxycholate Agar – ISO/TS 10272-2 Mandatory medium) for the enumeration of thermotolerant *Campylobacter* in poultry samples.

Methods: The specificity of both media (CASA® and mCCDA) was compared by testing 50 *Campylobacter* species (Inclusivity) and 100 non-*Campylobacter* species (Exclusivity). 87 naturally contaminated poultry samples were analyzed according to the ISO/TS 10272-2 protocol to evaluate the relative accuracy, sensitivity and linearity of both media. Typical colonies of *Campylobacter* spp. were enumerated on CASA®

Poster

and mCCDA after 40–48 h of incubation at 41.5°C under micro-aerophilic conditions. Additionally to the ISO/TS 10272-2 required confirmation tests, a *Campylobacter* Latex Test was carried out on one dark red typical colony isolated on CASA®.

Results: Exclusivity data demonstrated a real improvement of the CASA® selectivity compared to the mCCDA. Thirty-six of the non-*Campylobacter* strains grew on mCCDA whereas they were completely inhibited on CASA®. This strong selectivity associated with the growth properties of the CASA® allowed to enumerate *Campylobacter* spp. in 51 positive samples while the reference method only detected 39. For the positive samples detected by both methods, the linearity was shown to be equivalent.

Significance: The use of the CASA® medium is a reliable method for the enumeration of thermotolerant *Campylobacter* in poultry samples. The dark red typical coloration of the colonies and the high level of selectivity allow an easy screening of *Campylobacter* spp. and improve the sensitivity compared to the mCCDA. CASA® associated with the *Campylobacter* Latex Test will reduce significantly the workload in the laboratory for the confirmation of *Campylobacter* species.

P2-29 ISO 16140 Validation of a New Method for Detection of *Campylobacter*

JEAN-LOUIS R. PITTET, Virginie Ewe and Franck Montigon
bioMérieux, R&D, Marcy L'Etoile, France

Introduction: To release food products at the production stage, international rules require use of a reference method or a validated method, certified by a third party organization such as AFNOR, Microval or AOAC.

Purpose: The goal of this study was the validation of the VIDAS *Campylobacter* method for detection of *Campylobacter* in food products and environmental samples, according to ISO 16140 guidelines.

Methods: Food samples, 1/10 diluted in a new ready to use proprietary broth without blood were enriched for 48 hours at 41.5°C prior to immunoassay detection. Microaerobic atmosphere was generated directly into a specific stomacher bag, by addition of a gas generator in a small pocket in the top of the bag. All positive results were confirmed after streaking of the enrichment broth on Campy Food agar. In this study the new method was compared to the ISO 10272-1 reference method.

Results: Good results were obtained for the inclusivity and exclusivity studies with 50 *Campylobacter* and 30 non-*Campylobacter* strains. The 50% detection limit was found to be between 0.2 and 1.8 CFU/25 g for the new method and between 0.2 and 2.4 CFU/25 g for the reference method. A comparative study between the two methods was conducted on 208 products, 64 poultry products, 82 meat products and 62 environmental samples. 97 were found positive by the immunoassay method and 85 by the reference method. The difference in sensitivity (98%/85.9%) was found to be statistically significant.

Significance: The VIDAS *Campylobacter* method showed better performance than ISO 10272-1 method for the detection of *Campylobacter* in poultry and meat products and in environmental samples. It allows release of negative products in 2 days compared to 4 days for the cultural method. The use of a ready-to-use broth without blood and a specific bag to promote the microaerobic atmosphere greatly simplifies and enhances the practicality of the method.

P2-30 Development of a Multiplex Real-time PCR Assay for the Detection of *Campylobacter* Species for Use on a Food Security System Platform

KELLY WINTERBERG, J. Elijah Powell, Sarah Fowden, Wenhua Li, Stephanie Thatcher and Katy Andrews
Idaho Technology Inc., Salt Lake City, UT, USA

Introduction: It is estimated that campylobacteriosis affects over 2.4 million people each year. The United States Department of Agriculture's Food Safety and Inspection Service is currently in the process of setting standards for acceptable levels of *Campylobacter* species.

Purpose: In an effort to meet the upcoming *Campylobacter* species testing regulations, Idaho Technology has developed a quantitative, multiplex, real-time PCR assay for the detection of *Campylobacter* species for use on the R.A.P.I.D.® LT Food Security System (FSS) platform.

Methods: A real-time PCR assay using hybridization probes was designed to detect *Campylobacter* species. This multiplexed assay detects a target and an amplification control in separate fluorescent channels. Assay sensitivity was evaluated on serial ten-fold dilutions of purified nucleic acid. Live organism quantification was evaluated using an external standard curve made from PCR amplified *Campylobacter jejuni* subsp. *jejuni* nucleic acid.

Results: The multiplex PCR assay is sensitive to approximately 10 copies of genomic DNA (85% success and 90% confidence). The linear range of the external standard curve was between 100,000 and 1,000 copies. Standard curves specific to relevant sample matrices are being incorporated into the R.A.P.I.D.® LT FSS software.

Significance: The FSS software is flexible and can be adapted to any regulations that will be established. Incorporation of relevant standard curves into the R.A.P.I.D.® LT FSS software minimizes set-up time and provides a tunable method to detect relevant levels of *Campylobacter* species. Final development of this assay will provide customers with an easy, rapid-testing method for *Campylobacter* species.

P2-31 Simultaneous Detection of *Cronobacter* (*Enterobacter sakazakii*) and *Salmonella* Using a Chromogenic Plating Medium

Jeremy Chenu and JULIAN M. COX
University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Introduction: *Cronobacter* is an occasional contaminant of powdered infant formula (PIF), which has been associated with morbidity and mortality in neonates. It has been recommended that methods be developed for the improved isolation of *Cronobacter* and other PIF-associated organisms, such as *Salmonella*, from dairy powders. During a more general study of foods, *Cronobacter* was found to grow well on CHROMagar Salmonella (CAS).

Purpose: The aims of this study were to evaluate the isolation of *Cronobacter*, as well as the simultaneous detection of *Salmonella* and *Cronobacter*, on CAS.

Methods: The colonial appearance of a collection of food, clinical and environmental *Cronobacter* isolates as well as a range of non-*Cronobacter* isolates on CAS was determined. Growth in various enteric enrichment broths of *Cronobacter* alone or in co-culture with *Salmonella* Typhimurium was assessed. Predominantly dried foods, including PIF, were artificially inoculated with low levels (1–10 CFU) of *Cronobacter* and the detection on CAS compared to the recommended FDA method.

Results: All *Cronobacter* isolates produced 1–2 mm dark (Prussian) blue colonies after 24 h at 37°C with development of a diffuse purple halo between 24 and 48 h. Some related enteric bacteria were similarly pigmented, but only after 48 h. *Cronobacter* grew well in EE, RV and

TT broths, alone or in co-culture with *S.* Typhimurium. Both organisms were recovered on CAS after pre-enrichment in BPW and selective enrichment in RV and TT from a range of co-inoculated foods, including PIF. This method was slightly better than that advocated by the FDA for detection of *Cronobacter*.

Significance: The results indicate that CAS is suitable not only for the isolation of *Cronobacter*, but also for the simultaneous detection of *Cronobacter* and *Salmonella* from selected foods using enrichment protocols designed for the latter bacterium. Use of the same enrichment and a single plating medium greatly facilitates the screening of high-risk food products such as dairy powders and their derivatives for two significant pathogens.

P2-32 Growth of *Cronobacter sakazakii* Monitored by Microplate Luminometer

FEI YUAN, Ying Chen, Gui M. Zhao and Hai R. Yang
Food Safety Institute, Chinese Academy of Inspection and Quarantine, Beijing, China

Introduction: *Cronobacter sakazakii* is a member of the family *Enterobacteriaceae*, which used called *Enterobacter sakazakii*. A mortality rate of 50% to 75% has been reported in the literature. Little information exists with respect to the growth characteristics of this organism. In most reports, the growth of *C. sakazakii* was detected by the traditional plating method. The method is time consuming and labor consuming.

Purpose: Experiments were undertaken to determine the growth of *C. sakazakii* under different temperatures by microplate luminometer and plate counting method. 16 strains of *C. sakazakii* of 17 strains (including ATCC 29544) used in this study were isolated from dried infant formula available on the China retail market. And *C. sakazakii* firstly should be divided by Multilocus sequence typing method (MLST). Then a new method was developed to rapidly monitor the growth of *C. sakazakii* in brain heart infusion broth.

Methods: In this paper, ninety-six well microtiter plates were used to cultivate the microorganism, and the growth of *C. sakazakii* were monitored by microplate luminometer based on chromogenic detection as a result of the growth and α-glucosidase production from *C. sakazakiiin* broth containing 5-bromo-4-chloro-3-indoxyl -α-D-glucopyranoside (X-α-D-glucopyranoside.

Results: The correction between *C. sakazakii* counts by conventional plating and microplate luminometer was highly agreeable (R2 = 0.92). From these results, the new rapid method can quickly monitor *C. sakazakii* in brain heart infusion broth, required much shorter incubation times (no more than 10 h) than the conventional plating method (at least 24 h). Although at the end of incubation the amount of *C. sakazakii* strains was similar under 36°, but the growth curve was different. And the further results of 17 *C. sakazakii* strains incubated at 42° indicated that the microplate luminometer method provided a not only more convenience and rapid but also more real-time way to monitor the growth of *C. sakazakii*.

Significance: A new method was developed to rapidly monitor the growth of *C. sakazakii* in brain heart infusion broth. And the new rapid method can quickly monitor *C. sakazakii* in brain heart infusion broth, required much shorter incubation times (no more than 10 h) than the conventional plating method (at least 24 h).The results suggested that this microorganism might be divided into different phenotypes according to their character of growing, just as this microorganism can be divided into different phenotypes according to biochemistry reaction.

P2-33 Efficient Isolation and Identification of Enterotoxigenic *Bacillus cereus* Group

KRISTIN KOTEWICZ, Sandra M. Tallent and Reginald W. Bennett
US-FDA, ORISE, College Park, MD, USA

Introduction: *Bacillus cereus* is a group of ubiquitous facultative aerobic sporeforming Gram positive rods commonly found in soil. The spores frequently contaminate a variety of foods including produce, meat, eggs and dairy products. Foodborne illnesses associated with toxins produced by *B. cereus* result in self-limiting diarrhea or vomiting. The current methods recommended in the FDA Bacteriological Analytical Manual (BAM) to detect the presence of *B. cereus* from potentially contaminated food products include cultivation using differential plating media mannitol egg-yolk polymyxin (MYP) agar and an ELISA based method that identifies the diarrheal toxin.

Purpose: The aim of this study was to improve the method for the isolation of enterotoxigenic *B. cereus* from food products. Confirmation of the bacteria from incriminated foods is complicated by the presence of background flora since *B. cereus* is not competitive with other organisms. Therefore, the challenge of this study was to recover viable bacteria in less time.

Methods: A pilot study evaluated and compared the growth of 50 *Bacillus cereus* isolates grown on MYP to growth of the same isolates on four different media formulations including PEMBA, BCM, Brilliance, and Bacara. Two of the agar formulations, Brilliance and Bacara, satisfactorily inhibited competitive organisms. Further assessments comparing Brilliance and Bacara to MYP included an inclusivity and exclusivity study as well organism enumeration from artificially adulterated food matrices.

Results: Brilliance and Bacara were both selective and differential for *B. cereus*; however, Bacara was the most efficient. Cultivation and detection of *B. cereus* using Bacara was achieved 24 hours earlier than with Brilliance due to a delayed color development. Additionally, Brilliance was inhibitory even against *B. cereus*.

Significance: The BAM method is laborious and time to results can be as long as two weeks. Use of chromogenic agar for routine testing of food samples will simplify detection, quantification and identification of *B. cereus*. Ultimately, consumers will be protected since contaminated food products will be identified quickly.

P2-34 Evaluation of Various PCR Assays for the Detection of Emetic Toxin-producing *Bacillus cereus*

JUNG-BEOM KIM, Jai-Moung Kim, Jae-Ho Choi, Syed M. E. Rahman, Tian Ding, Uranchimeg Purev, Kang-Hyun Choi, Na-Jung Choi and Deoghwan Oh
Kangwon National University, Dept. of Food Science and Biotechnology, Chuncheon Si, Republic of Korea

Introduction: Emetic toxin-producing *B. cereus* has been detected by laborious and costly methods such as boar sperm assay, rat liver mitochondria assay, Hep-2 cell culture assay and HPLC/MS analysis. Recently, the PCR assays, easy and cheap than laborious methods abovementioned, have been reported based on the detection of emetic toxin synthesis related genes.

Purpose: The aims of this study were to estimate and compare the various PCR assyas for the detection of emetic toxin-producing *B. cereus* Korean isolates.

Methods: The PCR assays were performed for entire 160 *B. cereus* strains including 40 emetic toxin producing strains. The emetic toxin producing reference strain used for the positive control was *B. cereus* F4810/72. The enterotoxin producing reference strains used for the negative control were *B. cereus* ATCC 11778, ATCC 21772, and ATCC 14579. PCR primer such as CER, EM1, RE234, CES and Ces3R/CESR2 primers were used to detect emetic toxin-producing *B. cereus*.

Results: All of the species-specific PCR assays showed to be highly specific, but the sensitivity of the assays varied greatly. The accuracy of each primers presented 97.5% (CER), 95.6% (EM1), 96.3% (RE234), 89.4% (CES) and 83.1% (Ces3R/CESR2), respectively.

The sensitivity of CER primer showed the most highly sensitive (100%) than the other primers tested and CES primer had a specificity of 100%.

Significance: The PCR assays tested in this study for identification of emetic toxin producing *B. cereus* should be confirmed using other methods such as a boar sperm assay, rat liver mitochondria assay, Hep-2 cell culture assay and HPLC/MS analysis.

P2-35 Independent Evaluation and Validation of the TEMPO™ STA for Quantitation of *Staphylococcus aureus*

ERIN S. CROWLEY, Patrick Bird, Marianne Torontali, Kiel Fisher, Katherine Goetz, Eric Questel, James Agin and David Goins
Q Laboratories, Inc., Microbiology R&D, Cincinnati, OH, USA

Introduction: The TEMPO™ STA method is an automated system that enumerates coagulase-positive *Staphylococcus aureus* present in food products in 24–27 hours with no need for confirmation. The method works by combining the sample with a selective culture medium. The inoculated medium is introduced into a test card containing 48 wells across 3 different volumes. The culture medium contains an indicator which, when neutral, emits a signal detected by the TEMPO reader. The *S. aureus* present utilize nutrients in the medium during incubation, resulting in a decrease in pH and extinction of the fluorescent signal. The TEMPO™ system enumerates the *S. aureus* present according to a calculation based on the Most Probable Number (MPN) method.

Purpose: The purpose of this AOAC™ Research Institute Independent Study was to compare TEMPO™ STA to the AOAC OMA 975.55 Method for the enumeration of *S. aureus*.

Methods: Three matrices were tested including fresh ground beef, cooked diced chicken and vanilla ice cream. Twenty test portions of each food were analyzed by both methods and included 5 replicates of 3 lots that were artificially contaminated at low, medium and high levels with *Staphylococcus aureus* as well as 5 uninoculated controls. A 1:10 dilution of sample was prepared and stomached for 2 minutes. Two test cards per sample were prepared, 1.0 ml and 0.1 ml of diluted sample was added to 3.0 and 3.9 ml of sterile distilled water in the STA medium vial representing 1/40 and 1/400 dilutions, respectively. Cards were filled and sealed by the automated filler and incubated for 24–27 h at 35 ± 1°C. For the reference method, 1 mL for each sample dilution was spread plated onto triplicate plates of BP agar and incubated 45–48 h at 35 ± 1°C. Counts were obtained from typical colonies that were then confirmed for coagulase production according to AOAC OMA 987.09. Final counts were obtained by adding the number of coagulase-positive colonies and multiplying by the dilution factor to obtain the number *S. aureus*/g.

Results: In 9 lots of food tested, there was no significant difference for the mean log counts between the test and the reference methods using a paired t-test at the 95% confidence level. The TEMPO STA had a higher repeatability value in 2 out of 9 lots and there was no difference in the repeatability for 1 lot of ground beef. The AOAC method had a higher repeatability value for 6 lots evaluated.

Significance: The TEMPO™ STA method has demonstrated reliability as a rapid, automated enumeration method of *Staphylococcus aureus* in foods by providing results in 24 hours compared to 48 hours for traditional methods.

P2-36 ISO 16140 MicroVal Evaluation of a Chromogenic Medium for the Enumeration of Coagulase-positive Staphylococci

Mieke Uyttendaele, Anja DeLoy-Hendrickx, Dirk Berkvens, Niko Speybroeck, Alastair Thomas and JONATHAN CLOKE
Oxoid, Basingstoke, United Kingdom

Introduction: Oxoid *Brilliance*™ Staph 24 Agar is a new chromogenic medium for the enumeration of coagulase-positive Staphylococci (CPS) from foods within 24 h. Traditionally, Baird-Parker Agar supplemented with Egg Yolk and Tellurite (BPA) has been used for enumerating CPS, but positive colonies are often difficult to interpret because of the presence of typical and atypical colonies of staphylococci, both of which require confirmation.

Purpose: Brilliance Staph 24 Agar was evaluated against BPA for the enumeration of CPS from five identified food categories detailed in ISO 16140:2003 and according to the MicroVal validation rules.

Methods: Brilliance Staph 24 Agar was evaluated against BPA for the enumeration of CPS from five identified food categories detailed in ISO 16140:2003. Testing was performed according to ISO 6888-1:1999. Subsequent to the expert laboratory study, an interlaboratory study was conducted across 11 laboratories using eight blind samples of pasteurized milk, in accordance with ISO16140:2003.

Results: Brilliance Staph 24 Agar showed equivalence to BPA with dairy, meat, seafood, bakery products and composite/Ready-to-Eat food samples. Results for inclusivity, limit of detection and quantification limit (LOD = 2, LOQ = 4) were equivalent for the reference and alternative methods. Exclusivity testing of *Brilliance* Staph 24 Agar showed it to be more specific than BPA, with no false positive results (0/48) compared to BPA, where 13/48 non-CPS gave typical/atypical colonies. Statistical analysis demonstrated that *Brilliance* Staph 24 Agar showed excellent linearity and accuracy. The relative accuracy of the reference and alternative methods were shown to be equivalent (R = 0.999) for all food categories analyzed. *Brilliance* Staph 24 Agar and BPA were demonstrated by the collaborative study to be comparable both in terms of repeatability (no significant Fr- value for all inoculum levels) and reproducibility (no significant FR- value for all inoculum levels).

Significance: Brilliance Staph 24 Agar proved to be a suitable alternative to BPA in both the expert and collaborative laboratory studies of this ISO16140 validation. The new medium showed greater specificity than BPA and enabled accurate counts to be obtained within 24 h.

P2-37 ISO 16140 Validation Study of the *Listeria* Precis Method for *Listeria monocytogenes* Detection in Foodstuffs and Environmental Samples: Interlaboratory Study

Danièle Sohier, Maryse Rannou, Yann Le Bihan, Alastair Thomas and JONATHAN CLOKE
Oxoid Ltd., Basingstoke, United Kingdom

Introduction: The Oxoid Listeria Precis™ method combines the benefits of: - ONE Broth-Listeria, a specific medium improving *Listeria* recovery and background microflora inhibition. - *Brilliance*™ Listeria Agar, a chromogenic and selective medium. Characteristic colonies are easily and rapidly confirmed with a simple O.B.I.S. Mono test or the tests outlined in the ISO 11290 standards.

Purpose: Comparison of this alternative method with the ISO 11290-1 standard was performed according to the ISO 16140:2003 standard and the AFNOR technical rules. The methods comparative study has clearly shown comparable relative accuracy, selectivity, specificity and detection limits of the Listeria Precis method with the standard method, as well as the inclusivity and exclusivity results.

Methods: In order to assess the variability of the results, a ring trial involving 12 laboratories was organized using pasteurized milk samples. 8 non-contaminated samples and 16 artificially contaminated samples were analyzed by both the Listeria Precis and ISO 11290-1 reference methods by each laboratory.

Results: Two hundred eighty-eight data points were generated, gathering 191 positive agreements, 96 negative agreements, 0 negative deviation and one positive deviation, which was confirmed using the ISO 11290-1 tests. The calculated accordance, concordance and odds ratio of the Listeria Precis and ISO 11290-1 reference methods are in agreement. According to the ISO 16140 standard, the relative accuracy, sensitivity and specificity were 100%, 99.7% and 100%, respectively, confirming the methods comparative study results.

Significance: The interlaboratory study clearly shows that precision in the Listeria Precis method is equivalent to the ISO 11290-1 standard and represents a valuable alternative and user-friendly method for *Listeria monocytogenes* detection in foodstuffs and environmental samples. The Listeria Precis method offers important economic savings to laboratories by minimizing the time taken to obtain results and reduces the number of experimental steps compared to other methods.

P2-38 Non-selective Short-term Enrichment and Real-time PCR Methodologies to Detect Low Levels of *Salmonella* spp. and Shiga-toxigenic *Escherichia coli* on Leafy Greens

FAITH CRITZER, Rebekah Turk, Ali Aydin, Walid Alali and Jennifer Cannon
University of Georgia, Center for Food Safety, Griffin, GA, USA

Introduction: Outbreaks involving fresh produce have highlighted the need for improved detection methodologies for low levels of foodborne pathogens on these commodities. Methodologies should seek to improve sensitivity as well as speed in which results are obtained.

Purpose: A study was conducted to determine non-selective short-term enrichment protocols that could be used to detect Shiga toxigenic *Escherichia coli* and *Salmonella enterica* on leafy greens using real-time PCR detection.

Methods: Salmonella Tennessee and *E. coli* serotypes O145, O157, O26, O111, and O103 were separately inoculated onto 25 g of Romaine lettuce at a population of 1 to 9 CFU/25 g and allowed to attach for 1 h. The 25 g samples were then mixed with 225 ml of Buffered Peptone Water (BPW) or Universal Preenrichment Broth (UPB) and allowed to incubate at 37°C for 10 h before DNA extraction utilizing the standard protocol for the Food Extraction Pack (Pall GeneSystems; Bruz, France). Afterwards, DNA samples were analyzed with the GeneDisc Cycler™ (Pall GeneSystems; Bruz, France) using the *Salmonella* spp., *E. coli* O157 and STEC GeneDisc plate™ followed by analysis with the EHEC Identification GeneDisc plate™. Three samples were analyzed for each treatment combination and each experiment was replicated three times (n = 9).

Results: Low levels of contamination (1 to 9 CFU/25 g lettuce) of each target pathogen were detected after 10 h of enrichment. BPW was able to support growth of *E. coli* O157, O26, O111, O103, and O145 and *Salmonella* Tennessee. Subsequent molecular detection using the GeneDisc platform was able to identify positive samples.

Significance: This methodology can be used for detection of low levels of five serotypes of Shiga-toxigenic *E. coli* as well as *Salmonella* on leafy greens, and results were obtained within 12 h of beginning the enrichment procedure.

P2-39 Validation of PCR Assays for Screening *Salmonella* and *Listeria monocytogenes* in Breaded Stuffed Poultry Products

LIHONG L. WU and Andrew Farnum
DuPont Qualicon, Wilmington, DE, USA

Introduction: Salmonella and *Listeria monocytogens* are well known human pathogenic organisms that cause outbreaks of foodborne disease. With the increasing variety of poultry products, there is a corresponding increased demand for the food industry and relevant government bodies to apply effective detection methods that are rapid, accurate and easy to use.

Purpose: The objective of this study is to validate a PCR-based assay for detecting artificial *Salmonella* or *L. monocytogenes* contamination in breaded stuffed chicken products 48 h (*Salmonella*) or 24 h (*L. monocytogenes*) sooner than the reference culture method.

Methods: Twenty 25-g test portions of each food product, chicken cordon bleu and chicken breast stuffed with broccoli and cheese, were inoculated with either *Salmonella* or *L. monocytogenes* at target levels set to yield fractional positive results. Samples spiked with *Salmonella* were enriched in BPW for 22 h. Samples spiked with *L. monocytogenes* were enriched in Demi-Fraser broth for 24 h, then transferred to MOPS-BLEB for 20 h. Five unspiked portions of each food product were also included for each assay tested. Enrichment samples were evaluated using the PCR method and USDA-FSIS MLG method.

Results: For *Salmonella* spiked samples, the PCR assay detected 13 positives in the chicken cordon bleu and 10 positives in the chicken breast stuffed with broccoli and cheese. For *L. monocytogenes* spiked samples, the PCR assay detected 7 positives in the chicken cordon bleu and 6 positives in the chicken breast stuffed with broccoli and cheese. All positive results detected by PCR assays were confirmed by the reference method (USDA-FSIS MLG).

Significance: This study demonstrated the effectiveness of PCR assays for more rapid screening for the presence of *Salmonella* or *L. monocytogenes* in breaded stuffed poultry products with equal sensitivity and specificity to the USDA-FSIS MLG reference method.

P2-40 Validation of PCR Assays for Screening *Salmonella* in Pet Chews

LIHONG L. WU and Andrew Farnum
DuPont Qualicon, Wilmington, DE, USA

Introduction: Pet chews contaminated with *Salmonella* can be a serious health hazard. *Salmonella* is infectious to pets and can be transferred to persons handling pet treats. Effective assays for screening *Salmonella* contamination in pet treats are needed to improve product quality.

Purpose: The objective of this study is to validate a PCR-based assay for detecting artificial *Salmonella* contamination in pet chew products, including rawhides and pig ears, up to 48 h sooner than the traditional culture method (FDA-BAM).

Methods: Twenty 25-g test portions each of rawhides and pig ears were inoculated with *Salmonella* at target levels set to yield fractional positive results. Five unspiked test portions were also included in each test. Samples were incubated in lactose broth for 24 h. For the PCR method, lysates were prepared directly from primary enrichments and analyzed by the PCR method. For the FDA-BAM culture method, the primary enrichment was transferred to TT and RV broth and incubated for another 22 to 24 hours before streaking on BS, XLD and HE agar plates.

Results: The PCR method detected 5 positives in the 20 spiked rawhide samples, and 14 positives in the 20 spiked pig ears samples. The results of the PCR method were 100% identical to the results of the reference FDA-BAM method.

Significance: The results strongly support the effectiveness of PCR method for detecting *Salmonella* contamination in rawhides and pig ears. It offers a rapid, specific and user-friendly assay for the pet industry to monitor and limit contamination issues in pet treats.

P2-41 Evaluation of a High Throughput DNA Extraction Protocol Followed by Real-time PCR Detection of *Salmonella* and *Listeria monocytogenes*

MICHAEL CLARK and Matthew Fuller
Chestnut Labs, Springfield, MO, USA

Introduction: The iQ-Check kits are ready-to-use, real-time polymerase chain reaction (PCR) kits used for the detection of several foodborne pathogens. The high throughput DNA extraction protocol uses a 96-well deepwell microplate, reducing the overall time involved with high sample volumes compared to processing samples in a single tube format.

Purpose: The objective of this study was to compare a high throughput DNA extraction protocol for *Salmonella* spp. and *Listeria monocytogenes* to the appropriate US reference method for each food matrix. Matrices included deli turkey for *L. monocytogenes* and raw chicken breast, raw ground beef, raw pork and fresh spinach for *Salmonella*. An 8-h enrichment for *Salmonella* in buffered peptone water was also tested with the high throughput extraction.

Methods: Each matrix was spiked with the target organism and an MPN determined. Twenty inoculated and five uninoculated samples were tested using the high throughput extraction protocol followed by real-time PCR detection and an appropriate standard reference method. Confirmation followed the standard reference protocol. For each *Salmonella* matrix, with the exception of fresh spinach, the samples were paired and a Chi-square analysis was calculated according to McNemar. The *L. monocytogenes* and *Salmonella* fresh spinach samples were unpaired, and a Chi-square analysis was calculated according to Mantel-Haenszel.

Results: For each matrix tested there were no significant differences between the high throughput extraction protocol followed by real-time PCR detection and the reference methods using the Chi-square test at a 95% confidence level.

Significance: The high throughput DNA extraction protocol is able to meet the demands of high sample volumes by reducing manual transfer times with the utilization of multichannel pipetting and combining steps to eliminate potential bottleneck areas.

P2-42 Comparison of RNA Extraction Methods for the Detection of Noroviruses from Ready-to-Eat Foods

MARYLINE GIRARD and Julie Jean
Université Laval, Food Science and Nutrition, Quebec, QC, Canada

Introduction: Enteric viruses are more and more involved in foodborne outbreaks in humans worldwide with ready-to-eat foods being one of the major vehicles of transmission. Because enteric viruses are often present in very low concentration in foods, the development of methods allowing a low detection limit is still urgently needed. However, the complexity of food matrices as well as the presence of inhibitors make this objective not easily achievable.

Purpose: The objectives of this study were (1) to evaluate different methods for the extraction of viral RNA from various ready-to-eat foods and (2) to evaluate the compatibility of the recovered RNA with RNA amplification methods.

Methods: Strawberries, lettuce, sliced turkey, soft shell clams and potato salad samples were experimentally contaminated with murine norovirus (surrogate of human norovirus), at high (10^5 PFU/ml) and low (10^3 PFU/ml) concentrations. Viruses were then recovered after PEG concentration and their RNA were extracted using miniMAG, Basic kit or TriReagent. The viral RNA was detected by real-time RT-PCR.

Results: A better yield was achieved with the miniMAG for lettuce and sliced turkey, while there was no significant difference between the three methods for the potato salad. For strawberries, the basic kit and the miniMAG offer higher efficacy for the extraction of viral RNA compared to TriReagent which seems to be significantly affected by the presence of inhibitors. No difference was observed between TriReagent and miniMAG for the extraction from soft shell clams. No significant difference was observed between the methods between the low and high viral concentrations.

Significance: The RNA extraction methods tested did not allow us to choose an universal method for the detection of norovirus from various food matrices and the extraction method is food dependent.

P2-43 Evaluation of the 3M™ Petrifilm™ High-sensitivity Coliform Count Plate for Enumerating Coliforms in Pasteurized Milk Products

ADRIANA R. TASSINARI, Juliana Webster and Cristina Abreau
3M do Brasil Ltda, Microbiology, Sumare, Brazil

Introduction: Traditional methodologies used for enumeration of coliforms in dairy products are time consuming, usually taking up to four days. According to the American Public Health Association [APHA], the choice of method will depend on limits for the number of coliforms in which the analyst is interested, as well as materials required, laboratory facilities and labor available to process the required volume of work. In Brazil, the Ministry of Agriculture, through Normative Instructions IN 51 and IN 62, establishes that pasteurized milk should be analyzed by MPN methods to assure sensitivity in the results.

Purpose: This study was initiated to evaluate the efficacy of the 3M™ Petrifilm™ High Sensitivity Coliform Count plate (HSCC) for enumerating coliforms in naturally-contaminated pasteurized milk samples compared to traditional MPN techniques, according to IN 62 requirements from Brazilian Ministry of Agriculture that follow APHA recommendations for the MPN method.

Methods: Different brands of pasteurized milk samples were collected in the São Paulo state market and simultaneously analyzed using both methods: MPN techniques for coliforms, using LST and BGB broth at 35°C/48 h for each broth and 3M Petrifilm HSCC plates at 35°C/24 h.

Results: The results were compared and showed that the 3M™ Petrifilm™ HSCC plate was 99% (112/113) equivalent to the MPN method, considering the upper and lower 95% confidence limits. The main advantage was that the results were obtained in 24 hours instead of 96 hours, showing high sensitivity and accuracy. Another important consideration is that, about 19% (21/113) of the pasteurized milk products were sold out of the Brazilian Ministry of Agriculture specifications (considering MPN results alone), probably because they were delivered before the results were obtained.

Significance: The 3M Petrifilm HSCC plate is an alternative method to the traditional enumeration of coliforms in pasteurized milk products. This method would help the dairy industry obtain results in a more timely and easy manner, thereby meeting the Brazilian Ministry of Agriculture requirements.

P2-44 Rapid Enumeration Methods for Fungi in Fruit by the Most Probable Number Method

Maiko Watanabe, YUKIKO HARA-KUDO, Fumiyuki Tsutsumi, Ken-ichi Lee, Susumu Kumagai, Yoshiko Sugita-Konishi, Kosuke Takatori and Hirotaka Konuma
National Institute of Health Sciences, Division of Microbiology, Tokyo, Japan

Introduction: Fruit in agricultural fields is often contaminated by fungi and becomes rotten due to fungal contaminants. Determination of the fungal contamination level before significant growth is important for controlling fruit spoilage. However, the determination of fungi in foods by surface plating methods requires a long period of incubation.

Purpose: The purpose of this study was to evaluate rapid enumeration methods for detecting fungi in fruit using the most probable number (MPN) method.

Methods: The surface skins (5 g) of 27 commercial domestic fruits were homogenized with 45 ml of potato dextrose broth (PDB). The samples were tested by the standard MPN method with PDB in tubes, the plate-MPN method with potato dextrose agar (PDA) medium, and the surface plating method with PDA medium. After incubation for 2, 4, 7 and 10 days at 25°C, the fungal counts were performed.

Results: The standard MPN method resulted in a slow recovery of fungi in tubes and lower counts than the surface plating method and plate-MPN method. The fungal count on the fourth day of the incubation was approximately the same as the tenth day using the surface plating method or the plate-MPN method. Because the quantitative values are estimated based on the number of plates with one or more growing colonies in the plate-MPN method, the statistical procedure in this method can provide more accurate counts than counting the number of colonies in the surface plating method. Moreover, the plate-MPN method is a less laborious process.

Significance: These results demonstrate that the plate-MPN method is a rapid and effective method for the quantification of fungi.

P2-45 Evaluation of the TEMPO® YM Automated MPN Method for the Enumeration of Yeasts and Molds in Foods

JOHN MILLS, Judith Colon-Reveles, Ron Johnson and Gregory Devulder
bioMérieux, Inc., Hazelwood, MO, USA

Introduction: The TEMPO® YM method was developed for the automated enumeration of yeasts and molds in foods. The method utilizes a selective dehydrated culture medium and a Yeast and Mold (YM) enumeration card containing 48 wells across three dilutions for the automatic determination of the Most Probable Number (MPN).

Purpose: As part of the AOAC® Research Institute validation process, the alternative method was compared to the FDA Bacteriological Analytical Manual (BAM) for all foods.

Methods: Ten naturally and artificially contaminated foods were tested including fruits, dairy, dry products, and nuts. Five replicates of three lots for each food were tested for a total of 150 samples. A 1:10 dilution of each sample was prepared and stomached for 2 min. For each diluted and stomached sample, 1.0 ml of diluted food sample was added to a medium vial that had been reconstituted with 3.0 ml of sterile distilled water. The inoculated medium in the vial was then transferred and sealed into the YM card by the automated filler. Inoculated cards were incubated for 72 – 76 h at 25 ± 1°C and then read using the automated reader. Standard method testing was performed as detailed in the BAM.

Results: The enumeration values obtained from both methods were converted into $\log_{10}$ and an overall bias was determined. A 95% confidence interval of the bias was then calculated. The calculated confidence interval contains the integer 0; therefore, it can be concluded that no significant bias was observed between the methods evaluated in this study.

Significance: Traditional YM methods take five or more days to complete and often are the limiting factor in release of raw ingredients of finished products. The TEMPO YM provides an automated method for the enumeration of yeasts and molds in foods, which can be completed in three days with considerable time, and labor savings when compared to the reference method tested in this study.

P2-46 Development of a Plate-pair Strategy for the Identification of Lactic Acid Bacteria from a Mixed Population of Kefir Bacteria and Yeasts

CHERYL A. BAXA, Edmund M. Powers and Greg M. Burnham
U.S. Army Natick Soldier Research, Development and Engineering Center, DoD Combat Feeding Directorate, Food Safety and Defense Team, Natick, MA, USA

Introduction: Kefir is a fermented dairy drink produced by bacteria (primarily lactic acid bacteria) and yeasts in kefir grains. The symbiotic association between the bacteria and yeasts in the grains has resulted in a microbial population with similar growth characteristics, making isolation and identification of distinct genera difficult. We have observed that media purported to be selective for lactobacilli (MRS) and lactococci (M17) in fact support the growth of kefir lactococci and lactobacilli, respectively, as well as kefir yeasts.

Purpose: To identify a method using different selective and or differential media to ensure accurate identification of kefir lactic acid bacteria and yeasts from a mixed population.

Methods: Representative kefir bacteria and yeasts were plated as pure isolates or cocktails on nine types of selective and or differential media [MRS, M17, Potato Dextrose (PD), MRS + Cycloheximide (MRS+C), M17 + Cycloheximide (M17+C), Raka-Ray No. 3 Medium, Lactobacillus Selection (LBS), Lactobacillus Anaerobic MRS with Vancomycin and Bromocresol Green (LAMVAB), and Lactobacillus Streptococcus Differential Medium (LS)]. Growth was evaluated after two and four days incubation at three isothermal temperatures (25, 30, and 37°C) in the presence and absence of oxygen.

Results: Several yeasts were resistant to the antifungal cycloheximide at concentrations that inhibited growth of lactobacilli (MRS+C). LS did not differentiate between lactobacilli and lactococci; additionally it supported growth of yeasts. Raka-Ray, like MRS, supported growth of lactococci. Both LBS and LAMVAB were completely selective for lactobacilli; however, both supported growth of yeasts. Yeasts presented on LAMVAB as blue colonies, differentiating them from lactobacilli. PD was completely selective for yeasts. These results, in combination with specific incubation conditions, lead to the development of a plate-pair strategy whereby kefir lactobacilli and lactococci are effectively differentiated from kefir yeasts.

Significance: Our plate-pair strategy enables accurate identification of specific genera from mixed microbial populations in complex food matrices like kefir.

P2-47 Validation of New Sensitive ELISA Test Kits for Food Allergens

DONNA HOUCHINS, Elisabeth Halbmayr and Michael Zheng
Romer Labs, Inc., Union, MO, USA

Introduction: Food Allergy, typically an immune system response to a protein present in food that the body mistakenly believes is harmful, represents an important health problem in modern society. Cross-contamination during the production process may occur so that residues of food allergens in different products may be present. Worldwide labeling regulations lead to more accurateness for food manufacturers, although hidden allergens continue to be the largest single cause of global product recalls.

Purpose: With the aim of preventing health hazards by food allergy, Romer Labs® offers AgraQuant® Allergen ELISA Test Kits to sensitively detect food allergens in a wide range of processed foods and raw materials.

Methods: The AgraQuant® Allergen Test Kits are sandwich enzyme-linked immunosorbent assays (ELISA). Food allergen proteins, extracted from food products with an extraction buffer, bind to specific polyclonal antibodies pre-coated on the surface of a microwell. After a washing step an enzyme-conjugated antibody binds to captured specific food allergen proteins. The applied enzyme substrate develops a blue color. The reaction is then stopped by adding an acidic stopping solution, turning the color into yellow. Using a microwell reader the color intensity is determined and is directly proportional to the concentration of the food allergen in the sample.

Results: AgraQuant® Peanut and AgraQuant® Hazelnut have quantitation ranges of 1–40 ppm and detection limits of 0.1 ppm peanut and 0.3 ppm hazelnut. The quantitation range of AgraQuant® Gluten is 4–120 ppm gluten and limit of detection was determined to be 0.6 ppm gluten. AgraQuant® Soy has a quantitation range of 40–1000 ppb and a limit of detection of 16 ppb. AgraQuant® Almond and AgraQuant® Egg white have quantitation ranges of 0.4–10 ppm and detection limits of 0.2 and 0.05 ppm. AgraQuant® Walnut and AgraQuant® Beta-Lactoglobulin have quantitation ranges of 2–60 ppm and 10–400 ppb and detection limits of 0.35 ppm walnut and 1.5 ppb beta-lactoglobulin.

Significance: Extensive validation studies indicated low detection limits, good accuracy, precision and recovery of the Test Kits.

P2-48 Comparison of Different Magnetic Nanobeads for Simultaneous Immunoseparation of Multiple Foodborne Pathogens

HONG WANG, Yanbin Li and Michael Slavik
University of Arkansas, Poultry Science, Fayetteville, AR, USA

Introduction: Sample preparation is critical in simultaneous detection of multiple pathogenic bacteria in foods. Recently, immunoseparation have been applied with nanomaterials.

Purpose: The objective of this study was to compare two different sizes of magnetic nanobeads (MNBs) for their effectiveness in simultaneous immunoseparation of multiple foodborne pathogens.

Methods: 150 nm and 30 nm streptavidin conjugated MNBs were separately coated with anti-*E. coli*, anti-*L. monocytogenes*, or anti-*Salmonella* antibodies. The conjugated MNBs then were mixed with the food samples containing *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium to capture the three target bacteria. With a magnetic field applied for immunomagnetic separation, the three target bacteria were separated from the food matrix. Then, the MNBs-bacterium conjugates were mixed with the streptavidin modified quantum dots (QDs) that had been conjugated with biotinylated anti-*E. coli*, anti-*L. monocytogenes*, and anti-*Salmonella* antibodies with three emission wavelengths at 530, 580 and 620 nm, respectively. Unattached QDs were removed when a magnetic field applied. Finally, a spectrometer was used to measure the fluorescence of the complexes of MNBs-bacterium-QDs.

Results: The results showed that both sizes of MNBs could specifically and simultaneously capture and separate *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* at concentrations as low as < 10 CFU/ml in different food samples including chicken, beef, and lettuce. When the magnetic field at 0.4 tesla was applied, 150 nm and 30 nm MNBs took 2 min and 40 min to finish the magnetic separation, respectively. The capture efficiency of all three target pathogens in foods was more than 75% and 90% using 150 nm and 30 nm magnetic nanobeads, respectively.

Significance: This research provided very valuable information to enhance the immunoseparation of multiple foodborne pathogens using nanomaterials.

P2-49 Impact of Sample Preparation and Analytical Method for *Escherichia coli* Recovery from Strawberry and Mangetout Pea

CAROLINE CÔTÉ, Mylène Généreux, Michèle Grenier and Karine Seyer
IRDA, St-Hyacinthe, QC, Canada

Introduction: Many preparation and analytical methods are available for the detection of indicator and pathogenic microorganisms on fruits and vegetables, which can affect the recovery of microorganisms.

Purpose: An experiment was conducted to evaluate the impact of sample preparation and analytical method on the recovery of *E. coli* from strawberry and mangetout pea at two inoculum levels.

Methods: A mixture of five *E. coli* strains was used to spot-inoculate strawberry and mangetout pea at final levels of 129 and 1455 CFU/g. Four sample preparation methods were evaluated: washing of whole and cut (1-g pieces) product, stomaching and blending for each inoculum level. *E. coli* populations were determined by Petrifilms™ *E. coli* count plates and direct plating using membrane filters and TBA medium.

A Poisson regression model was fitted to *E. coli* counts and contrasts were performed to evaluate the impact of the variables on *E. coli* populations.

Results: Statistical analysis showed that for the inoculum of 129 CFU/g, the preparation method was the most important variable affecting *E. coli* recovery. Washing of cut product gave significantly higher counts than the other preparation methods for strawberry. For mangetout pea, blending gave the highest counts, but the results were not statistically different from washing of whole and cut product. At high inoculum level, the product and the preparation method had a statistically significant impact on *E. coli* counts. Higher counts were obtained on strawberry compared to mangetout pea. Washing of cut products gave statistically higher results than stomacher, but results were not different from washing of whole product and blending.

Significance: Results emphasize the importance of taking into account the preparation and analytical methods for the determination of *E. coli* populations in fruits and vegetables.

P2-50 Assessing the Performance of Modified Moore Swabs as a Low-cost Method of Large Volume Irrigation Source Surveys

ADRIAN SBODIO, Sharyn Maeda, Mark Trent and Trevor V. Suslow
University of California, Plant Sciences, Davis, CA, USA

Introduction: To improve the predictive value of irrigation source analysis in both research and commercial practice, we utilized a capture-swab similar to the Moore Swab approach used by researchers monitoring sewer effluent, manure run-off, and pathogen-impacted irrigation water. Modified Moore Swabs (MMS) were developed with the intent to analyze for the presence of *E. coli* O157:H7 and *Salmonella* using nonselective enrichment and immunomagnetic separation system (IMS) or membrane filtration coupled with PCR and confirmation plating on selective media.

Purpose: A modification of this sampling strategy was evaluated to re-assess regional irrigation sources and modes of delivery (such as overhead irrigation) to trap the bacteria, either free or attached to suspended solids, by surveying larger volumes of source water than typical grab-samples used for indicator organism (IO) evaluations.

Methods: After filtration of reservoir or irrigation district canal water with MMS, pathogen recovery was accomplished by extracting the MMS from the cassette and placing the saturated swab in a sterile Whirl-Pak® bag holding 200 ml double strength (2X) Universal Pre-enrichment Broth (UPB) followed by an overnight incubation (O.N.) at 37°C. For *E. coli* O157:H7 recovery, 10 ml of enrichment was transferred to 90 ml of mEHEC and incubated at 42°C for 24 h. Biocontrol GDS®-O157 was used for detection. For *Salmonella* detection, 10 ml of 2X UPB enrichment was transferred to 90 ml Tetrathionate Broth Base (TBB) followed by 6-h incubation at 42°C. After incubation 20 ml of sample was transferred into 180 ml of M Broth for a final O.N. incubation at 37°C. BAX®-*Salmonella* and GDS®-*Salmonella* were used for detection.

Poster

Results: Prior conventional surveys were always negative for pathogen presence. With MMS capture in a lab or on-site, *E. coli* O157:H7, 1 out of 31 samples tested positive by GDS®-O157. Culture confirmation was obtained after plating GDS®-O157 immunomagnetic beads on CHROMagar O157 followed by 24-h incubation at 37°C. For *Salmonella*, 7 out 31 samples were positive by BAX®-*Salmonella* but only 3 out 7 were confirmed positives with GDS®-*Salmonella*. Three colonies were isolated following recovery via immunomagnetic separation by GDS®-*Salmonella* beads plated on XLT4.

Significance: MMS are an effective low-cost method for filtration capture of large water volumes for pathogen screening of sources and conveyances.

P2-51 Microbial Testing of the Consumer Environment in Foodservice Establishments: A Pilot Study

MARGARET BINKLEY, Snehal Nanivadekar, Leslie Thompson and Mindy Brashears
The Ohio State University, Consumer Sciences, Columbus, OH, USA

Introduction: In the United States more than 70 billion meals are served at 945,000 commercial food establishments each year. Americans spend almost 50% of their food dollars on restaurant meals, and approximately 44% of adults eat at a restaurant each day. The trend of eating out has increased tremendously in recent years due to challenges faced in working and social life. When people patronize a foodservice establishment, they expect that the food they are receiving is safe and served in a clean and sanitary environment.

Purpose: To determine microbial loads on environmental surfaces in the consumer environment in fast food and casual dining facilities.

Methods: Fast food (20) and casual dining restaurants (14) both chain and independent were used in this study. Five surfaces in the consumer environment were sampled for *S. aureus*, coliforms, and aerobic plate counts (APC) both pre- and post-lunch on two separate days. Sterile 3M Quick swabs or RODAC plates were used. In fast food restaurants, table tops, serving trays, serving counter, door handles inside the women's restroom, and the beverage machine push bar were sampled. In casual dining restaurants, samples were taken on table tops, menus, diaper changing tables or door handles inside women's restroom, high chairs, and condiment bottles.

Results: Microbial loads for *S. aureus* and APCs were similar pre- and post-lunch for most surfaces regardless of facility type. For table tops both pre- and post-lunch, the average APC count in fast food and casual dining was 147.4 CFU/50 cm. Higher microbial loads on table tops in fast food (*S. aureus* = 62 CFU/50 cm, coliforms = 12 CFU/50 cm) were observed compared to table tops in casual dining (*S. aureus* = 37.6 CFU/50 cm, coliforms = 1.2 CFU/50 cm, $P \le 0.05$). Aerobic plate counts on high chairs in casual dining were high, averaging 808.0 CFU/50 cm. *S. aureus* and coliforms were frequently found on diaper changing tables. Menus and condiment bottles tended to have lower bacterial counts.

Significance: Results indicated that sanitation practices in the consumer environment in both fast food and casual dining restaurants could be improved. These results provide valuable baseline information on microbial loads that could be used for future studies evaluating interventions designed to improve the sanitation practices related to the consumer environment.

P2-52 Further Validation of the Specificity of a Real-time PCR MPN Enumeration Method for Foodborne *Listeria monocytogenes*

ANTHONY D. HITCHINS
FDA-CFSAN, HFS-711, College Park, MD, USA

Introduction: A real time polymerase chain reaction assay (RTiPCR) for MPN enumeration of foodborne *Listeria monocytogenes* was developed using primers from a published PCR method for *L. monocytogenes* detection. The RTiPCR method was initially validated with respect to primer pair target specificity by testing inclusivity with *L. monocytogenes* (15 strains) and exclusivity with 15 strains of other *Listeria* species. The observed 100% specificity corresponded to that reported for the original method.

Purpose: In order to fortify the validity of the claimed primer specificity of the RTiPCR, it was necessary to study additional strains because one of four laboratories reported significant cross-reactivity.

Methods: Inclusivity was tested with *L. monocytogenes* (25 strains) and exclusivity with 15 near neighbor strains: *L. innocua* (2), *L. welshimer*i (2), *L. seeligeri* (4), *L. ivanovii* (4) and *L. grayi* (2). Seven strains from other Gram positive genera were also tested. DNA was extracted from the cells by heating at 98°C for 10 min. The amounts of cells to be extracted were standardized turbidometrically. RTiPCR was conducted with a Cepheid Smart Cycler instrument using a SYBR Green PCR mix and the L1 and L2 primers of Wang et al. RTiPCR reaction efficiencies were determined from the slopes of C_t versus log cell level curves.

Results: Inclusivity was 100%. Exclusivity was 100% too but only if a new criterion for the lower C_t limit for a negative test result was conservatively reset at 35 cycles instead of 45 cycles. Some non-*monocytogenes* strains of *Listeria* species behaved as if they virtually contained 1 *L. monocytogenes* target per 10^4 or more test targets. However, strain re-purification did not eliminate this very weak cross reaction. The efficiency of amplification of this cross-reaction was the same as *L. monocytogenes*. The presence of non-*monocytogenes* target 16S-rDNA mutants, while possible, was considered an improbable explanation. The remaining possibility of RTiPCR system errors was supported by studies with combinations of LI and L2 primers and their corresponding alleles.

Significance: It was concluded that the extreme cross-reactivity in the outlier study was due to the very high DNA levels it used. The weak cross reactivity in this study can be easily counteracted by adopting the new C_t criterion and by use of calibrated positive and negative controls. This study suggests that weak cross reactivity may be a factor to consider in validating primer specificity with other RTiPCR targets if there are target alleles in near neighbor species.

P2-53 Factors Affecting the Determinacy of Reproducibility Assessments of Qualitative Foodborne Pathogen Detection Methods

ANTHONY D. HITCHINS
FDA-CFSAN, HFS-711, College Park, MD, USA

Introduction: An important feature of the validation of a qualitative microbiology method is the reproducibility of the method's performance between different laboratories (ideally ≥10). Such a study is financially and logistically burdensome. In addition, analytical portion replication (≥ 6), several target microbe levels (3), and food matrix numbers (1 or more types) add to the burden. Ways to ameliorate this burden invariably meet statistical hurdles.

Purpose: There is apparently no explicit description of the mechanistic basis of the qualitative microbiological method reproducibility validation. The purpose of this study is to define the mechanistic basis in the hope that it will provide way(s) to reduce the experimental design burden.

Methods: Factors involved in interlaboratory studies were reviewed and evaluated for their relative contributions to inter-laboratory method reproducibility. Data from a typical published study were re-examined and reinterpreted using the factors considered to be most important. Experimental 3-tube MPN data were compared with the mean number of CFU per analytical portion calculated using the Poisson equation. Using the MPN and the Poisson counts, the corresponding expected binomial distribution of values for positives per replicate

set among laboratories were calculated. Expected distributions were compared with the observed distribution. Results were statistically evaluated for significance using the 1-sample binomial or Chi-square tests.

Results: Ideally reproducibility studies use sub-samples of a centrally prepared spiked sample. The studies are conducted around the qualitative method's limit of detection, typically 1–2 CFU per analytical portion, which means that the spike level cannot be constant across portions. The determined mean spike level, after equilibration in the matrix, has a broad confidence interval when obtained by a 3 or 5-tube MPN. The interval can be narrowed by using the control method data. Based on such considerations the distribution of the numbers of laboratories obtaining each possible number of positives per replicate set is the best reflection of the mechanics of the validation. This distribution can be compared with the expected binomial distribution for the given mean spike level. If significantly different, inter-laboratory irreproducibility can be suspected.

Significance: This explicit mechanistic interpretation of qualitative microbial method reproducibility data potentially allows the empirical burden of such studies to be reduced substantially since a standard method comparison is not obligatory. This approach requires accurate and precise spike enumeration.

P2-54 Removal of Biofilms Formed by the Cells of Shiga Toxin-producing *Escherichia coli* Using Treatments with Organic Acids and Commercial Detergents

JINRU CHEN

The University of Georgia, Griffin, GA, USA

Introduction: Biofilms are a mixture of bacterial cells and extracellular products secreted by the cells. The biofilms are a great concern of the food industry because they offer physical, mechanical, and biological protection to bacterial cells.

Purpose: This study was undertaken to evaluate whether treatments with organic acids and commercial detergents could significantly remove the biofilms formed by the cells of STEC on their contact surfaces.

Methods: Cells of six STEC strains producing different amounts of cellulose were allowed to form biofilms on a polystyrene or stainless steel surface at 28°C for 7 d. The two types of surfaces with formed biofilms were treated with 2% acetic or lactic acid, as well as a manufacturer-recommended concentration of an acidic and alkaline detergent, respectively. The residual biofilms on the surfaces were determined after the treatments.

Results: Treatments with 2% acetic and lactic acid significantly removed the biofilms formed by the cells of two tested STEC strains on the polystyrene surface. The 2% lactic acid also significantly removed the biofilms formed by the cells of one of the STEC strains on the stainless steel surface. Treatments with the two commercial detergents significantly removed the biofilms formed by the cells of all tested STEC strains on the polystyrene surface. However, the detergents only significantly removed the biofilms formed by the cells of some tested STEC strains on the stainless steel surface. Lactic acid was numerically more effective than acetic acid in removing the biofilms formed by the cells of all tested STEC strains on both polystyrene and stainless steel surfaces. The commercial detergents were either significantly or numerically more effective than the organic acids in removing the biofilms formed on the two surfaces.

Significance: The study revealed that the biofilms formed by the cells of selected STEC strains could not always be significantly removed by treatments with the evaluated organic acids and commercial detergents.

P2-55 The Influence of Non-lethal Temperature on the Rate of Inactivation of Vegetable Bacteria in Inimical Environments May be Independent of Bacterial Species

DONGLAI ZHANG, Olivia J. McQuestin, Lyndal A. Mellefont and Thomas Ross

Shanghai Academy of Agricultural Science, Institute of Agri-Food Standards and Testing Technology, Shanghai, China

Purpose: The study reported here sought to assess the extent of the above observation by testing whether non-lethal temperature has a similar effect on the inactivation of other species of bacteria when they are precluded from growth.

Methods: The influence of non-lethal temperature on the survival of two species of foodborne bacteria under growth-preventing pH and water activity conditions was investigated. Specifically, inactivation rates of four strains of *Escherichia coli* and three strains of *Listeria monocytogenes* were determined in culture broth adjusted to pH 3.5 and water activity 0.90, to prevent growth of both species, and for temperatures in the range 5 to 45°C at 5°C intervals.

Results: Sixty-three inactivation rates were obtained, plotted on Arrhenius co-ordinates, and lines of best-fit determined by simple linear regression. Differences in the mean inactivation rate of each species at a given temperature were not significant ($P < 0.05$) with the exception of the rates at 25°C. The inactivation rate responses of both species were comparable to those reported by McQuestin et al. (Appl. Environ. Microbiol., 75:6963–6972, 2009) for a variety of *E. coli* strains under a wide range of growth-preventing pH and water activity conditions.

Significance: The results support the hypothesis that non-lethal temperature is a key factor governing the rate of inactivation of vegetative bacteria in foods when other hurdles prevent their growth and indicate that the temperature effect may also be independent of bacterial species.

P2-56 Reduced-temperature Growth Studies of *Bacillus cereus* and *Bacillus weihenstephanensis*

SARAH M. MARKLAND, Kalmia E. Kniel, Peter Setlow and Dallas G. Hoover

University of Delaware, Animal and Food Sciences, Newark, DE, USA

Introduction: Bacillus cereus is a Gram-positive sporeforming bacterium found ubiquitously in nature. Some strains of the species are considered psychrotrophic pathogens and consequently represent a food safety risk in refrigerated foods. Psychrotrophic *Bacillus weihenstephanensis* is a newly identified species very similar to *B. cereus*, including its ability to produce cereulide toxin at temperatures below 15°C.

Purpose: Genetic sequencing has shown a high level of similarities between mesophilic and psychrotolerant strains of *B. cereus*; however, comparison studies on the actual growth behaviors of these bacteria are lacking. This study compared the growth behaviors of three different strains of *B. cereus* and one strain of *B. weihenstephanensis*.

Methods: B. cereus T and 569 were originally identified as mesophilic strains, and *B. cereus* 6A16 and *B. weihenstephanensis* 6A23 were originally identified as psychrotolerant strains. Cultures were grown at 30, 20, 15 and 10°C in TSB with agitation. Samples were taken at appropriate time intervals, plated onto TSA and incubated at 37°C for 24 h. Growth curves were generated to compare lag time and growth rates.

Results: Strains T and 569 grew similarly at both 30 and 10°C ($P = 0.999$; $P > 0.05$). Growth rates of *B. cereus* T and 569 were 0.745 and 0.51 h with a lag time of 2 h at 30°C and at 10°C 5.07- and 5.4-h growth rates, respectively, both strains with a lag time of 90 h. Strains 6A16 and *B. weihenstephanensis* 6A23 grew significantly faster at 30°C than strains T and 569 with growth rates of 0.48 and 0.49 h and no growth at 10°C ($P = 0.004$; $P > 0.05$).

Poster

Significance: Strains T and 569 grew after 90 h at 10°C and then declined after 10 days. Strains 6A16 and 6A23 showed no population growth after 14 days at 10°C. The psychrotrophic and mesophilic varieties demonstrated substantial variability in growth and lag times at refrigeration temperatures, indicating potential ability for both types to cause foodborne illness in refrigerated foods.

P2-57 Comparison of the Microbial Contamination Associated with Fresh Herbs and Edible Flowers Using DNA Sequencing Analysis

KAEDRA WETZEL, Jiyoung Lee, Chang Soo Lee and Margaret Binkley
The Ohio State University, College of Public Health, Tallmadge, OH, USA

Introduction: As the use of fresh herbs and edible flowers as ready-to-eat foods grows, so does concern over their ability to harbor microbial pathogens with the potential to cause foodborne illness. By identifying the types of microbial contamination present on these raw foods it is possible to locate possible sources of contamination that require control in order to reduce the chance of foodborne outbreak. Microbial contamination on loose and pre-packaged samples, and conventionally and organically grown samples, was assessed using DNA sequencing analysis.

Purpose: The purpose of this study was to identify microbial contaminants present on loose and packaged samples, and also on conventionally and organically grown samples of basil, cilantro, and edible flowers.

Methods: Samples, purchased at local grocery stores, were homogenized, and then enriched in BHI broth. Isolation of bacteria was carried out on TSA and subsequent DNA extraction was performed. A universal primer set targeting the 16S rDNA gene was used for PCR amplification and the PCR products were purified and sent to the Plant Microbe Genomic Sequencing Facility at The Ohio State University for DNA sequencing. Sequencing results were compared to results available in the NCBI BLAST database in order to determine bacterial isolate identity.

Results: Sequencing results indicated a high level of bacteria species unique to the genus *Enterobacter* (38%) and also the presence of *Salmonella* spp. (4%). The sequencing results were used to construct phylogenetic trees and linear-per-time plots. Linear-per-time plots indicated that the microbial diversity among packaged basil, cilantro, and edible flower samples is higher than loose samples and the microbial diversity among organic samples is higher than conventionally grown samples.

Significance: The identities of bacterial isolates indicate fecal contamination in all samples and a high level of microbial diversity from packaged ready-to-eat samples and also from organically grown samples. This is the first study to apply the linear-per-time-plot method to compare genetic diversity in food samples between food types, package types, and growing methods. The presence of frank pathogen, *Salmonella enterica*, suggests that special care be given when preparing these types of raw foods in order to prevent foodborne illness.

P2-58 Pathogen Inactivation in Animal Manure-based Compost Systems Containing Different Carbon Amendments

MARILYN C. ERICKSON, Jean Liao, Chris Smith, Michael P. Doyle and Xiuping Jiang
University of Georgia, Center for Food Safety, Griffin, GA, USA

Introduction: To ensure that animal manures are not harbingers of zoonotic pathogens prior to their application to produce fields, it is recommended that they undergo thermal aerobic composting. This process is facilitated by adding to the nitrogen-rich manure a carbon amendment that serves as an energy source for indigenous thermophilic microorganisms.

Purpose: Using variable compost formulations, the survival of *Salmonella* spp. and *Listeria monocytogenes* was examined in systems simulating internal (bioreactors) and surface (trays) sites of composting piles in an effort to understand whether the type of carbon amendment could influence either the thermal or non-thermal inactivation of pathogens.

Methods: Cow manure, contaminated with 7 log CFU/g of either *Salmonella* or *L. monocytogenes*, was mixed with either wheat straw, peanut hulls, or pine needles prior to adding to bioreactors whereas chicken manure, contaminated with 5 log CFU/g of *Salmonella*, was mixed with wheat straw, peanut hulls, pine needles, or rice hulls prior to holding in trays at 25°C. In both systems, pathogen populations and pH were monitored while volatile acid and ammonia levels were also measured in the bioreactor systems.

Results: The carbon amendment did not have a significant effect on inactivation of *L. monocytogenes* in bioreactors whereas *Salmonella* was inactivated the fastest when compost mixtures contained wheat straw. In tray systems, *Salmonella* could not be detected within a week in compost mixtures containing wheat straw or peanut hulls whereas the pathogen survived for more than 4 weeks in mixtures containing either pine needles or rice hulls.

Significance: Understanding the differences that occur in pathogen survival with different formulations of animal manure-based compost mixtures is essential to providing guidelines that would ensure the inactivation of zoonotic pathogens.

P2-59 A Numerical Approach for Predicting Volumetric Inactivation of Foodborne Pathogens by Pulsed Light

LILLIAN HSU and Carmen I. Moraru
Cornell University, Food Science, Ithaca, NY, USA

Introduction: Pulsed light (PL) uses short, high-energy pulses of UV-rich broad spectrum light to inactivate spoilage and pathogenic microorganisms. Beverages could be an attractive application of PL, but the optical properties of the fluid, shape and size of the substrate, and relative location to the PL source might limit the effectiveness and uniformity of the treatment. Understanding the spatial distribution of inactivation in a substrate will allow processors to design appropriate applications of PL treatment.

Purpose: The goal of this study was to predict the spatial distribution of microbial inactivation for liquid substrates of known optical properties and geometry (cylinder and rectangular prism), and then validate these predictions.

Methods: Butterfield's Phosphate Buffer (BPB), Trypticase Soy Broth (TSB), and apple juice were used as substrates. Total fluence (F_{total}) measurements were performed in a RS-3000C SteriPulse unit (Xenon Corporation; MA) using a pyroelectric head (Ophir Optronics, MA). UV fluence (F_{UV}) was determined by first using a High Resolution Fiber Optic Spectrometer (Ocean Optics, FL) attached to a 1000 µm fiber to measure total irradiance as a function of wavelength. Integrating the irradiance curve from λ = 200 to 400 nm and dividing by total area under the curve gave F_{UV}. Equations describing the spatial distribution of F_{total} and F_{UV} within each substrate were developed. The Weibull model was used to describe the microbial inactivation kinetics of *E. coli* ATCC 25922 and *L. innocua* FSL C2-008, surrogates for the foodborne pathogens *E. coli* O157:H7 and *L. monocytogenes*.

Results: Coupling the fluence equations with the Weibull kinetics equations, and integrating over the volume for each substrate yielded values for the expected microbial inactivation, and maps of the spatial distribution of inactivation. Calculated levels of inactivation using F_{UV} were considerably more accurate than those based on F_{total}. The spatial distribution of inactivation was not uniform throughout the substrate, a direct artifact of the non-homogeneous light penetration.

Significance: The results of this study clearly emphasize the substantial possibility for non-uniform treatment by PL, and the need for thorough understanding of light behavior when designing commercial applications for PL.

Poster

P2-60 Survival of *Salmonella* Newport, *Escherichia coli* O157:H7 and *Escherichia coli* K12 in Irrigation Water Samples from the Lower Colorado River Basin

SADHANA RAVISHANKAR, Libin Zhu and Jorge M. Fonseca
University of Arizona, Veterinary Science and Microbiology, Tucson, AZ, USA

Introduction: Irrigation water is a potential source of microbial contamination to vegetables. Irrigation water can be contaminated by manures used in agricultural operations, runoff from areas used for animal husbandry operations and wildlife. Once introduced into irrigation water, foodborne pathogens can survive over relatively long periods, potentially posing health risks to consumers, since they can contaminate the crops grown and irrigated using contaminated water.

Purpose: The objective of this study was to investigate the survival of *Salmonella* Newport, *Escherichia coli* O157:H7 and *E. coli* K-12 in irrigation water samples collected from the Lower Colorado River Basin (Yuma valley).

Methods: Three microorganisms were used for this study: *Salmonella* Newport, *E. coli* O157:H7 and *E. coli* K-12. Water samples from four different locations in the Yuma valley were inoculated with one of the test organisms and stored at room temperature or 12°C for 1 month. Two initial inoculum levels were used: 10^5 and 10^3 CFU/ml. Samples were taken at regular intervals, diluted, plated on appropriate media, and enumerated for the test organisms.

Results: At room temperature, there was 1 log population increase for all 3 tested microorganisms at day 1. The population levels decreased eventually after day 1. At day 30, there were about 2–3, 3–5 and 3–4 log reductions for *S.* Newport, *E. coli* O157:H7 and *E. coli* K-12, respectively. At 12°C, bacterial population decreased after day 3, and there were 3, 3–5 and 3–4 log reductions for *S.* Newport, *E. coli* O157:H7 and *E. coli* K-12, respectively. There were differences in the survival among water samples from various locations. In general, *S.* Newport survived better than *E. coli* O157:H7.

Significance: The results showed that foodborne pathogens can survive in irrigation water for long periods and that survival can vary based on the location. These results can provide a basis for risk assessment of irrigation water.

P2-61 The Regrowth Potential of *Salmonella* spp. in Super-heated Chicken Litter Pellets

JINKYUNG KIM, Cortney Miller and Xiuping Jiang
Clemson University, Food Science and Human Nutrition, Clemson, SC, USA

Introduction: Poultry production in the U.S. generates enormous amounts of chicken litter. To better recycle the nutrients in poultry wastes, the poultry industry has developed a process to dry the feces with heat and press them into pellets which can then be used as organic fertilizers. Although studies revealed that *Salmonella* regrowth could occur in biosolids and finished composts under favorable conditions, there is no study regarding regrowth potential of *Salmonella* in dried chicken litter pellets.

Purpose: The purpose of this study is to evaluate the possibility of *Salmonella* regrowth in super-heated chicken litter pellets under different environmental conditions.

Methods: Commercially available super-heated chicken litter pellets was acquired and ground into powder. A three-strain mixture of rifampin-adapted *Salmonella* spp. was inoculated into the chicken litter powder (ca. 2 log CFU/g). Moisture was adjusted by fine-misting sterile tap water to desired levels (17-original, 30, 40, and 50%). Samples were stored at 7, 20, and 30°C, and analyzed microbiologically on days 1, 3, 5, and 7.

Results: Original super-heated chicken litter pellets were absent of *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* by enrichment. The populations of background microorganisms were ca. 5.4 log CFU/g. At 7°C, *Salmonella* survived through day 7 at all moisture levels, whereas it was detectable up to day 7 at 20°C with only 50% moisture. When the samples were held at 30°C, *Salmonella* died off before day 7. Overall, *Salmonella* did not regrow in chicken litter at any of the moisture levels or storage temperatures. However, *Salmonella* persisted longer in heat-treated chicken litter with high moisture contents at lower temperatures.

Significance: These data suggest that the commercial super-heated chicken litter pellets as organic fertilizers don't support the growth of *Salmonella* spp. However, certain combinations of temperature and moisture level may aid in survival/persistence of the pathogen in chicken litter pellets.

P2-62 *In vivo* Passage Modulates Acid Tolerance Response in *Listeria monocytogenes*

HIROSHI ASAKURA, Sou-ichi Makino, Yumiko Okada, Fumiko Kasuga, Shigeki Yamamoto and Shizunobu Igimi
National Institute of Health Sciences, Division of Biomedical Food Research, Tokyo, Japan

Introduction: In an outbreak of *Listeria monocytogenes* due to cheese products in Japan, 2001, we have previously demonstrated that *L. monocytogenes* isolates from the causative foods and patients exhibited high genetic similarities, based on the PFGE and ribotyping analysis.

Purpose: All causative foods (cheeses) were manufactured through fermentation, while the contamination rates in each food products were in variety. We thus aimed to clarify the association between the contamination rates and acid tolerance of *L. monocytogenes*.

Methods: Acid sensitivity test: *L. monocytogenes* were incubated in PBS supplemented with lactic acid (pH 4.0) at 37°C. Aliquots of the suspensions were spread on BHI agar to enumerate CFUs. Mice passage: BALB/c mice were orally inoculated with *L. monocytogenes*. At 2 days post infection, *L. monocytogenes* were recovered from intestine by plating on selective agar. Macrophage survival assay: RAW264.7 cells were infected with *L. monocytogenes*. Intracellular bacteria were enumerated by gentamycin protection assay. Annealing Control Primer (ACP)-PCR assay: After incubation under low acidity (pH 4.0), total RNA was isolated from *L. monocytogenes*, and subjected to ACP-PCR (Seegene). Each transcripts were estimated by RT-PCR.

Results: There were in variety for the acid tolerance among *L. monocytogenes* isolates. Their phenotype was correlated to their contamination rates in foods. Among them, one food- and one patient-isolate showing identical PFGE pattern, exhibited a distinct phenotype toward acid tolerance (food isolate was acid-tolerant, while the patient isolate was sensitive to it). It remains unclear whether the phenotypic modulation of acid tolerance occurs during host passage. Mice experiment demonstrated that the food-isolate (acid tolerant) increased acid-sensitivity after recovery from mice, whereas the patient isolate did not alter its acid tolerance. Thus, it is conceivable that *in vivo* passage trigger to increase acid-sensitivity in *L. monocytogenes*. The decreased acid tolerance of the patient isolate was appeared to the shortened time of survival inside macrophages. Upon acidic pH, the food isolate expressed significantly higher levels of gadD gene than the patient isolate, and which corresponded to their GAD (glutamate decarboxylase) activities. These suggested that the distinct phenotype of the two isolates might be due to the genetic alteration during host passage.

Significance: Acid tolerance test proposed this phenotype was one of the most important factor for the occurrence of that outbreak. Furthermore, this is the first report to demonstrate the phenotypic change in *L. monocytogenes* during host passage.

Poster

P2-63 Comparison of Gene Expression of *Listeria monocytogenes* 10403s following Exposure to Pulsed Light and Ultraviolet Light

AARON R. UESUGI and Carmen I. Moraru
Cornell University, Dept. of Food Science, Ithaca, NY, USA

Introduction: Pulsed Light technology uses intense, short pulses of broad spectrum light, in the range 200 to 1,100 nm, to inactivate microorganisms. This treatment has been successfully used both in clear liquid solutions and on food and food contact surfaces. The light is generated by Xenon gas discharge lamps and is rich in germicidal UV light (200 to 280 nm). While it is generally accepted that UV plays a major role in microbial inactivation by Pulsed Light, the response of microbial cells to Pulsed Light as compared to continuous UV treatment is not known.

Purpose: The objective of this study was to compare the gene expression of *Listeria monocytogenes* 10403s exposed to Pulsed Light and conventional germicidal UV light (254 nm).

Methods: L. monocytogenes 10403s were grown to early stationary phase in Defined Media for *L. monocytogenes*. 15 ml of culture were exposed to 6.8 J/cm^2 of PL (2s) or 33 J/cm^2 of UV (120s), shaken at 750 RPM, which resulted in a comparable reduction. After exposure, cells were held in the dark at 37°C for 5 min shaken at 750 RPM. RNA was extracted and gene expression of these cells was compared by microarray analysis.

Results: Reductions of *L. monocytogenes* 10403s ranged from 2.5 to 2.7 log CFU/ml following each treatment. Gene expression of both Pulsed Light and UV treated cells showed similar SOS and DNA repair response. The gene patterns were different between the Pulsed Light and UV exposed cells with respect to heat shock proteins, i.e., Pulsed Light showed increased expression in both GroES and GroEL.

Significance: The results of this study show the similarities and differences in gene expression between Pulsed light treatments and continuous UV treatment, indicating that in Pulsed Light other factors besides UV may be responsible for the inactivation of microbial cells.

P2-64 Laboratory Proficiency Testing as a Measure of National Food Safety and Biosecurity Preparedness

LACEY M. GUILLEN, Ravinder M. Reddy, Chrissy M. Leopold Wager, Shannon M. Dugan, Christopher Conway, Vishnu Y. Patel, Wei Zhang, Lauren Yeung, Terri T. McConnell and Glenn Tillman
Illinois Institute of Technology, NCFST, Summit-Argo, IL, USA

Introduction: The Food Emergency Response Network (FERN) was created to form a nationally integrated system of regulatory laboratories to respond to instances of food safety and biosecurity. The FERN proficiency testing (PT) program is vital to routinely assess each laboratory's capabilities by sending test samples consisting of a variety of microorganisms or other analytes in different food matrices.

Purpose: Participating FERN laboratories were evaluated on their ability to isolate, identify, and enumerate low levels of *Listeria monocytogenes* (10 and 100 CFU/ml). In this PT exercise the laboratories had to be able to differentiate between pathogenic and non-pathogenic *Listeria* spp. in a sample with a complex background microflora.

Methods: Five milk samples were sent to each of 70 FERN laboratories. Three of the 5 samples were inoculated with 10 or 100 CFU/ml *L. monocytogenes*. *L. inoccua*, *L. welshemerii*, and *L. grayii* were added as confounding microorganisms. All 5 of the samples were inoculated with typical raw milk microflora including non-pathogenic *E. coli*, *E. aerogenes*, *S. epidermidis*, *S. aureus*, *P. aeruginosa*, *E. faecalis*, *S. agalactiae*, and *B. cereus*. Laboratories analyzed each sample with approved standard procedures and reported both results and detection methods. The laboratories were graded based upon the accuracy of analytical results.

Results: Most laboratories (67%) correctly analyzed all 5 samples. The laboratories reported the use of a variety of multi-step protocols which included cultural methods (79%), chromagenic media (27%), biochemical assays (87%), immunoassays (37%), and PCR methods (63%). Laboratories with incorrect results were required to complete a corrective action questionnaire and may also receive a second set of samples containing *L. monocytogenes* depending on the nature of the deficiencies recognized.

Significance: The FERN PT program has been vital in evaluating the readiness and capability of FERN member laboratories to respond to any threat in our food supply.

P2-65 Application of Weibull Type Distribution to Model Inactivation of *Listeria innocua* by Thermo-sonication at Selected Vanillin Concentrations

GABRIELA G. GASTÉLUM-REYNOSO, Enrique Palou and Aurelio López-Malo
Universidad de las Américas Puebla, Departamento de Ingeniería Química, Alimentos y Ambiental, Cholula, Mexico

Introduction: There is a growing interest in combining food preservation techniques such as heat, ultrasound, and natural antimicrobials in order to inactivate microorganisms. The right combination of preservation techniques could diminish the intensity of traditional thermal processes, thus improving the quality and safety of foods.

Purpose: In this experiment, the combined effect of simultaneous application of heat (40, 50, or 60°C), low frequency ultrasound (20 kHz, at 60, 75 or 90 μm amplitude), and vanillin concentration (200, 350, or 500 ppm) on *Listeria innocua* viability was evaluated.

Methods: Media adjusted at a_w 0.96 and pH 3.5 were inoculated with 10^6 CFU/mL of *L. innocua*. Vanillin alcohol solution (10 g/L) previously filtered was added to adjust the systems to 200, 350, or 500 ppm. Then thermo-sonication treatments were applied. Samples were taken periodically and survivors determined by surface platting. Survival curves were adjusted with Weibull model. Statistical parameters (mean, mode, variance and coefficient of skewness) that describe the frequencies were calculated.

Results: When the highest studied temperature value (60°C) was applied, a rapid microbial decay was observed while inactivation at 40°C presented remarkable non-linear kinetics. A combination of moderate temperatures, low ultrasound amplitudes, and intermediate concentrations of vanillin enhanced *L. innocua* inactivation as demonstrated by the change in the Weibullian distributions of inactivation times yielding narrower frequency shapes, lower variance and mode values, slightly skewed to the right, meaning that the majority of the bacterial population died at an early stage of the combined treatment.

Significance: In general, a multi-target inactivation effect was observed at a temperature range from 45 to 55°C achieving four log-cycle reductions of *L. innocua*. The use of hurdle technology combining mild thermal treatments, ultrasound, and vanillin could be an alternative to achieve microbial inactivation. Further studies are needed in order to scale up thermo-sonic pasteurization to an industrial level.

P2-66 Quantitative Analysis on the Growth and Contamination of *Penicillium nalgiovense* on Licorice Extract and Its Derivatives

BIN LIU
Mafco Worldwide, Camden, NJ, USA

Introduction: Penicillium Nalgiovense and its family have been widely distributed in the environment and isolated from raw materials and finished products in food and pharmaceutical industries.

Poster

Purpose: Investigate the factors influencing the growth of *Penicillium nalgiovense*, and fit the data gap of its statistical distribution on food commodities in bulk, containers, or individual packaging.

Methods: Penicillium Nalgiovense cells were grown for 18 hours at 37°C with shaking (150 rpm) in tryptic soy broth. One-gram seed samples were taken randomly and incubated for 24 hours at 37°C. Total plate counts were obtained from plate count agar. Licorice extracts and its derivatives at three Loss-on-Drying (LOD) levels (5%, 15%, and 40%), were inoculated at three levels (~3 CFU/g, ~30 CFU/g, and ~300 CFU/g), then transferred into either atmospheric or vacuum environments. Microbial concentrations were determined from air and product at various times over a one-year period. Data were fit to logistic regression models, and growth rates and maximum concentrations were compared using the generalized linear model procedure of SAS statistical software.

Results: Higher LOD levels resulted in higher microbial growth rates. No statistically significant differences in growth rates were observed in samples taken from the air or the product itself, but were constant irrespective of inoculum level. Microbial concentrations in air showed a linear relationship to samples taken directly from the samples at corresponding time points, for all three inoculum levels.

Significance: 1. Higher moisture level provides a better environment for the growth of *P.* Nalgiovense. 2. Air sampling could be an easy substitute to direct sampling in licorice, and microbial levels could be derived from the mathematical model generated from this research.

P2-67 PCR Detection and Identification of Tyramine-producing Bacteria and Partial Cloning of Tyrosine Decarboxylase Gene

MIN-JU KIM, Jae-Hee Jeong, Bo-Mi Lee, Ji-Eun Kang and Keun-Sung Kim
Chung-Ang University, Food Science and Technology, Anseong-Si, Gyeonggi-Do, Republic of Korea

Introduction: Biogenic amines (BA) are low molecular weight organic bases that can be detected in raw and fermented food. Several toxicological problems resulting from the ingestion of food containing BA have been described. BA are mainly produced by the decarboxylation of certain amino acid by microbial action. Some lactic acid bacteria contain a tyrosine decarboxylase (tdc) which converts tyrosine to tyramine.

Purpose: To develop PCR methods of lactic acid bacteria producing tyramine in fermented foods and to determine sequence of the tdc gene.

Methods: A total of 16 strains were used in this study, including indicator strains and tyramine-producing bacteria isolated from Kimchi. Tyramine-producing bacteria were identified using PCR-based specis-specific primer set, and the sequences of partial 16s rRNA genes of them were determined and compared with those reported in the Genebank database. Two target primer sets were used for the detection of tdc gene. Primer TDC-F and TDC-R, P2-for and P1-rev were used respectively as forward and reverse primer in the PCR tests as previously reported.

Results: Tyramine-producing isolates were identified as four species, i.e., *Lactobacillus brevis, Leuconostoc mesenteroides, Lactobacillus* spp. or *Staphylococcus* spp. when they were tested by PCR method. At present, two primer sets to amplify the tdc gene of 14 strains isolated from kimchi. The analysis of the PCR products showed 825 or 924 bp single fragment respectively.

Significance: The methods can help to better control and to improve fermented food making conditions, in order to avoid biogenic amine production.

P2-68 Melamine Contamination of Milk Powder and Infant Formula on the African Market — A Significant Global Issue with Public Health and International Trade Implications

DAGMAR SCHODER
Universitiy of Veterinary Medicine, Institute of Milk Hygiene, Milk Technology and Food Science, Vienna, Austria

Introduction: Chemical food safety has emerged as a significant global issue with public health and international trade implications. The most recent example was the epidemic of melamine poisoning in China. Locally sold contaminated milk powder and infant formula originating from China have been identified in several countries worldwide, with, until now, the exception of Africa.

Purpose: The present study aimed to determine the incidence of melamine in milk powder and infant formula imported to the East African market and the distribution of the contaminated products either through formal/legal or informal/illegal channels. The study took place in Dar-es-Salaam, Tanzania, East Africa. Dar-es-Salaam is one of the fastest growing African megacities, the largest seaport and the center of international trade in East Africa. For this reason Dar-es-Salaam is a suitable representative location for sampling milk powder products imported to the East African market.

Methods: Melamine determination was carried out using the commercially available AgraQuant® Melamine Sensitive Assay (Romer Labs®, Singapore-Pte-Ltd., Jalan Bukit Merah, Singapore). Two categories of samples were collected: (i) market brands of all international companies supplying the East-African market and (ii) illegally imported unlabelled products from the informal channels.

Results: Despite the national import prohibition of Chinese milk products and unlabelled milk powder in Tanzania, 11% (22/200) of the inspected micro-retailers sold milk powder on the local black market. Manufacturers could be identified for only 55% (27) of the 49 investigated batches. Six percent (3/49) of all samples and 11% (3/27) of all international branded products tested, revealed melamine concentrations up to 5.5 mg/kg milk powder. This amount represents about twice the tolerable daily intake (TDI) as suggested by the U.S. Food and Drug Administration (FDA). All melamine-contaminated batches revealed production dates from September 2007 to May 2008.

Significance: Our study demonstrates for the first time that, despite official regulations, melamine contamination of milk powder is a significant problem in Africa and clearly indicates that melamine-contaminated milk powder and infant formula had been processed and exported to Africa long before the melamine scandal became a real topic of international attention.

P2-69 The Influence of Sample Preparation Chemistry on Bacterial Target Cells Analyzed by Transmission Electron Microscopy of *Listeria monocytogenes* and *Salmonella* Typhimurium

Sabine Fuchs, Patrick Mester, Martin Wagner and PETER ROSSMANITH
University of Veterinary Medicine Vienna, Christian Doppler Laboratory for Molecular Food Analytics, Vienna, Austria

Introduction: Adequate sample preparation is the key to the successful broad range application of molecular biological methods in food analysis. Therefore a major research topic is the development of sample treatment methods and novel approaches based on selective chemical digestion or solubilization of the foodstuffs with subsequent separation of the intact bacterial target cells were developed recently.

Purpose: The purpose of this study was to evaluate the implications of various chemical compounds which are used in food-solubilization-based sample preparation methods on bacterial cells by transmission-electron-microscopy (TEM).

Methods: Negative stained and ultra thin section samples of cultures of *Listeria monocytogenes* and *Salmonella* Typhimurium were analyzed in a Zeiss EM 900 by TEM after incubation in reagents used for solubilization of foodstuffs. Detergents, chaotropic substances, ionic liquids, salts, organic solvents, acids and combinations thereof were applied as published for each protocol. Negative staining of the samples for TEM was performed with phosphor tungsten acid. For ultra thin section samples a prefixation with glutaraldehyde and a fixation step with osmium tetroxide was used for staining.

Results: The influence of the various tested chemical compounds on the bacterial target cells corresponded to the respective chemical features. The chemicals affected cell morphology as well as the cell wall and membrane and the morphology of the cytoplasm. The published survival rates of the bacteria during the various protocols were clearly reflected in the mechanisms of cell destruction as caused by the various chemicals and determined by TEM.

Significance: The investigation of the bacterial cells after sample preparation using TEM enabled the determination of the impact of the used chemicals on the structure of the cells. The alteration of the cell morphology and the impact on the prokaryotic cell wall and membrane as well as the cytoplasm clearly reflected the loss of cell counts by means of recovery values as published for respective methods. The study adds new and sufficient information to an important topic in food pathogen detection useful for further development of sample preparation methods.

P2-70 Antibiotic-resistance Class 1 Integrons in Commensal Bacteria from Various Environments and Their Transferability to Foodborne Pathogens

HUA YANG, Oleksandr A. Byelashov, Ifigenia Geornaras, Lawrence D. Goodridge, Kendra K. Nightingale, Keith E. Belk, Gary C. Smith and John N. Sofos
Colorado State University, Animal Sciences, Fort Collins, CO, USA

Introduction: Mobile integrons are common vectors for the dissemination of antimicrobial resistance genes among diverse bacteria.

Purpose: This study assessed the distribution of Class 1 integrons in commensal bacteria isolated from various environments, and the transferability of Class 1 integrons to foodborne pathogens.

Methods: Real-time PCR was used to screen 1,870 commensal bacterial isolates for Class 1 integrons. The isolates were obtained from soil, fecal floor and water samples collected from cattle operations (a beef ranch and a dairy farm), the city of Fort Collins (city parks, hospital areas and water reclamation facilities), and the Rocky Mountain National Park in Colorado, and from manure, compost, unused feed and unused bedding samples collected only from cattle operations. The antibiotic resistance gene cassettes within Class 1 integrons were determined through DNA sequence analysis. Class 1 integron-positive commensal bacteria were identified through 16S rRNA gene sequencing and tested for their susceptibility to clinically important antibiotics. Transferability of Class 1 integrons from commensals to *Salmonella* and *Escherichia coli* O157:H7 strains were tested by filter mating experiments.

Results: A total of 26 isolates were found to be Class 1 integron-positive by real-time PCR among fecal samples from cattle operations and a city park, water samples from a beef ranch and city lakes, and soil, feed (unused), manure and compost samples from a dairy farm. Most of the Class 1 integron genes had one gene cassette belonging to the *aadA* family which confers resistance to streptomycin and spectinomycin. All of the Class 1 integron-positive isolates from city locations displayed multi-resistance to 7 to 13 of the antibiotics tested, while Class 1 integron-positive isolates from cattle operations displayed diverse resistance patterns to 1 to 21 of the antibiotics tested. One isolate from a dog fecal sample collected from a city park transferred its Class 1 integron to a strain of *E. coli* O157:H7 at a frequency of 10^{-7}. Class 1 integron-positive isolates from city locations were taxonomically close to gamma-proteobacteria, and showed multiple resistances and self-transferability of Class 1 integrons. However, Class 1 integron-positive isolates from cattle operations displayed diverse taxonomic relationships and the transferability of their Class 1 integrons needs to be further confirmed.

Significance: Our findings emphasize the possible role of environmental commensals in serving as reservoirs of antibiotic resistance genes and indicate that antibiotic resistance is a complex problem that cannot be simply linked only to agricultural activities.

P2-71 Behavioral Influences and Temperature Profiling of Reconstituted Powdered Infant Formula Feeds Stored in Day Nurseries

ELIZABETH C. REDMOND and Christopher J. Griffith
University of Wales Institute Cardiff, Food Industry Centre, Cardiff School of Health Sciences, Cardiff, United Kingdom

Introduction: The use of day nurseries for infant care has increased in recent years and the majority store bottles of reconstituted powdered infant formula (PIF) feeds. PIF can become contaminated with microorganisms, including pathogens, during production, handling and preparation. Inadequate temperature control may increase risk of microbial growth. Relatively little is known about how day nursery staff (DNS) manage the safety of reconstituted PIF storage.

Purpose: The aims of this study were to determine behavioral influences of PIF storage practices implemented by DNS and to track time-temperature profiles of reconstituted PIF stored in nurseries.

Methods: DNS knowledge, attitudes and beliefs were determined using a postal questionnaire, administered to 10% (n = 830) of UK nurseries with infants aged < 6 months. Time-temperature profiling of PIF feeds (n = 55) occurred using a miniature-datalogger (accuracy ± 0.5°C) and validated methods. Temperature tracking commenced from PIF reconstitution in nurseries or arrival at nursery, until feeding.

Results: On arrival at nurseries, temperatures of PIF feeds were 14 to 51°C (± 1°C) and the length of time between arrival at nursery and refrigeration ranged from 0 to 135 minutes (mean 39 minutes) with the storage duration lasting for > 7 h. No reconstituted PIF brought to nursery achieved < 5°C during storage prior to feeding. Warm/hot feeds were initially stored in nursery kitchens (23 to 25°C) for > 2 h before refrigeration. PIF made-up in nurseries were stored for < 6 h; such feeds stored for > 60 min achieved < 5°C. The majority (63%) of DNS were unaware of specific microbiological risks associated with PIF and 76% believed PIF is a sterile product. Knowledge of correct refrigeration temperatures was lacking and 87% reported never being trained regarding microbiological risks associated with PIF.

Significance: Findings indicate time-temperature abuse of reconstituted PIF feeds stored in day nurseries. This could have a major impact on PIF quality. Cumulatively, data will help development of targeted training and national policies that address the microbial risks of storage of PIF in day nurseries.

P2-72 Natural Occurrence of Type B Trichothecenes in Cereals and Cereal Product in South Korea

Hyun Ee Ok, Dong Min Kim, Hee-Ra Park, Dong-Sul Kim and HYANG SOOK CHUN
Korea Food Research Institute, Sungnam, Republic of Korea

Introduction: A variety of *Fusarium* fungi produce a number of different mycotoxins of the class of trichothecenes. The major type of trichothecenes are type A (T-2 toxin, HT-2 toxin, etc.) and type B (deoxynivalenol (DON), nivalenol (NIV), 3-acetyl DON (3ADON), 15-acetyl DON (15ADON), fusarenon-X (Fus-X)). The *Fusarium* fungi are commonly found on cereals grown in the temperate regions of Asia, Europe and America.

Purpose: The aim of this study was to examine the occurrence and the health risk associated with exposure for type B trichothecenes in cereals and cereal products marketed in 2009 in Korea.

Poster

Methods: A total of 315 samples including rice, barley, corn, wheat, wheat flour, mixed minor rice and breakfast cereals were randomly collected from variable retail outlet during 2009 year. After extraction with acetonitrile/water (86/14, v/v), the toxins were purified with MycoSep® column and determined by gas chromatography with electron capture detection after derivatization with trifluoroacylation. The analytical method was validated according to EURACHEM method validation guidance.

Results: The overall incidence of contamination was 58% for NIV and 63% for DON, respectively. The ranges of mean level in samples were 4.3 – 106.6 µg/kg for NIV and 1.8 – 123.8 µg/kg for DON. The maximum levels found were 822.6 µg/kg for NIV in breakfast cereals and 491.9 µg/kg for DON in corn. DON was tended to be more frequently contaminated in corn, wheat, wheat flour and mixed minor rice. The occurrence and contamination levels of 3ADON, 15ADON and Fus-X were very low. The estimated daily intake (EDI) of NIV and DON for an average Korean consumer on basis of analysis data and consumption of food was found to be 51.2 and 20.8 ng/kg bw/day. The EDI for NIV and DON were far below the (temporary)-tolerable daily intake, and the exposure to NIV and DON through intake of cereals and cereal products marketed in 2009 in Korea was considered as not serious.

Significance: These results can be used as basic data for managing food risk posed by contamination with NIV and DON in Korea.

P2-73 Thermal Inactivation of Shiga Toxins in Orange Juice

FRANCISCO DIEZ-GONZALEZ, Wang Na, Neal R. Fredrickson and Theodore P. Labuza
University of Minnesota, Dept. of Food Science and Nutrition, St. Paul, MN, USA

Introduction: Shiga toxins are produced by enterohemorrhagic *Esherichia coli* and cause hemolytic uremic syndrome by affecting human vascular cells. The potential use of Shiga toxins (*Stxs*) as bioterror weapons in the food supply is a recent concern for homeland security. Denaturation by heat is expected to reduce the toxicity of *stx*s, but the effectiveness of thermal treatments in food matrices is largely unknown.

Purpose: The objective of this project was the characterization of thermal inactivation kinetics of *stx*s in buffer and orange juice.

Methods: Enterohemorrhagic *Escherichia coli* strains were used to produce Shiga toxin 1 (*stx*1) and Shiga toxin 2 (*stx*2). *Stxs* were added into phosphate buffered saline (PBS) or orange juice and samples (50 µL) were transferred into capillary tubes. The thermal stability of *Stxs* was determined by incubating capillary tubes at temperatures from 72 to 90°C. The residual concentration of active *Stxs* was determined by an enzyme-linked immunosorbent assay. Kinetic parameters were determined by linear regression.

Results: The heat inactivation of *stx*s in PBS and orange juice followed first-order reaction kinetics. The concentration of active *Stxs* decreased with increasing temperature and heating time. Both *Stxs* in PBS and orange juice would reach the concentration that was not detectable using ELISA within 30 s at 90°C and 120 s at 85°C. D values of *stx*1 were 2 to 6-fold greater than those of *stx*2, which suggested that *stx*1 is more heat stable. The Z values for *stx*1 and *stx*2 were 6.7 and 7.2°C in PBS, and 8.7 and 6.9°C in orange juice, respectively.

Significance: This study generated the first series of parameters that could serve as a basis for recommendations for treating orange juice subjected to intentional adulteration with *stx*s in an orange juice plant with typical pasteurization equipment so it can be safely disposed.

P2-74 Validation of Immunodetection of Ricin Using a Biological Activity Assay

STEPHEN E. LUMOR, Aaron Hutt, Francisco Diez-Gonzalez and Theodore Labuza
University of Minnesota, Food Science and Nutrition, St. Paul, MN, USA

Introduction: Recent publications have questioned the suitability of the enzyme-linked immunosorbent assay (ELISA) for residual ricin activity determination since ELISA is not a measure of the toxic biological activity (TBA) of ricin, but a measure of ricin concentration. Accurate determination of TBA is necessary before a decontaminated food, equipment or surface is deemed free of residual ricin activity. Ricin inhibits protein synthesis by removing adenine residues from polynucleotides.

Purpose: To investigate the correlation between residual ricin concentration and TBA.

Methods: Ricin activity (TBA) was determined by digesting a 2551 bp DNA (from pBR322 plasmid) with ricin to release adenine. The released adenine was converted to 1,N6-ethenoadenine (EDN), which was quantified by fluorescence spectroscopy using an excitation wavelength of 275 nm and an emission wavelength of 410 nm. The amount of EDN detected was indicative of TBA. Subsequently, ricin was thermally treated at 80, 85 and 90°C for different times, and residual ricin concentration by means of ELISA, and TBA were determined.

Results: Semi-log plots of residual ricin concentration and TBA at the different temperatures against time showed that inactivation of ricin followed first-order kinetics. Half-lives determined at 80, 85 and 90°C were 1.98, 0.58 and 0.27 min, respectively, for the TBA assay, whereas half-lives determined by ELISA were 3.08, 0.81 and 0.41 min, respectively. A plot of half-life against temperature showed that the Z-value, Q10 and Arrhenius activation energy were similar for both assays. They were 11.4°C, 7.15 and 50.0 kcal/mol, respectively, for ELISA; and 11.6°C, 7.3 and 50.7 kcal/mol for the TBA assay. A plot of half-lives determined by ELISA against half-lives determined by TBA assay produced an R^2 of 1, indicating that there was perfect correlation between ELISA and TBA assay.

Significance: These results strongly support the application of ELISA for detecting the toxicity of heat-treated ricin.

P2-75 Assessment of the Transfer Rate of Ochratoxin A Into Decoction of Herbal Medicines

Kyeongyeol Kim, JEONG-EON J. SONG, Qing Liu, Neema Kassim, Won-Bo W. Shim and Duck-Hwa D. Chung
Gyeongsang National University, Division of Applied Life Science, Jinju, Republic of Korea

Introduction: Ochratoxin A (OTA), toxic fungal metabolites, has been reported to be found in herbal medicines which are consumed for health benefits in most of Asian countries.

Purpose: The objective of this study was to determine the transfer rate of OTA into the decoction of herbal medicines by boiling and autoclaving after soaking and non-soaking in water.

Methods: Herbal medicines (Trichosanthis Semenm, Eucommiae Cortex, Rubi Fructus) spiked with OTA at 100 and 200 ng/g and soaked or not soaked in distilled water were individually boiled at 100°C for 3 h or autoclaved at 121°C for 1 h. The decoction and remainder of herbal medicines were separated, and respectively extracted with 70% MeOH in 1% $NaHCO_3$ then cleaned up using immunoaffinity column. The elutes were analyzed by HPLC system [mobile phase; acetonitrile : water : acetic acid (99:99:2 v/v/v), wavelengths; excitation 333 nm, emission 460 nm].

Results: As a result, the transfer rates of OTA in the decoctions were 16.03–61.33% (with soaking) and 12.72–54.87% (without soaking). Among herbal medicines, trichosanthis semenm with and without soaking showed the highest transfer rate in the ranges of 41.97–61.33% and 31.48–54.87%, respectively. Especially, the OTA levels in the decoction by boiling at 100°C for 3 h were lower than those of the decoction by autoclaving at 121°C for 1 h.

Significance: These results indicated that OTA in herbal medicines could be transferred into the decoction. It is necessary to the prevention of mycotoxin contamination in herbal medicines and the attention for intake of herbal medicines.

P2-76 Heavy Metal Contamination of Use Water and Street-vended Foods in Uganda

CHARLES MUYANJA, Leontina Nayiga and George Nasinyama
Makerere University, Food Science and Technology, Kampala, Uganda

Introduction: Uganda is no exception among developing countries where street-food vending is a common economic activity in urban and peri-urban areas. Street-vended foods raise concern as a potential source of food poisoning outbreaks due to environmental contaminants, rudimentary cooking utensils and improper food handling practices.

Purpose: This study was set out to evaluate heavy metal contamination of selected street-vended foods, use water in Kampala, Jinja and Masaka districts in Uganda.

Methods: Total Cadmium, Lead, Zinc, Copper and Nickel were determined using Flame Atomic Absorption Spectrophotometer (FAS). Data was analyzed using SPSS statistical package version 16.0 (SPSS Inc., 2007) at significant level of $P \leq 0.05$.

Results: The heavy metals concentrations ranged from 0.01 to 9.4 ppm. Cadmium levels in bean stew and ground nut sauce were significantly different ($P < 0.05$) among the study districts. Cadmium was also detected in Amaranthus (0.76 ppm, Jinja) and beef stew (0.93 ppm, Masaka). Lead was noted in Amaranthus at a level of 7.6 ppm (Jinja) and bean sauce at 9.4 ppm, (Kampala). Groundnut sauce mixed with smoked fish also had high levels of Lead. Zinc level in Matooke, bean stew, and chapatti was significantly different ($P < 0.05$) among the districts. Groundnut sauce from Jinja district had the highest levels of Zinc (1.23 ppm). There was significantly different ($P < 0.05$) in Copper concentration in foods (Bean sauce and chapatti) among the districts. Use water in Kampala district had the highest concentration of Cadmium (0.36 ppm), Zinc (1.68 ppm), Lead (7.6 ppm) and Copper (2.08 ppm). Final rinse water from Kampala district had higher Lead level (7.2 ppm) than other districts. Nickel was not detected in all samples tested.

Significance: The presence of heavy metals above the recommended maximum limits suggests possible health risk to consumers and environmental pollution.

P2-77 Modeling Effect of Gamma Irradiation on Production of a Sterile Korean Space Food (Kochujang) under Accelerated Storage Condition

YOHAN YOON, Beom-Seok Song, Jae-Nam Park and Ju-Woon Lee
Korea Atomic Energy Research Institute, Jeongeup, Republic of Korea

Introduction: Kochujang is a traditional Korean red pepper paste which nicely appetite Korean astronauts. Because it is consumed without additional cooking and space foods should be sterile, irradiation would be appropriate to produce sterile Kochujang as a non-thermal treatment. In addition, predictive models can be useful to predict irradiation dose to have sterile Kochujang.

Purpose: This study modeled the effect of irradiation on production of space Kochujang.

Methods: Kochujang samples were irradiated at 0, 5, 10, 15 and 20 kGy, and stored under accelerated condition (35°C for 10 days). During storage, total bacterial populations in Kochujang samples were enumerated on plate count agar (PCA) on day 0, 1, 3, 5, 7, and 10. For calculation of maximum specific growth rate (μmax; log CFU/g/day), lag phase duration (day), low asymptote (Y_0; log CFU/g) and upper asymptote (Y_{max}; log CFU/g), surviving cell counts recovered on PCA were fitted to the equation developed by Dr. Jozsef Baranyi. The parameters then were further expressed as a function of irradiation dose, and bias (B) factor, accuracy (A) factor, R^2 and root mean square error (RMSE) were calculated to validate the developed models.

Results: Total bacterial populations in Kochujang were below detection limit (1 log CFU/g) after irradiation (5-20 kGy). The samples irradiated at 5, 10 and 15 kGy then had bacterial recovery, but no growth was observed in the 20-kGy irradiated samples during storage. After validation of models, acceptable model performances (B factor = 1.15, A factor = 1.29, RMSE = 1.044, $R^2 = 0.862$) were observed.

Significance: The developed models may be useful in predicting irradiation dose to produce space Kochujang.

P2-78 Effect of Gamma Irradiation on Shelf-life Extension and Sensory Characteristics of Dak-galbi (Marinated Diced Chicken)

YOHAN YOON, Jae-Hun Kim and Ju-Woon Lee
Korea Atomic Energy Research Institute, Jeongeup, Republic of Korea

Introduction: Dak-galbi is a popular Korean food prepared by stir-frying diced chicken after marination in a gochujang (pepper paste) based sauce containing various spices which may introduce spoilage bacteria into the sauce.

Purpose: This study examined effect of gamma irradiation on shelf-life extension and sensory characteristics of dak-galbi.

Methods: Commercial dak-galbi sauce was gamma-irradiated at 0–30 kGy. The dak-galbi sauce was then added to diced chicken for cooking, and the cooked dak-galbi samples in vacuum bags were stored under accelerated condition (35°C, 5 days). The samples were analyzed on day 0, 1, 2, 3, 4 and 5 for microbial analysis (plate count agar), thiobarbituric acid reactive substance (TBARS) and volatile basic nitrogen (VBN) measurement, and on day 0 for sensory evaluation.

Results: On day 0, total bacterial populations were below detection limit after dak-galbi marinated with the gamma-irradiated (≥ 15 kGy) sauces were cooked, and the samples marinated with higher dose irradiated dak-galbi sauce had lower ($P < 0.05$) bacterial populations during storage. TBARS values of the dak-galbi marinated with non-irradiated sauce were not different ($P < 0.05$) with those marinated with irradiated sauces on day 0, and TBARS values increased ($P < 0.05$) during storage at 35°C, regardless of irradiation dose. There was no difference ($P \geq 0.05$) in VBN values among irradiation doses on day 0, but VBN values decreased ($P < 0.05$) as irradiation dose increased during storage. Moreover, there were no differences ($P \geq 0.05$) in sensory characteristics among irradiation doses.

Significance: These results indicate that use of gamma irradiation on dak-galbi sauce may be useful in shelf-life extension without compromising the sensory characteristics of dak-galbi.

P2-79 Effects of Heating, Autoclave, Gamma and Electron-beam Irradiation on Reducing Spoilage Bacteria, and Sensory Properties of Ready-to-Eat Bulgogi

YOHAN YOON
Korea Atomic Energy Research Institute, Jeongeup, Republic of Korea

Introduction: Ready-to-Eat (RTE) Bulgogi is marinated ribeye with various spices, which may introduce spoilage bacteria into the food. Therefore, a decontamination technology is necessary to destroy spoilage bacteria in Bulgogi.

Purpose: This study compared the effects of various decontamination technologies to have long-term RTE Bulgogi storage.

Methods: Vacuum-packaged Bulgogi samples (3 × 3 × 1 cm) were treated with (i) nothing (control), (ii) heat at 100°C for 15 min (HT), (iii) autoclave at 121°C for 15 min (AC), (iv) gamma ray at 20 kGy (GR-20), (v) gamma ray at 40 kGy (GR-40), (vi) electron beam at 20 kGy (EB-

Poster

20), and (vii) electron beam at 40 kGy (EB-40). Total bacterial populations were then enumerated on plate count agar on day 0, 30, 60 and 90 during storage at 35°C. TBARS (thiobarbituric acid reactive substances) and VBN (volatile basic nitrogen) values in samples were also determined, and sensory evaluation was performed during storage.

Results: On day 0, the total bacterial populations in control, HT, GR-20, and EB-20 samples were 3.2, 2.5, 1.2 and 1.3 log CFU/g, respectively, and AC, GR-40 and EB-40 treated samples had below detection limit (1 log CFU/g). However, total bacterial populations in GR-20 and EB-20 treated samples decreased below detection limit during storage. In addition, AC, GR-40 and EB-40 treated samples did not have bacterial growth during storage. TBARS values were in order of control<HT<AC<EB-20<GR-20<EB-40<GR-40, and VBN were not different ($P \geq 0.05$) among treatments except for control and HT. Sensory properties of control and irradiated samples were acceptable, but AC treatment deteriorated the texture and taste of samples during storage.

Significance: These results indicate that GR and EB treatments at 40 kGy may ensure the long-term storage of RTE Bulgogi without compromising the sensory properties.

P2-80 Quantifying the Efficacy of X-ray for Reducing Initial Microbial Population on Dates

IBRAHIM GREIBY, Gordon R. Davidson, Sanghyup Jeong and Kirk D. Dolan
Michigan State University, Biosystems and Agricultural Engineering, East Lansing, MI, USA

Introduction: Dates (*Phoenix dactylifera*) are a major source of carbohydrates in many desert areas around the world. In the United States, California is a major producer of dates. Due to the microbial contamination throughout the pre- or post-harvest process of dates, microbial, non-thermal and non-chemical methods to decrease microbial loads are becoming more desirable.

Purpose: The objective of this study was to evaluate the efficacy of X-ray for the reduction of an initial microbial population on dates.

Methods: California sun dates were purchased from a local grocery market. Dates were randomly selected and irradiated at doses of 0, 0.221, 0.441, 0.662 and 0.882 kGy in triplicate. Phosphate buffer solution was added to each sample. Samples were placed in the Pulsifier for 3 minutes, and the wash-solution was used for microbial analysis with serial dilutions. For total plate counts (TPC) of bacteria, trypticase soy agar containing 0.6% yeast extract was used. Subsequently, plates were incubated at 30°C for 48 ± 2 h. Potato dextrose agar was used for the enumeration of molds and yeasts and the plates were incubated at room temperature (25°C) for 3 days.

Results: Mean total count results showed one log reduction in bacterial population from about 3.9×10^4 CFU/g for the control to 4.0×10^3 CFU/g at 0.882 kGy ($R^2 = 0.87$). For molds and yeasts, it was countable after three days of incubation The change in mold and yeasts was from 1.0×10^4 CFU/g to 2.9×10^3 CFU/g at 0.882 kGy ($R^2 = 0.53$).

Significance: X-ray irradiation at low doses offers a potential option to reduce microbial loads by one log on partially dried dates. X-ray irradiation may be combined with other treatments as hurdle technology.

P2-81 Surface Pasteurization and Sterilization of Low–moisture Food Crops by the Controlled Condensation Process CCP

RAINER PERREN and Jürgen Fischer
RPN FOODTECHNOLOGY Ag., Sursee, Switzerland

Introduction: Low moisture food crops such as nuts, oil seeds or spices contaminated with pathogenic microorganisms are a major hazard for food production. Recently, elevated *Salmonella* contamination was reported in almonds, pistachios and peanut products causing outbreaks of foodborne salmonellosis and resulting in extended product recalls. While conventional heat decontamination technologies increase moisture content considerably degrading the initial and authentic quality of food crops, the proprietary controlled condensation pasteurization technology CCP pasteurizes and sterilizes particulate food items controlling surface condensation and actual moisture increase by creating thermal equilibrium conditions. This humid heat pasteurization and sterilization process controls surface temperature, surface condensation and moisture increase maintaining the default moisture level.

Purpose: It was the aim to investigate the influence of varying CCP treatment conditions on selected food crop quality attributes.

Methods: Almonds, pecans, sesame seeds and other crops were inoculated with *Enterococcus faecium* NRRL B 2354, a heat resistant surrogate for *Salmonella* Enteritidis SE PT 30, and pasteurized with the humid heat process at varying conditions. Highly contaminated spice crops (such as pepper or chili as well as powdered turmeric, fennel or jeera) were sterilized at temperatures >100°C. Inactivation trials were repeated and inactivation results were statistically evaluated.

Results: At pasteurization conditions of 81°C and 5 min in a vapor atmosphere, inoculated almonds, pecans and sesame seeds were inactivated by more than 5 logs. In case of blanched nuts and pistachios the pasteurization temperature had to be slightly increased to 86°C in order to realize a comparable inactivation rate. Pasteurization at these conditions did not cause relevant changes in moisture content, flavor attributes, color and shelf life. Sterilization of spices at 105 to 120°C during 5 to 12 min in a vapor atmosphere lead to a complete inactivation of the microflora (initial natural contamination up to 10^8 CFU/g, detection level < 10 CFU/g). Depending on the temperature-time conditions applied, the indigenous and authentic properties of the raw material were preserved and changes in moisture, color and volatile oil fraction were little.

Significance: These results show that with the proprietary pasteurization and sterilization process, food crops are pasteurized or sterilized at equilibrium conditions securing inactivation of pathogens and preserving the authentic quality of the food crop.

P2-82 Genetic Diversity of Strains of *Leuconostoc* Isolated from Sausage Processing Environment

Sofía M. Arvizu-Medrano, J. JESUS PADILLA-FRAUSTO and Alma G. Pérez-Rico
Universidad Autonoma de Queretaro, Departamento de Investigacion y Posgrado en Alimentos, Querétaro, Mexico

Introduction: Lactic acid bacteria are the dominant microflora in processed meat products packaged under modified atmosphere. The microbial spoilage in Vienna sausages is frequently caused by *Leuconostoc*. Deficient sanitation processes could permit the persistence of spoilage microorganisms in processing environments.

Purpose: The objectives of this work were to investigate the genetic diversity of *Leuconostoc* spp. isolated from sausage processing environment and to determine the strains' persistence in the equipment.

Methods: Sixty-eight strains of *Leuconostoc* spp. previously isolated from the sausage processing environments (equipment, work surfaces and products) were identified as *L. mesenteroides* (41), *L. fallax* (20), *L. lactis* (4) and *L. plantarum* (3), using the Biolog™ system. *Leuconostoc* strains were differentiated by a randomly amplified polymorphic DNA (RAPD) protocol using a specific primer 1299 ((AGCT)

Poster

CC(AG)TC(CT)TG(ABGT)CCAG)AA(AG)TA(AGCT)ACCCA) designed from the conserved regions of dextransucrase genes. The genetic profiles were compared using the Dice similarity coefficient. Dendrograms were generated by the Statistica ver. 4.3 (1993) program.

Results: *Leuconostoc mesenteroides* exhibited less genetic diversity than the other species. Dendrograms showed that eight strains, isolated from diverse materials, surfaces along the production process and spoiled sausages, constitute a group of *L. mesenteroides* subspecies Mesenteroides with more than 70% genetic similarity. *L. mesenteroides* strains isolated during November exhibited different genetic profile (0% of similarity) as compared to the rest of the strains. Two different groups of *Leuconostoc fallax* genotypes were identified. Each is related to specific sampling intervals (April—August, and August—November). Some *Leuconostoc* genotypes showed persistence over time. It is the case of *L. mesenteroides* isolated from spoiled sausage in December 2007 and in April 2008.

Significance: Our results suggest the existence of *Leuconostoc* reservoirs in the processing equipment determined by deficient sanitation practices.

P2-83 Isolation and Characterization of Causative Organisms of "Blown-pack" Phenomenon in Ground Beef Chubs

TAM MAI, Rico Suhalim and Mansour Samadpour
IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA

Introduction: "Blown-pack" spoilage of ground beef chubs is characterized by the accumulation of copious amounts of gas in the package, which leads to pack distention and spoilage and consequently results in substantial economic losses in the beef industry.

Purpose: The objective of this study was to enumerate and identify the microflora associated with blown appearance of spoiled ground beef chubs obtained from commercial beef processing plants in the United States.

Methods: Ground beef samples in blown-pack packaging were analyzed for the following microflora using selective and non-selective media: total mesophilic bacteria, total psychrotrophic bacteria, lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* and *Brochothrix*. In addition, samples were enriched in gas assay media containing Durham tubes at 7°C and 23°C until visual signs of gas production were observed. Gas producing organisms were isolated and identified using phenotypic characterization and 16s rRNA sequencing.

Results: Total mesophilic plate counts ranged from 3 to 5 log CFU/g, while psychrotrophic bacterial counts ranged from 5 to 7 log CFU/g. There was a predominance of lactic acid bacteria with counts ranging from 6 to 7 log CFU/g. The highest counts of *Pseudomonas*, *Brochothrix*, and *Enterobacteriaceae* were 3 log CFU/g, 3 log CFU/g and less than 2 log CFU/g, respectively. *Leuconostoc gasicomitatum*, *L. gelidum*, *L. mesenteroides*, *Hafnia alvei* and *Clostridium* sp. were identified as the most common organisms from the samples.

Significance: This study presents the bacterial populations in the "blown-pack" spoilage of ground beef chubs and identified potential gas-producing spoilage organisms.

P2-84 Mathematical Indices for Microbial and Physico-chemical Characteristics of Biltong Product during Storage

KARABO SHALE
Central University of technology, Free State, Environmental Health, Bloemfontein, South Africa

Introduction: Food is considered to be a habitat for microbial growth and the survival and growth of microbes in food are determined by factors such as temperature, moisture availability (water activity, a_w), pH, the oxidation-reduction potential and the nature of the gaseous environment to which the organisms are exposed, not excluding the availability of nutrients.

Purpose: The purpose of the study was to simulate storage environment of biltong and conduct shelf-life study over a period of three months.

Methods: Ninety samples of biltong product were collected from randomly selected supermarkets, butcheries, mini markets and kiosks. Samples were analysed for the presence of total viable counts, staphylococci, *Bacillus cereus*, *Listeria monocytogenes*, yeasts and molds, as well as the physico-chemical parameters such pH, and NaCl, water activity and moisture content.

Results: Microbial counts decreased with time although the counts remained relatively high throughout the study (in the range of 10^5 and 10^6 CFU.g-1) with some correlations amongst some of the studied micriobiota. *Listeria monocytogenes* was not detected throughout the study period. Furthermore, the differences on the pH, water activity, moisture content and NaCl% content throughout the study were insignificant. Mathematical indices were also generated in order to circumvent any hazardous risks associated with biltong product in the future.

Significance: The hurdles technology employed in the processing of biltong were not efficient in keeping the microbial loads low. The study suggests proper quality control measures such as GMP and HACCP to decrease microbial load from the slaughtering floor throughout biltong production process.

P2-85 Evaluation of the Antibacterial and Antioxidant Properties of *Coptis chinensis* and *Glycyrrhiza uralensis* among 35 Medicinal Plants

HYUN SUK KIM
Dongguk University, Seoul, Republic of Korea

Introduction: Medicinal plants have been spotlighted as a resource of complementary and alternative medicine due to their outstanding biological and physiological properties. Also the extracts from medicinal plants can be used in food industry to prevent food spoilage or food poisoning.

Purpose: The purpose of the present study was to determine the presence of antibacterial and antioxidant activities in the crude extracts of some of the medicinal plants.

Methods: In this investigation, the ethanolic extracts of 35 medicinal plants were subjected to screening against three strains of bacteria species, *Escherichia coli, Bacillus cereus*, and *Staphylococcus aureus*, by following standard procedure of antimicrobial susceptibility test. In addition, the screened extracts were tested for their antioxidant activity using DPPH free radical scavenging activity assay and for their cytotoxic activity using assessment of cell viability against mouse embryonic fibroblast cells.

Results: The ethanolic extracts of *Coptis chinensis* and *Glycyrrhiza uralensis* have potential antibacterial activity to both Gram-negative *E. coli* and Gram-positive *B. cereus* and *S. aureus*. The ethanolic extracts of *C. chinensis* and *G. uralensis* showed good antioxidant potential at low concentrations. Our results showed that *C. chinensis* and *G. uralensis* can be promising sources of natural products with potential antibacterial and antioxidant activity.

Significance: The ethanolic extracts of *G. uralensis* showed the strongest efficiency of inhibition of *E. coli* and *B. cereus* growth, and especially, the antibacterial activity against *E. coli* was almost similar to that of kanamycin. From our results, the ethanolic extracts of *Coptis chinensis* showed remarkable DPPH radical scavenging activity.

P2-86 A Study to Determine the Thinning of Soups by *Bacillus* spp. (*B. subtilis, B. licheniformis, B. megaterium* and *B. pumilus*) and Amylases Produced by *Bacillus licheniformis*

ANDRES RODRIGUEZ LOZANO, Helen Brown, Matteo Campagnoli and Joy E. Gaze
Campden BRI, Microbiology, Chipping Campden, United Kingdom

Introduction: Thinning of soups is an important spoilage problem to the food industry. This is believed to be caused by the presence of heat resistant amylases or the growth of *Bacillus* spp. that have survived the heating process.

Purpose: The purpose of this work was to evaluate the thinning of soups during *Bacillus* spp. germination and growth and by *Bacillus* amylases.

Methods: Three different types of soups (cream of tomato, pH = 4.1, potato and leek, pH = 4.9, and cream of chicken, pH = 5.9) were inoculated with the spores (approx. 10 × 2–10 × 3 CFU/ml) of *B. subtilis*, *B. licheniformis*, *B. megaterium* and *B. pumilus* or *B. licheniformis* alpha-amylase (3 different concentrations: high = 12U/ml, medium = 0.03U/ml and low = 0.003U/ml), and incubated at 37°C for 21 days or a maximum period of 40 days, respectively.

Results: The results showed that the 4 bacilli were able to grow in the potato and leek soup and cream of chicken to levels of 10 × 7 CFU/ml after 48 h, and remained to this level during the incubation period. For the cream of tomato, only *B. licheniformis* was able to grow 1.5 log CFU/ml after 21 days. Visual thinning was observed in the potato and leek and cream of chicken soups even after 24 h incubation and became obvious after 21 days. No thinning was observed in the cream of tomato. The potato and leek, and cream of chicken soups inoculated with the alpha-amylase showed obvious thinning for all the concentrations tested after the 40 days incubation and with initial signs of thinning with the highest enzyme concentration even after 4 h. For the cream of tomato, thinning was only observed at the highest enzyme concentration starting after 48-h incubation.

Significance: The results of this work show that controlling the presence and growth of bacilli to avoid the production of heat-resistant amylases is critical to the reduction of the problems of thinning.

P2-87 ADIAFOOD® *Listeria* spp. Environment Detection Kit, a Complete Solution Adapted to Laboratory Workflow

TONY ROUILLARD, Anita Padham, Jerome Thepaut, Louisiane Giovanetti and Emmanuel Bertrand
AES CHEMUNEX, Molecular Biology, Bruz, France

Introduction: Foodborne pathogens cause millions of gastrointestinal diseases each year over the world killing millions of people and generating big losses for the food industry. Among these foodborne pathogens, *Listeria* is part of the most widespread in the world. Its presence is prevalent in the environment and generates many problems in food processes.

Purpose: AES CHEMUNEX decided to develop two detection protocols (21-h and 48-h time to result, respectively) intended for environmental monitoring of surface sampling; that would fit to any laboratory workflow. The detection of *Listeria* spp. in environmental samples will help prevent further contaminations in food processes in the agri-food industry.

Methods: ADIAFOOD® *Listeria* spp. duplex detection kit is based on real-time PCR technique providing results within 21 or 48 hours for up to 88 samples per run. After swabbing the environmental surface (9 surfaces have been tested, such as stainless steel, ceramic, plastic, etc.), we proceed with a selective enrichment step in our Environmental Listerboost® broth (18-24 h or 42-48 h). Then DNA is extracted from the samples, with or without a centrifugation step, in a thermocycler. Finally, the extracted DNA is amplified, detected and analyzed within 1.5 hours in a dedicated thermocycler and its associated Sentinel software.

Results: The 21-h and 48-h environmental sampling protocols for ADIAFOOD® *Listeria* spp. duplex detection kit are in process of validation by AOAC-RI and Health Canada, respectively. The test of 71 targeted and 51 non-targeted strains shows molecular markers used in the kit are highly specific with 100% inclusivity and exclusivity rates. The limit of detection is 1–10 CFU per PCR. These data prove that ADIAFOOD® detection kit yields results as good as or better than the reference method.

Significance: ADIAFOOD® *Listeria* spp. duplex detection methodology appears to be the one which fits best to laboratory workflow, compared to other methods currently available in the market since it provides laboratories with results either in 21 or 48 hours. The AOAC-RI and MFLP certifications are pending for these two protocols.

P2-88 Behavior of Shiga Toxin-producing *Escherichia coli* in Acid Media and in Acerola and Tamarind Pulps

MARIZA LANDGRAF, Katia L. Souza-Silva, Maria T. Destro and Adriana Lucatelli
Universidade de São Paulo, Alimentos e Nutricao Experimental, São Paulo, Brazil

Introduction: *E. coli* O157:H7 is the most studied strain of Shiga toxin-producing microorganisms (STEC). However, many cases of infections are emerging around the world due to non-O157 serogroups, such as O103, O111 and O26. An interesting characteristic of *E. coli* O157:H7 is its resistance to acid stress known as acid tolerance.

Purpose: This study aimed at verifying the behavior of non-O157:H7 STEC strains in fruit pulps (acerola and tamarind) and in acid culture medium.

Methods: Aliquots of overnight culture of *E. coli* strains O103, O111 and O26 were inoculated in trypticase soy broth (TSB) pH 7.0 (non acid-adapted strains) and in TSB pH 5.0 (acid-adapted strains), both incubated at 37°C/18–24 h. Acid tolerance test was done individually inoculating 27 mL of fruit pulp with 3 mL of non acid-adapted and acid-adapted strains with concentrations varying from 10^6 to 10^8 CFU/mL. On days 0, 1, 4, 6, 11, 13, 18, 20, 26 and 30 of storage at 4°C, 1 mL aliquots were diluted in 0.85% saline, and streaked onto MacConkey sorbitol agar (SMAC) and trypticase soy agar (TSA). The same assay was done using TSB with pH similar to the studied pulps: 3.5 for acerola and 2.4 for tamarind.

Results: Non acid-adapted and acid-adapted strains inoculated in acerola and tamarind pulps were recovered up to day 30 when streaked onto TSA. On the other hand, when streaked onto SMAC, recovering was restricted to the first day. When these microorganisms were inoculated in acidified TSB pH 2.4 they could be recovered on day zero when streaked onto TSA but not when streaked onto SMAC. When inoculated in TSB pH 3.5 (acerola pH), the cells could be recovered up to day 6 when streaked onto SMAC and day 18 when streaked onto TSA.

Significance: These data indicate that non-O157 STEC strains can survive in acid environment but it is necessary to use adequate media for recovery of acid stressed cells. They also show that acid conditions should not be considered a barrier concerning these bacteria.
Acknowledgment: Fapesp 08/58726-9.

P2-89 Genetic Characterization of Atypical *Vibrio parahaemolyticus* Isolates from Imported Raw Shrimp Detected with a Real-time PCR Assay for *Vibrio cholerae/parahaemolyticus/vulnificus*

TIMOTHY R. DAMBAUGH, Julie Kraynak, Hawley Pruitt, Bridget Andaloro, Morgan Wallace and George Tice
DuPont Qualicon, R&D, Wilmington, DE, USA

Introduction: Classic screening for pathogenic *Vibrio* species uses enrichment and presumptive identification on selective agar plates (TCBS, mCPC and chromogenic media like CHROMagar™ *Vibrio*). Atypical metabolic responses and reduced plating efficiencies of target species on selective plating media can yield false negative results. In contrast, PCR screening has significant advantages in sensitivity, time-to-result, specificity and detection of atypical target species.

Purpose: To demonstrate the value of PCR screening in reducing false negatives in seafood testing, BAX®-positive *V. parahaemolyticus* (Vp) isolates from raw shrimp with both typical (sucrose-negative; green colonies on TCBS) and atypical (sucrose-positive; yellow on TCBS) morphologies on TCBS and CHROMagar™ *Vibrio* plates were analyzed with a battery of genetic identification and molecular typing methods to establish taxonomy.

Methods: Purified DNA from freshly isolated and reference cultures of *V. parahaemolyticus*, *V. alginolyticus* and other vibrios were characterized by 16S rRNA sequence analysis and Multi-Locus Sequence Typing (MLST) using published protocols. EcoRI and PvuII ribotypes of all isolates were compared with the RiboPrinter® System. Isolated colonies were screened by PCR for Vp-specific hemolysin genes (*tdh, trh* and *tlh*) as specified in the FDA BAM.

Results: Molecular genetic characterizations of PCR-positive atypical and typical morphology isolates confirm the identity of *V. parahaemolyticus*. All PCR-positive typical and atypical isolates were positive for the pan Vp-specific thermolabile hemolysin (*tlh*). *Vibrio alginolyticus* isolates were negative in the PCR and FDA Vp-specific *tlh, tdh* and *trh* gene assays. Typical and atypical BAX® Vp-positive isolates shared EcoRI and PvuII ribotypes patterns discrete from other *Vibrio* species.

Significance: Atypical Vp isolates can readily be overlooked in *Vibrio* screening by selective plating methods. The BAX® Real-Time PCR assay for *Vibrio cholerae/parahaemolyticus/vulnificus* detects typical and atypical Vp isolates with equal sensitivity and specificity, removing subjective judgment as a potential source of false negative results.

P2-90 Diversity and Distribution of *Salmonella* Shed by Asymptomatic Dairy Cattle and in Dairy Farm Environments

LORRAINE D. RODRIGUEZ-RIVERA, Emily M. Wright, Karin Hoelzer, Julie D. Siler, Mara Elton, Kevin J. Cummings, Lorin D. Warnick and Martin Wiedmann
Cornell University, Food Science, Ithaca, NY, USA

Introduction: While it is well established that clinically sick livestock represents an important reservoir of *Salmonella* associated with human infection, the importance of asymptomatic shedders as sources of human salmonellosis is less well defined.

Purpose: The aims of this study were to assess the subtype diversity and distribution of *Salmonella* isolates from asymptomatic dairy cattle and dairy farm environment in New York, and to compare isolates from these sources with *Salmonella* isolates from clinical human cases collected in the same geographic area.

Methods: To characterize the on-farm diversity of *Salmonella* and its relationship to human infection we used serotyping, antimicrobial resistance patterns, and pulsed-field gel electrophoresis (PFGE). From a total of 1,362 *Salmonella* isolates from asymptomatic dairy cattle and farm environments, 437 isolates from 46 farms were selected for PFGE. The isolates were selected based on isolation date, farm, source, serovar and antimicrobial resistance pattern in order to avoid over-representation of strains that were re-isolated on the same farm.

Results: Twenty-seven *Salmonella* serovars were detected among the 437 isolates, with the five most common serovars being Cerro (177 (40%) isolates from 21 farms), Kentucky (68 (16%) isolates from 12 farms), Typhimurium (40 (9%) isolates from 7 farms), Newport (28 (6%) isolates from 8 farms), and Meleagridis (27 (6%) isolates from 3 farms). One hundred-one of the 437 isolates (23%) were resistant to between 1 and 11 antimicrobial agents, representing 54 different antimicrobial resistance patterns. A total of 65 *Xba*I PFGE patterns were detected among the *Salmonella* isolates; 12 of those patterns, representing 9 different serovars, exactly matched PFGE patterns from human isolates obtained in New York.

Significance: Our data indicate that *Salmonella* subtype diversity on dairy farms is high, and that *Salmonella* isolates from clinically healthy cows and farm environments are frequently multidrug resistant. These findings suggest that asymptomatic dairy cattle and farm environments must be considered as potential sources of human disease-associated *Salmonella* subtypes.

P2-91 Non-O157 Enterohemorrhagic *Escherichia coli* with AB_5 Subtilase Cytotoxin Found in Commercial Ground Beef and Spinach Products

Viktoriya Beskhlebnaya and MANSOUR SAMADPOUR
IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA

Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) strains are a subset of Shiga toxin-producing *E. coli* (STEC) that are able to cause hemorrhagic colitis and in severe cases hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura. EHECs are usually identified by the presence of two virulence genes, *stx* (encoding Shiga toxin) and *eae* (encoding intimin). AB_5 subtilase cytotoxin (SubAB) was found in some non-O157 *eae*-negative STEC associated with HUS.

Purpose: This study surveyed the incidence of non-O157 EHECs with SubAB in commercial ground beef products and spinach enrichments received for confirmatory testing in the laboratory.

Methods: A total of 1800 raw ground beef samples purchased prepackaged from different retail stores in the Seattle, WA area were analyzed from September, 2008 through June, 2009. Samples were enriched overnight in a selective medium and tested by multiplex PCR for STEC detection. Enrichment cultures yielding EHEC presumptive positive bands (corresponding to *stx*, *eae* or *subAB* were streaked onto MacConkey agar and incubated at 35°C overnight. Isolated colonies of pink color were analyzed by PCR to confirm the presence of the virulence factor genes and submitted for Pulsed Field Gel Electrophoresis (PFGE).

Results: Thirty-two ground beef samples tested positive for EHEC contamination. Of those, 28 showed unique PFGE patterns. Twenty-five strains (78.1%) had *subAB* with stx_1, stx_2. All of the strains with subAB were non-O157. Seven strains (21.9%) had eae with either stx_1, or stx_1 and stx_2. No strains were positive for both *subAB* and *eae*. Additionally, from spinach samples sent for confirmation of EHEC contamination, five of seven EHEC-positive *E. coli* strains contained the *subAB* gene, and none of these were positive for *eae*.

Significance: Currently, *E. coli* O157:H7 is the only EHEC declared to be an adulterant in raw ground beef and is the only serotype for which USDA Food Safety and Inspection Service (FSIS) routinely tests. Certain strains of non-O157 EHECs such as those containing the intimin or subtilase gene may be as virulent as *E. coli* O157 hence identifying these strains would be of value in improving the safety of food supply.

Poster

P2-92 Phenotypic and Genotypic Diversity of Emetic Toxin-producing *Bacillus cereus* Korean Isolates

JUNG BEOM KIM, Ki-Cheol Kim, Hong-Rae Jeong, Dae-Hwan Kim, Suk-Ho Kang, Yong-Bae Park, Mi-Hye Yoon and Jong-Bok Lee
Gyeonggi-do Institute of Health and Environment, Health Research, Suwon, Republic of Korea

Introduction: *Bacillus cereus* is a Gram-positive and endospore-forming opportunistic human pathogen. *B. cereus* was divided into emetic toxin- and enterotoxin-producing strains. However, no specific study of the phenotypic and genotypic characterization of emetic toxin-producing *B. cereus* Korean isolates has been conducted.

Purpose: The objective of this study was to investigate the phenotypic and genotypic diversity of emetic toxin-producing *B. cereus* strains Korean isolates.

Methods: A collection of 39 emetic toxin-producing *B. cereus* was used for this study. The emetic toxin-producing reference strain used for the positive control was *B. cereus* F4810/72. HPLC/MS analysis was performed to confirm emetic toxin production. The RAPD assay was carried out using primer 6 (5-CCCGTCAGCA-3). Also, PFGE analysis digested with the NotI enzyme was performed according to the previous report. The Kirby-Bauer disk diffusion method was used to evaluate the antibiotic susceptibility pattern of emetic toxin-producing *B. cereus*.

Results: A total of 17 distinct pulsotypes were obtained from the RAPD banding patterns and the majority cluster consisted with clinical and food strains belong to group 3. The PFGE banding patterns were divided into 17 distinct pulsotypes and groups 2, 3 and 4 were dominated by clinical strains. Emetic toxin-producing *B. cereus* Korean isolates showed diverse pulsotypes based on the RAPD and the PFGE banding patterns. All strains were resistant to β-lactam antibiotics such as penicillin and ampicillin. Combining phenotypes, PFGE types, RAPD types and antibiotic resistance types, a total of 7 composite groups were found.

Significance: Emetic toxin-producing *B. cereus* Korean isolates showed phenotypic and genotypic diversity comparing the previous studies.

P2-93 Genotyping Environmental, Fresh Produce, Meat and Clinical Isolates of *Salmonella* Saintpaul

ALICE E. HAYFORD, Mark K. Mammel, Jayanthi Gangiredla, Shaohua Zhao, Joseph E. LeClerc and Chris Elkins
FDA, Laurel, MD, USA

Introduction: The ability to differentiate foodborne bacteria is important and often critical for epidemiological investigations of outbreaks associated with foods. *Salmonella* Saintpaul continues to present significant health and economic threats in the United States. In 2008, *Salmonella* Saintpaul was associated with a multi-state outbreak linked to jalapeno peppers and possibly tomatoes, and in 2009 it was associated with illness from consumption of alfalfa sprouts. Generally, PFGE profiles indicate that *S.* Saintpaul is homogeneous among this serovar, and strains are difficult to differentiate using this method.

Purpose: The aim was to investigate the genomic relatedness of *S.* Saintpaul isolates using a molecular method based on single nucleotide polymorphisms (SNPs), to link food sources and outbreak isolates.

Methods: We tested 113 *S.* Saintpaul isolates from environmental, food (ground turkey), and foodborne outbreaks using a panel of 30 SNPs. The SNP patterns of these isolates were analyzed using Pyrosequencing.

Results: Eight different SNP patterns were found among the isolates in this study. A common SNP pattern was generated with 75% of these isolates with ground turkey source most common. Eighteen environmental isolates from Mexico had two different SNP patterns. The jalapeno outbreak isolates shared a SNP pattern with that of one of the two environmental SNP patterns. The five remaining SNP patterns were represented by one isolate each from ground turkey.

Significance: SNP differences are proving to be useful genetic markers for exploring the genomic diversity of closely related microbial strains. The ability to distinguish closely related isolates using SNPs will aid in determining if a cluster of clinical strains belong to the same outbreak, and to match an outbreak strain to a food isolate. We have discovered SNP differences in a population of *S.* Saintpaul isolates from diverse sources. We are currently comparing these SNP profiles and their associated PFGE patterns to assess the contribution and added value of this technique to improve epidemiological linkages. Using this information, the search for additional SNPs will continue in an effort to improve such linkages.

P2-94 Co-occurrence of *Escherichia coli* Shiga Toxin $2d_{activatable}$ Variant and AB_5 Subtilase in STEC in Commercial Ground Beef Products

Viktoriya Beskhlebnaya, Kay Greeson, Songhai Shen and MANSOUR SAMADPOUR
IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA

Introduction: Shiga toxin-producing *E. coli* (STEC) is a food- and waterborne pathogen that causes hemorrhagic colitis, and in severe cases hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. Shiga toxin (*Stx*) has two forms: $Stxt_1$ and Stx_2. A variant Stx_2, called $Stx_{2dactivatable}$, is more virulent than other subtypes. STEC associated with severe clinical outcome usually express in addition to *Stx* the other virulence factors - such as intimin (Eae) and AB_5 subtilase cytotoxin (SubAB).

Purpose: The purpose of this study was to investigate the presence of $Stx_{2dactivatable}$ in raw ground beef samples by PCR, PFGE analysis and sequencing of the stx_{2A} subunit.

Methods: A total of 1800 raw ground beef samples purchased prepackaged from different retail stores in the Seattle, WA area were analyzed from September, 2008 through June, 2009. Samples were enriched overnight in IEH media and tested by multiplex PCR for STEC detection. Presumptive positive enrichment cultures (i.e., showing the *stx* and *eae* or *subAB* bands) were streaked onto MacConkey agar and incubated at 35°C overnight. Pink colonies were picked and analyzed further by PCR and Pulsed Field Gel Electrophoresis (PFGE). One representative strain per sample batch with a common PFGE pattern was taken for further analysis and submitted for determination of Shiga toxin variants by PCR and by sequencing of the stx_{2A} subunit.

Results: Thirty-two ground beef samples tested positive for STEC contamination. Of these, eight (25%) samples contained the $stx_{2d\text{-}activatable}$ variant. Two of these were positive for the stx_{2c} variant as well. All of the strains containing $stx_{2d\text{-}activatable}$ were also positive for the *subAB* gene and were not of the O157 serotype. Seven strains (21.9%) had *eae* with either stx_1, or stx_1 and stx_2, none of these were positive for $stx_{2d\text{-}activatable}$ or sub_{AB}. Of the 32 isolates, 28 STEC strains showed unique PFGE patterns.

Significance: This study showed that certain strains of non-O157 EHECs such as those containing the variant $stx_{2d\text{-}activatable}$ or *subAB* may be missed when testing for O157 only. These non-O157 STECs may be as equally virulent as the O157 strains, hence equal importance in detecting these strains be given to ensure consistent quality of beef supply.

Poster

P2-95 Virulence Gene and CRISPR Sequence Typing Method for Subtyping the Three Most Common Serovars of *Salmonella enterica* Subspecies *enterica*

FENYUN LIU, Efrain Ribot, Peter Gerner-Smidt, Edward Dudley and Steve Knabel
Penn State University, Food Science, University Park, PA, USA

Introduction: *Salmonella enterica* subsp. *enterica* is the leading cause of bacterial foodborne disease in the United States. Molecular subtyping methods are powerful tools for tracking the farm-to-fork spread of foodborne pathogens during outbreaks. Pulsed-field gel electrophoresis is currently the "gold standard" molecular subtyping method for *Salmonella*; however it has certain limitations, such as inadequate epidemiologic concordance and low discriminatory power for typing *S.* Enteritidis.

Purpose: The objective of this study was to develop a multilocus sequence typing (MLST) method that has both good discriminatory power and epidemiologic concordance for *S. enterica* subspecies *enterica*.

Methods: Virulence genes *sseL*, *fimH2* and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) regions were sequenced from 50 clinical isolates of *S.* Typhimurium, *S.* Enteritidis and *S.* Newport.

Results: MLST correctly clustered epidemic clones and outbreak clones together, which is reflected by the high epidemiologic concordance of 0.94. Epidemiologic concordance of PFGE, on the other hand, was 0.90. Discriminatory power of the MLST scheme was 0.98, which is equal to combined discriminatory power of PFGE using *Xba*I and *Bln*I.

Significance: In conclusion, our MLST scheme may be an alternative or complementary method to PFGE for subtyping certain *S. enterica* subspecies *enterica* serovars during outbreaks. We are currently testing this hypothesis with a larger number of *Salmonella* isolates from the most prevalent clinical serovars of *S. enterica* subsp. *enterica*.

P2-96 Development of Rapid Enzyme-linked Immunosorbant Assay for the Detection of *Cronobacter* sp. (*Enterobacter sakazakii*) in Infant Formula

YESOL KIM, Myunghee Kim and Sejong Oh
Chonnam National University, Division of Animal Science, Gwangju, Republic of Korea

Introduction: *Enterobacteriaceae* are Gram-negative bacilli that normally inhabit the intestines of humans or animals. While most species are harmless, some produce exotoxins. Especially, *Cronobacter* sp. former named *Enterobacter sakazakii*, is an opportunistic pathogen that causes meningitis, sapremia, and necrotizing enterocolitis in neonates and small children.

Purpose: This study was aimed at developing an indirect immunoassay using microtiter plates for the detection of *Cronobacter* sp. (*E. sakazakii*) in infant formula.

Methods: We injected *E. sakazakii* cells into laying hens for 12 weeks after immunity. Booster injections began to have an effect 2 weeks after the first injection. Subsequently, we isolated IgG from the sera of the chickens and purified it by dialysis. We then performed indirect enzyme-linked immunosorbant assay (ELISA) to determine the effectiveness of chicken IgG against *E. sakazakii*.

Results: The results of ELISA showed that the antibody titer at 2 weeks after the first injection was 10 times greater than that after the first injection. Between the third and fourth weeks, the titer increased substantially; it remained constant thereafter and then decreased. We defined that titer increased 1.2 times after first immune, and titer increased more than 1.5 times after the third injection. The IgG and IgY titers were similar. Water dilution is the most effective method of IgY purification. The isolated chicken IgG was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a medium-range molecular weight marker (10~173 kDa), and its molecular weight was determined to be 38~49 kDa. Indirect competitive ELISA revealed that the lowest detection limit was 10^5 cells/well in the case of infant formula.

Significance: We determined that the antibody had high specificity for *E. sakazakii* and low cross-reactivity with other pathogens. On the basis of our results, we can develop a liposome-based kit for the rapid detection of *E. sakazakii*, and thus help minimize the outbreak of *E. sakazakii* at food industry.

P2-97 Virulence Factors of *Salmonella* Recovered from Pre- and Post-chill Whole Broiler Carcasses

SALINA PARVEEN and Tagelsir Mohamed
University of Maryland Eastern Shore, Princesse Anne, MD, USA

Introduction: *Salmonella* causes 1.4 million cases of salmonellosis and over 500 deaths annually in the United States. Salmonellosis is mainly caused by food of animal origin, especially poultry. *S.* Typhimurium and *S.* Kentucky have been isolated from pre- and post-chill whole broiler carcasses. However, little information is available about the association between the presence of virulence factors in these serotypes and their potential of causing human illness.

Purpose: The purpose of this study was to evaluate virulence factors of *S.* Typhimurium and *S.* Kentucky recovered from pre- and post-chill whole broiler carcasses.

Methods: A total of 309 (146 pre- and 163 post-chill) isolates were tested for the presence of *Salmonella* virulence genes *inv*A, *pag*C, and *spv*C by PCR. Bioassays were used to evaluate aerobactin and colicin production.

Results: All isolates contained *inv*A and *pag*C but only 1.3% contained *spv*C. All *spv*C positive isolates were *S.* Typhimurium, one of them was recovered from pre-chill and the other three were recovered from post-chill. Overall, 30.7% of the isolates were aerobactin producers, and 41.1% were colicin producers. Among the 21.4% of aerobactin producers, 10.4% of *S.* Kentucky isolates were from pre-chill and 11.0% were from post-chill. Five percent of pre- and 4.2% of post-chill *S.* Typhimurium isolates were aerobactin producers. Among the 38.5% colicin producers, 20.7% of *S.* Kentucky isolates were from pre-chill and 17.8% were from post-chill. Two percent of pre- and 1.0% of post-chill *S.* Typhimurium isolates were colicin producers. There was no significant difference ($P > 0.05$) in the presence of virulence factors between pre- and post-chill isolates.

Significance: The results suggest that *Salmonella* isolates recovered from pre- and post-chill whole broilers carcasses can possess virulence factors and thus have the potential to cause salmonellosis. The research also indicates that chilling had no effect on virulence factors of *Salmonella*.

P2-98 Effects of Titanium Dioxide-UV Photocatalytic Reaction on DNA Damage and Disinfection of Foodborne Pathogenic Bacteria

KASHIF GHAFOOR, Soohyun Kim, Younjung Lee, Jongkun Lee, Sangyoul Na and Jiyong Park
Yonsei University, Dept. of Biotechnology, Seoul, Republic of Korea

Introduction: Titanium dioxide (TiO_2) photocatalytic reaction under ultraviolet (UV) irradiation provides high rates of microbial disinfection. TiO_2-UV photocatalysis is an effective method due to no toxicity, chemical stability, sustained catalytic activity and the absence of waste materials. The hydroxyl radicals generated by TiO_2-UV reaction have much stronger oxidizing power than other oxidizing agents and this property can be effectively used for disinfection in foods.

Purpose: The objective of this study was to evaluate the effects of TiO_2-UV treatment on DNA damage, morphology and disinfection of foodborne pathogenic bacteria (*E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium).

Methods: Different UV light domains (A, B and C) were used in this study for TiO_2 photocatalysis. Super-coiled plasmid DNA (pUC19) and genomic DNA of *E. coli* were used to demonstrate photodynamic DNA strand-breaking activity and the effects were measured by gel electrophoresis. The counts of foodborne bacteria (*S.* Typhimurium, *E. coli, L. monocytogenes*) were determined after TiO_2-UV and UV irradiation alone for 20 min. Morphological studies were performed using scanning electron microscopic (SEM).

Results: Regardless of the bacterial strains and TiO_2 use, the UVC light resulted in an earlier bactericidal phase (within 90 s) than UVA and UVB (initial counts of approximately 6 log CFU/ml). TiO_2-UVC treatment showed better photocatalytic effect on plasmid DNA than UVC irradiation alone. After TiO_2-UVC photocatalytic treatment for 6 min, all plasmid DNAs were changed to linear DNAs, but under UVC irradiation alone, super-coiled DNAs remained. An increase in TiO_2-UVC irradiation time also increased the photocatalytic effect for genomic DNA destruction. SEM observations suggested that bacterial cells were severely damaged by TiO_2-UVC photocatalytic reaction.

Significance: This research signifies the importance of TiO_2-UV photocatalytic treatment, particularly in association with UVC irradiation, as an effective method to disinfect foodborne pathogenic bacteria.

P2-99 Phylogenetic Analysis of *Arcobacter* Species by Sequencing of the Gyrase B Gene

SUSUMU KAWASAKI, Pina M. Fratamico, Irene V. Wesley and Shinichi Kawamoto
National Food Research Institute, Japan, Tsukuba, Japan

Introduction: The genus *Arcobacter* spp. includes bacteria previously known as aerotolerant campylobacters and belongs to the family Campylobacteraceae. In our previous report, we demonstrated that gyrase B gene analysis is an effective tool for classifying several species of the genus *Campylobacter*. In this study, 6 strains of *Arcobacter* spp. and *Bacteroides ureolyticus* were analyzed. The gyrB gene was sequenced and the phylogenic tree was constructed, that was similar to the 16S rDNA gene sequence analysis.

Purpose: The purpose of this study was to identify sequence polymorphisms in the *Arcobacter* gyrB gene and to construct the phylogenic tree for *Arcobacter* spp.

Methods: Partial DNA sequences of the gyrB gene of 6 strains of *Arcobacter* species and *Bacteroides ureolyticus* were determined. A universal primer set, designed to amplify a 960-bp fragment of the gyrB gene of each strains, these sequence data were used for phylogenic analysis.

Results: The topology of the resulting phylogenic neighbor-joining tree based on the gyrB gene was similar to a previously reported phylogenic tree based on the 16S rDNA gene. However, gyrB provides a better resolution for *Arcobacter* species than the 16S rDNA gene. The gyrB gene was sequenced and the phylogenic tree was constructed, that was similar to the 16S rRNA gene sequence analysis. However, gyrB provides higher resolution for *Arcobacter* species, with lower interspecies sequence similarities (ranging from 88.2 to 80.5%) compared to 16S rRNA gene (ranging from 98.6 to 93.9%). The gyrB gene is useful as a molecular taxonomic marker for *Arcobacter* species.

Significance: The gyrB sequence information will facilitate taxonomic studies of novel *Arcobacter* species and provide tool for rapid identification of *Arcobacter* species.

P2-100 Expression of Curli by Enterohemorrhagic *Escherichia coli* as Influenced by Environmental Conditions

JINRU CHEN
The University of Georgia, Griffin, GA, USA

Introduction: Curli has been shown to assist the cells of *Escherichia coli* (EHEC) in attaching to solid surfaces. The protein fiber binds Congo red and coomassie brilliant blue, and curli expressing cells (C+) form purple, and non curli expressing cells (C-) form colorless colonies on Luria-Bertani no salt (LBNS) agar supplemented with the two dyes.

Purpose: This study was undertaken to examine the expression of curli by wild type EHEC strains and determine the stability of C+ cells and their C- counterparts under different growth conditions.

Methods: Wild type EHEC strains, 7–52 and 5–11, capable of forming both curli expressing and non curli expressing colonies, were used in the study. Percentage ratios of the colonies formed by the C+ and C- cells were determined. The stability of each colony morphotype was determined by growing the cells on LBNS, Beef Extract (BF) or Beef Extract Peptone (BEP) agar plates at 10, 15, 22, 28 or 37°C for 72 h. The influence of growth media and incubation temperature on curli expression was assessed.

Results: The percentage ratios of curli expressing vs. non curli expressing colonies formed by the wild type EHEC cells were significantly influenced by growth media and incubation temperature ($P < 0.05$). Curli was not expressed on the three growth media at 10°C and on LBNS and BE at 15°C. The C- cells of the two EHEC strains were stable. However, 2–4% of the C+ cells of 5–11 formed colorless colonies on the three media at 15°C, and 1–2% of the C+ cells of 5–11 formed colorless colonies on LBNS and BE agar at 22°C. For the C+ cells of 7–52, about 36% of the colonies were colorless on BEP agar plates incubated at 37°C.

Significance: The results of this study suggest that curli expression and its stability were affected by the tested growth conditions.

Poster

P2-101 Comparison of Four Molecular Typing Methods for *Vibrio parahaemolyticus* Isolated from the Middle-East Coastline of China

Wan yi Chen, Jing ye Xu, Yanping Xie, Ming Gu, Min Zhou and XIANMING SHI
Shanghai Jiao Tong University, Food Science and Technology and Bor Luh Food Safety Center, Shanghai, China

Introduction: *Vibrio parahaemolyticus* is one of the most important pathogens causing seafoodborne gastroenteritis. Due to the large number of reports of human disease outbreaks and the consequent economic loss in aquaculture, correct identification, classification, and tracing the transmission of this organism becomes an issue of great importance.

Purpose: Outbreaks of *Vibrio parahaemolyticus* gastroenteritis in China highlights the need to develop a rapid molecular typing method for strain differentiation of this pathogenic species. Molecular typing of *V. parahaemolyticus* may be specifically useful for tracking the source of infection and detection of virulent strains, as well as the study of the geographical and host distribution of possible variants.

Methods: Fifty-six *V. parahaemolyticus* epidemiologically-unrelated strains collected from various environmental sites, seafood, and clinical samples along the middle-east coastline of China were investigated, using four different molecular typing methods, including ribotyping, enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), pulsed-field gel electrophoresis (PFGE), and sequence analysis of the gyrB gene. UPGMA dendrograms were produced on the basis of the similarity analysis by the software (Bionumericus version 5.0). Distance dendrogram of the gyrB gene sequences determined in this study was generated by the Mega4.0 package using the neighbor-joining method. The discriminatory ability among four typing methods was calculated by Simpson's Index of Diversity.

Results: Discriminatory index of ERIC-PCR typing is maximal (0.931), discriminatory index of sequence analysis of the gyrB gene typing is minimal (0.702). Cluster analysis of genetic profiles from these molecular techniques more clearly showed that automated ribotyping has lower discriminatory ability compared to PFGE and is limited to application in *V. parahaemolyticus* subtyping and outbreak investigation. ERIC-PCR is more rapid and easier to perform than PFGE and is very useful for the analysis of large number of strains from an epidemiological monitoring stand point. The evolutionary relationship between the isolates from environment and seafood and the clinical pandemic isolates is clearly understood by aligning the gyrB gene sequence, despite its comparatively lower discriminatory ability.

Significance: By taking advantage of comparing the conjunction of two methods, these results suggested that combining ERIC-PCR with sequencing analysis will be a reliable, high-effective and high-resolution subtyping system for *V. parahaemolyticus*.

P2-102 Development of a Real-time PCR Assay with an Internal Amplification Control for the Rapid Detection of *Vibrio parahaemolyticus*

Xiaohua He, Bin Liu, Wanyi Chen, Shuijing Yu, Chunlei Shi, Jing Chen and XIANMING SHI
Shanghai Jiao Tong University, Dept. of Food Science, School of Agriculture and Biology, Shanghai, China

Introduction: Vibrio parahaemolyticus is an important foodborne pathogen, which is a cause of gastroenteritis associated with the ingestion of contaminated raw or partially-cooked seafood. Real-time PCR methods are being applied widely for the detection and quantification of foodborne pathogens. However, due to the presence of inhibitory food residues, organic solvents from nucleic acid extraction, or other unknown factors, this high level of sensitivity does not preclude the risk of false-negative results. Moreover, the detection targets of *V. parahaemolyticus* for real-time PCR methods, such as gyrB, tlh, tdh, trh, and toxR genes, may lack specificity.

Purpose: The aim of this study was to mine a specific target sequence and to develop a real-time PCR method with a competitive IAC for detection of *V. parahaemolyticus* in seafood.

Methods: Genomic comparison analysis was used to explore *V. parahaemolyticus*-specific targets, which were then evaluated for the specificity and the sensitivity both by on-line BLAST and PCR amplification. An IAC was constructed by the compound primer technology. PCR parameters were optimized, and its reaction system was developed. Then the real-time PCR detection system with an IAC for *V. parahaemolyticus* was evaluated for specificity, sensitivity, anti-interference ability and accuracy in examination of naturally-contaminated food samples.

Results: The specificity of this assay was evaluated using 390 bacterial strains, and only *V. parahaemolyticus* strains generated a fluorescent signal, while the non-*V. parahaemolyticus* strains generated a fluorescent signal only for the IAC, which was used to indicate false-negative results. The detection limit of this assay was 4.8 fg of purified genomic DNA (≈1 genome copy) of *V. parahaemolyticus* per reaction. Positive results were also obtained by this assay in the presence of 2.1 µg of genomic DNA or 10^7 CFU of non-*V. parahaemolyticus* bacteria per reaction. In addition, 94 copies of IAC per reaction had no influence on the detection limit of this assay. Ninety-six seafood samples were tested using real-time PCR assay, and 58 (60.4%) of the samples were positive.

Significance: This real-time PCR detection system with a competitive IAC was highly specific, sensitive, rapid, accurate and efficient method for the detection of *V. parahaemolyticus* in seafood.

P2-103 Correlations between Serotype and Genotype among *Salmonella* Serogroups Based on Comparative Genomic Analysis

Bin Liu, Lida Zhang, Xinna Zhu, Chunlei Shi, Jing Chen, Weibing Liu, Xiaohua He and XIANMING SHI
Shanghai Jiao Tong University, Dept. of Food Science, School of Agriculture and Biology, Shanghai, China

Introduction: Genotyping of *Salmonella* has become an increasingly important tool, which provides more information to identify the sources of infection during outbreak investigation and the recognition of particular strains. Genotyping in combination with serotype characterization will contribute to understanding the routes of foodborne transmission by means of epidemiological data from various prevalence studies and outbreak investigations. The comparative genomic approach provides abundant information to seek the correlations between serotype and genotype among *Salmonella* serogroups.

Purpose: The purpose of this study was to find out the different sequences among *Salmonella* serogroups and reveal the correlations between serotype and genotype among *Salmonella* serogroups based on comparative genomic analysis.

Methods: Twenty-five *Salmonella* whole genomic sequences were divided into 6 (A, B, C1, C2, D and others) serogroup-databanks. For each serogroup, the reference sequence was selected and split into 1,000-bp fragments in silico to align against all the other genomic sequences within the serogroup using BLASTN (basic local alignment search tool) program (version 2.2.9). The fragments that matched all genomic sequences in the same *Salmonella* serogroup with an E-value less than 10-200 were considered highly conserved, thus were used in the following alignment against all the genomic sequences from other *Salmonella* serogroups. The fragments with a very low similarity (E > 0.01) with the genomic sequences from other serogroups were considered as serogroup-specific fragments. The specificity of these fragments was verified by PCR assay.

Results: As a result, 2, 6, 7, 10 and 7 specific fragments were found for A, B, C1, C2 and D serogroups, respectively. Based on the gene annotations, these 32 serogroup specific fragments were divided into 3 categories (i.e., membrane protein genes, rfb gene clusters and fimbrial genes). All three groups of genes are conserved and closely connected with phenotypic characterization. To target these DNA fragments, several specific primer sets were designed for multiplex PCR to serotype 21 *Salmonella* standard strains and 85 additional food isolates. The PCR results demonstrated a good agreement with that of *Salmonella* serotyping.

Significance: This finding implied that the diversities of *Salmonella* serogroups are possibly resulted from these genes, which were especially correlated with sugar synthesis and metabolism or glycosyl and O-actetyl transfer.

P2-104 Evaluation of *Salmonella* Isolates from Various Regions and Reservoirs Using Multilocus Sequence Typing

Weibing Liu, Bin Liu, Xinna Zhu, Shuijing Yu and XIANMING SHI
Shanghai Jiao Tong University, Dept. of Food Science, School of Agriculture and Biology, Shanghai, China

Introduction: *Salmonella* is one of the most important foodborne pathogens, and it is found in the route from farm to table. Most surveillance data for molecular epidemiology on *Salmonella* are based on DNA banding pattern-based subtyping methods to elucidate its sources and reservoirs. In general, these methods such as PFGE might be difficult in comparison between different laboratories for the same analysis or between different runs within the same laboratory.

Purpose: The purpose of this study was to evaluate the population of *Salmonella* isolates collected from different sources and reservoirs using MLST (Multilocus Sequence Typing) method.

Methods: One hundred and twenty one *Salmonella* isolates from imported feed samples, and domestic chicken and food samples were collected from 1978 to 2008. MLST analysis was performed based on the standard method of *Salmonella*, so as the data obtained in this study could be compared with *Salmonella enterica* MLST Database.

Results: The overall *Salmonella* population was diverse as revealed by the MLST data, with 42 STs (sequence types) identified among the 121 *Salmonella* isolates tested. Six unique STs were found in chicken reservoir, 11 in food, and 35 in feed. ST-92 was the most common ST, represented by 16 *Salmonella* isolates from chicken and feed followed by ST-367 represented by 14 isolates from chicken and feed. Except a few common ST, seven new alleles were identified resulting in the assignment of nine new STs, which were observed in isolates originating from feed (mainly between 2006 and 2008). The dN/dS ratio (nonsynonymous to synonymous base substitution) was less than one for all the housekeeping genes and varied from 0.0000 for thrA to 0.1699 for hemD gene. The standardized IA for the whole population was 0.1464, which provides strong evidence of linkage equilibrium in this population.

Significance: The population of *Salmonella* isolates from imported feed and domestic chicken and food were different. Most STs of *Salmonella* isolates coming from imported feed were not found in domestic chicken and food. Furthermore, seven new identified alleles were contributed by the *Salmonella* isolates from imported feed samples, which is highlight to concern of the population of *Salmonella* in different reservoirs.

P2-105 Identification and Characterization of a Small Molecule That Inhibits the Quorum Sensing of *Vibrio vulnificus*

SEUNG MIN KIM, Su Yeon Kim and Sang Ho Choi
Seoul National University, Dept. of Agricultural Biotechnology, Seoul, Republic of Korea

Introduction: Quorum sensing (QS) has been implicated as an important global regulatory system controlling the expression of numerous virulence factors in bacterial pathogens. Therefore, identification of chemicals that inhibit QS is quite important in developing new approaches to combat bacterial infections.

Purpose: *Vibrio vulnificus* is a model pathogen for studying many other foodborne pathogens because it causes life-threatening septicemia and gastroenteritis with various potential virulence factors controlled by QS. So identification and characterization of small molecules that inhibit the QS of *V. vulnificus* are required for delineating novel strategies to control foodborne pathogens.

Methods: A high throughput screening of small molecule libraries was performed to identify inhibitors of the *V. vulnificus* QS. Using a reporter strain PVV2_1398::*luxAB* whose activity entirely depends on the SmcR, a QS master regulator of *V. vulnificus*, we identified a QS inhibitor named U-262.

Results: U-262 suppressed the exoprotease and elastase activities of *V. vulnificus* which are regulated by QS without having effect on bacterial growth itself. *V. vulnificus* revealed attenuated cytotoxic activity in the presence of the chemical. Western blot analysis demonstrated that the chemical decreases the cellular level of SmcR in a dose-dependent manner, indicating that upper QS signaling cascade is inhibited by the chemical. U-262 also decreased the luminescences of *V. harveyi* and the total protease activities of *V. anguillarum* which are regulated by QS, suggesting that it inhibits other QS of *Vibrio* spp. Derivatives of the U-262 presenting better QS inhibitory effects were developed and one of them has an IC_{50} value of ~0.05 μM.

Significance: These results suggested that U-262 is a novel anti-microbial agent inhibiting the QS of *Vibrio* spp. It will be useful to protect food from the *Vibrio* spp. and help to enhance public health.

P2-106 PCR Screening for Shigatoxin- and Intimin-encoding Genes of *Escherichia coli* and for *Salmonella* in Beef Products in Processing Establishments

WALTER HILL, Rico Suhalim, Hans C. Richter, Chad R. Smith, Andy Buschow and Mansour Samadpour
Institute for Environmental Health, Lake Forest Park, WA, USA

Introduction: Pathogenic *Escherichia coli* strains on raw or insufficiently cooked foods are of public health concern as serious disease may result from their ingestion. Therefore, most commercial producers of beef products screen for *E. coli* O157:H7 prior to shipment. While *Salmonella* is not considered an adulterant on raw beef products, it is used as an indication of process control. To detect these microorganisms, rapid screening methods are often used to provide results within 8–24 hours after sampling.

Purpose: Samples from several commercial beef production plants were tested using a rapid screening method based on the polymerase chain reaction (PCR) to determine if they were presumptively positive for bacterial cells carrying *E. coli* genes related to virulence, if the frequency of positive samples varied over time and if the contamination rates differed between trim, variety meat and ground beef samples.

Methods: Samples from 971,389 lots (trim, ground beef and variety meats) were analyzed during 2005 to 2008 by using an 8 h enrichment followed by PCR detection of the stx_1, stx_2 (Shiga toxins), *eae* (attaching and effacing encoding intimin), and *rfb* (encoding the O157 specific O side chain polysaccharide) genes plus a lateral flow antigen detection kit for *E. coli* O157, plus two *Salmonella*-specific genes. The significance of differences between frequencies of positive results was determined using Fisher's Exact Test.

Results: Of the lots sampled, 15% were positive for the stx_1 and/or stx_2, 9.1% for the *eae*, 3.0% for *rfb*, and 1.7% for *Salmonella* by the PCR assay. Generally, lots of ground beef showed the lowest frequency of contamination and variety meats the highest. Overall, 15%, 4.6%, 4.6% and 0.81% samples were screen-positive for Shigatoxin-producing *E. coli*, enteropathogenic *E. coli*, enterohemorrhagic *E. coli* and *E. coli* O157, respectively. Of the *E. coli* O157 positive samples, 14% were also *Salmonella* positive. The frequency of screen-positive samples increased during the summer months.

Significance: The higher incidence of non-O157 pathogenic *E. coli* and *Salmonella* in these beef products suggest that they could serve as indicator organisms signaling an increased risk for the presence *E. coli* O157.

P2-107 Isolation and Detection of *Listeria* spp., *Salmonella*, *Escherichia coli* O157:H7, and Non-O157 Shiga Toxin-producing *Escherichia coli* from Agricultural Environments in Northern Colorado

CLYDE S. MANUEL, Christina A. Ahlstrom, Kelly M. Horgan, Martin Wiedmann and Kendra K. Nightingale
Colorado State University, Animal Sciences, Fort Collins, CO, USA

Introduction: *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, and non-O157 shiga toxin-producing *E. coli* are important human foodborne pathogens. An understanding of the prevalence and diversity of these pathogens at pre-harvest level such as production agricultural areas is important for their reduction in the human food supply.

Purpose: The purpose of this study was to probe the presence of major foodborne pathogens from agricultural environments (i.e., livestock grazing pastures and produce fields) in northern Colorado.

Methods: A total of 580 soil, 115 drag swab, 266 fecal, and 190 water samples were collected on five grazing pastures and five produce farms in northern Colorado during three sampling trips in the spring, summer, and fall. Each location was divided into four areas for a total of 40 sample sites during each collection. Individual samples from each area were pooled prior to analysis. Samples were first pre-enriched using non-selective enrichment and partitioned for subsequent microbiological analysis using modified protocols derived from the Food and Drug Administration's Bacteriological Analytical Manual to detect each targeted pathogen. Up to four presumptive colonies representing each target pathogen from selective and differential media were confirmed using PCR.

Results: Of the 1,151 samples taken, three water and one surface soil sample were positive for *L. monocytogenes*, two water and two fecal samples were positive for *Salmonella*, and one soil and one fecal sample were positive for *E. coli* O157:H7. Fifteen non-O157 STEC were isolated and each carried stx_1 and/or stx_2. In addition to the pathogens of interest, two water samples were positive for *L. ivanovii* and six soil, three fecal, seven drag swab, and eight water samples were positive for non-pathogenic *Listeria* spp.

Significance: The data suggests the prevalence of human foodborne pathogens in production agriculture environments to be low. Future characterization of rare isolates from these environments through molecular subtyping will provide important insight into the epidemiology and transmission dynamics of these pathogens throughout the human food chain.

P2-108 Evaluation and Performance of a *Salmonella* Serotyping System on Pork and Poultry Isolates from Commercial Sources

J. E. Stratton, Y. MENESES, M. Hardin, R. van-Santen and M. Sanchez-Plata
University of Nebraska-Lincoln, The Food Processing Center, Lincoln, NE, USA

Introduction: *Salmonella* is one of the leading causes of foodborne illness worldwide, and it is estimated that 1.4 million infections occur annually in the U.S. alone. Serotyping is essential for surveillance, epidemiological investigations, and identification of sources of *Salmonella* in the food chain. The Premi®Test *Salmonella* (PTS), a recently developed commercial system based on genetic profiling, is a potential tool for rapid detection and identification of *Salmonella* serovars.

Purpose: The objective of this project was to evaluate the use of the PTS system as a serotyping tool to identify pork and poultry isolates obtained from vertically integrated operations and to compare the performance of the PTS system with traditional serotyping methods.

Methods: Eighty-six *Salmonella* isolates were obtained using a modified version of the USDA method and serotyped according to the traditional Kaufmann-White scheme. Isolates were then typed using the PTS system. The PTS system uses a select set of genetic markers that yield unique microarray hybridization profiles to identify and discriminate 100 different *Salmonella* serovars. Among the isolates, 63 different serotypes were represented, 36 of which were included in the PTS database and 27 were not present in it.

Results: Serotype identification using the PTS system was reproducible independently of the source (pork or chicken) or replication. Sixty-nine percent of the serotypes present in the PTS database were successfully identified as *Salmonella* and matched traditional serotyping. Thirty-one percent of the isolates were identified as *Salmonella* but did not match results from traditional serotyping. Further investigation indicates that these discrepancies may be due to mistyping of the original isolates by the traditional method or overlaps with known serotypes. Isolates not present in the PTS database were recognized as *Salmonella* genovars (profile unknown).

Significance: Traditional serotyping has deficiencies in that it is time-consuming and often results are not reproducible. The PTS system, with an analysis time of 8 h, is relatively simple to use and is highly specific for the serotypes present in the PTS database (100 in total). The use of this system can increase the accuracy of serotyping and decrease the time to result of analysis, which are important factors in responding to outbreaks and measuring the efficacy of reduction interventions.

P2-109 Validation of an *Escherichia coli* O157 Test System for the Detection of *Escherichia coli* O157 in Composite Meat Samples

MEREDITH SUTZKO, Bill Centrella, Ryan Welsh and Wendy Warren
Strategic Diagnostics, Newark, DE, USA

Introduction: Recent changes in beef industry sampling practices and regulatory testing concerns have required reevaluation of rapid *Escherichia coli* O157 methods for composite raw beef samples. The effects of compositing samples while maintaining shortened incubation times must be managed appropriately. Thus, sensitive, reliable, and validated methods are needed to monitor foods for *E. coli* O157 and ensure that safe food is being supplied to the consumer.

Purpose: The aim of the study is to evaluate the performance of the RapidChek® *E. coli* O157 test system against the USDA-FSIS reference method (Microbiology Laboratory Guidebook, Chapter 5.04) for the detection of *E. coli* O157:H7 from meat products.

Methods: Method comparison studies were conducted with 375 g composite samples of beef trim, ground beef, and ammoniated beef at a third party laboratory to determine accuracy, specificity, and sensitivity. One-hundred sixty (160) samples were analyzed by both methods. The ability to verify RapidChek *E. coli* O157 potential positive results with commercially available DNA-based methods directly from the same enrichment media followed by further confirmation with biochemical/serological procedures, as listed in the USDA MLG 5.04, was also analyzed.

Results: According to the Mantel-Haenszel chi-square analysis ($X^2 = 1.12$), there was no significant difference between the test method and reference method for the detection of *E. coli* O157 in composite raw beef samples. The test system demonstrated 86% relative accuracy. The sensitivity of the method was 100%. The specificity of the method was 100%.

Significance: The target pathogen can be detected at very low levels of contamination in 375 g composite raw beef samples in as few as 10 to 18 hours with the test system (depending on sample matrix). Verification of an H7 result can be accomplished without the need for further enrichment.

P2-110 Stability of Multidrug Resistance among *Campylobacter coli* from Turkeys

CAROL B. D'LIMA and Sophia Kathariou
University of California-Davis, Davis, CA, USA

Introduction: *Campylobacter* is a zoonotic pathogen that commonly infects animals grown for food. Turkeys grown conventionally were frequently colonized with multi-drug resistant (MDR) strains of *Campylobacter coli* resistant to tetracycline, streptomycin, kanamycin, erythromycin, ampicillin, nalidixic acid and ciprofloxacin. Such MDR strains may aggravate the public health threat associated with this zoonotic pathogen.

Purpose: The purpose of this study was to determine whether the MDR phenotype remained stable in the absence of antibiotics in a panel of genetically diverse MDR *C. coli* strains from turkeys. An additional objective was to determine overall genotypic stability of these strains.

Methods: The study employed four MDR *C. coli* strains that were derived from conventionally grown turkeys and represented different genotypes based on molecular subtyping, including pulsed-field gel electrophoresis (PFGE). Bacteria were grown microaerobially in Mueller-Hinton Broth at 42°C and transferred 60 times at daily intervals. Stability of the MDR phenotype was assed via minimum inhibitory concentration (MIC) determinations for tetracycline, streptomycin, kanamycin, erythromycin, ampicillin, nalidixic acid and ciprofloxacin. Bacteria from passage 60 were also analyzed with PFGE using *Sma*I and *Kpn*I.

Results: Resistance to all tested antimicrobials was stable after the 60 transfers for all four strains. However, for some of the strains MICs were slightly lower after the 60th transfer, even though they remained clearly above the threshold for resistance. PFGE and plasmid profiles of the strains following the 60th transfer were indistinguishable to those of the original strains.

Significance: The MDR phenotype appears to be stable following multiple transfers of the organisms in the absence of antibiotics. Thus, once arisen, such multidrug resistance may be difficult to eliminate. The apparent stability of the MDR phenotype may contribute to the observed high pre-harvest prevalence of MDR strains in conventionally grown turkeys.

P2-111 Surveillance of *Campylobacter* spp., *Escherichia coli* O157, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp. and Methicillin-resistant *Staphylococcus aureus* in Poultry

TAM MAI, Kristina Tenney, Truyen Nguyen, Michael McDowell and Mansour Samadpour
IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA

Introduction: Poultry meat has been implicated as a significant source of foodborne pathogens that can cause fatal illness among consumers.

Purpose: This study was undertaken to determine the level of contamination (*Campylobacter* spp., *Escherichia coli* O157, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp. and Methicillin-resistant *Staphylococcus aureus* (MRSA)) in poultry across 10 states in the United States.

Methods: A total of 100 samples (10 from each state) were shipped to the laboratory in cool packs and were immediately screened for the following pathogens: *Campylobacter* spp., *E. coli* O157, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp. and MRSA. Screening methods used were multiplex PCR, either for individual pathogens (*Campylobacter* spp., MRSA, and *Listeria monocytogenes*) or multiple pathogens in a single PCR analysis (*Salmonella* spp., *Shigella* spp. and *E. coli* O157). Initial reactive samples were further confirmed using a secondary PCR and cultural confirmation methods using USDA-FSIS or FDA-BAM procedures. Background microflora was also determined using an IEH MPN method that simultaneously enumerates total aerobic bacteria, total coliform and *E. coli*.

Results: The background flora for chicken parts and ground chicken were as follows (MPN/g): 67 to 106 aerobic bacteria, 3 to 105 total coliform and < 2.2. to 500 *E. coli*. *Salmonella* spp., *Listeria monocytogenes* and *Campylobacter* spp. were detected in 37, 26, and 72 samples, respectively. None of the samples tested positive for *E. coli* O157, *Shigella* spp. or MRSA. Multiple pathogens were detected in 32% of the positive samples. *Salmonella* incidence was higher in ground chicken than in chicken parts, while *Campylobacter* incidence was higher in chicken parts than in ground meat. All the positive results were confirmed using the USDA-FSIS methods specific for each pathogen.

Significance: Results from this study reaffirm earlier reports on the high incidence of *Campylobacter* and *Salmonella* in poultry products, despite quality control testing performed by the meat processors. A significant number of samples contained two or more pathogens, which can potentially cause serious human illness if not eliminated through proper handling and adequate cooking.

P2-112 Is Imported Raw Chicken a Source of Antimicrobial-resistant Strains of *Escherichia coli*, *Campylobacter* Species and *Salmonella* Species in the UK?

NIAMH MURPHY, Hiran Dhanji, Judith Richardson, Elizabeth De Pinna, Jon Averns, Neil Woodford and Susanne Surman-Lee
Health Protection Agency, Food, Water and Environmental Microbiology, London, United Kingdom

Introduction: It has been proposed that the use of antimicrobial agents in food animal production could select for resistant bacteria that could be transmitted to humans through the food chain. Chicken meat on sale in the UK has been shown to be a potential source of antibiotic-resistant bacteria.

Purpose: To investigate the potential of resistant organisms entering the food chain from contaminated chicken meat a survey was undertaken for the presence of antimicrobial-resistant *Salmonella* spp., *Campylobacter* spp. and *E. coli* in non-EU poultry.

Methods: The survey analyzed 47 different batches of raw chicken meat imported into the UK from South America using culture-based detection techniques. The samples were analyzed for the presence of *E. coli*, *Campylobacter* spp. and *Salmonella* spp. Antimicrobial-resistance types of *Campylobacter* spp. and *Salmonella* isolates was established. A novel method was developed for the detection of antibiotic-resistant *E. coli* in food samples. The method incorporated cultural enrichment, selective isolation and double disc diffusion. Full antimicrobial resistance typing was applied to isolates of *E. coli* that were resistant to cephalosporin-type antibiotics.

Results: *Campylobacter* spp. and *Salmonella* spp. were isolated from eight and two batches, respectively. *Salmonella enterica* Thompson, *S. enterica* Infantis and *S. enterica* Newport were isolated from the chicken samples. Only the *Salmonella enterica* Thompson was resistant to antibiotics. Twelve isolates of *Campylobacter coli* and five isolates of *Campylobacter jejuni* were detected, all of which were resistant to at least one antibiotic. *E. coli* was isolated from 94% of raw chicken batches from non-EU sources and 79% were contaminated with *E. coli* that was resistant to at least one type of antibiotic.

Significance: The survey found that low numbers of chicken samples were contaminated with *Salmonella* spp. and *Campylobacter* spp., however high numbers were contaminated with antibiotic-resistant *E. coli*. The results indicated that imported chicken was a source of antimicrobial-resistant bacteria, but these organisms did not correlate with the resistance types currently causing considerable illness in the UK. This study provides a baseline for the bacterial resistance types currently entering the UK, which may become established cause illness in the future.

P2-113 Prevalence of *Listeria* spp. and *Listeria monocytogenes* of On-farm Dairies Manufacturing Cheese Made of Raw Ovine and Caprine Milk

DAGMAR SCHODER, Daniela Melzner, Alois Schmalwieser, Abdoulla Zangana, Petra Winter and Martin Wagner
Universitiy of Veterinary Medicine, Institute of Milk Hygiene, Milk Technology and Food Science, Vienna, Austria

Introduction: Within Europe, Austria has the second-highest share in direct selling of milk and milk products. There is a small but growing group of people that consume non-pasteurized ovine or caprine milk, either for practical reasons, medical reasons or for perceived health benefits of natural and unprocessed food.

Purpose: The aim of this study was to (i) determine the prevalence of *Listeria* spp. and *Listeria monocytogenes* in on-farm dairies manufacturing fresh cheese made of raw ovine and caprine milk and (ii) to prove epidemiological links by typing the strains with pulsed-field gel electrophoresis.

Methods: Fifty-three on-farm dairies manufacturing fresh cheese made of raw ovine and caprine milk were screened for the prevalence of *Listeria* spp. and *Listeria monocytogenes* along the entire production chain. Samples were taken from the (i) animal and milk processing environment (including feces, feed, milk filters and swab samples from the cheese making facilities), (ii) half udder samples, and (iii) tank milk and cheese samples.

Results: Overall, 5,799 samples, including 835 environmental samples, 230 milk and milk products and 4,734 aseptic half-udder samples were collected. A total 153 samples were positive for *Listeria* spp. yielding an overall prevalence of 2.6%, whereas *L. monocytogenes* were found at a rate of 0.9%. Working boots and fecal samples showed a significantly ($P < 0.001$) higher overall prevalence of *L. monocytogenes* (15.7 and 13.0%) than swab samples from the milk processing environment (7.9 and 4.7%). Although tank milk samples were constantly negative, milk filter samples yielded a significantly higher prevalence of *Listeria* spp. of 8.4%. Interestingly, 28% (14/50) of all *L. monocytogenes* isolates belonged to one clonal type and could be isolated from four different farms. Three of four farms tested were located adjacently to each other and the animal health was managed by the same veterinarian.

Significance: Listeria monocytogenes may also reach bulk tank and raw cheese as a result of exogenous contamination via the milking equipment because of fecal contamination during milking, whereas work boots may act as significant vectors.

P2-114 *Salmonella* Survival and Migration in Soil with Presence of Poultry Litter

JIE WEI and Kalmia E. Kniel
University of Delaware, Animal and Food Sciences, Newark, DE, USA

Introduction: Pelletized poultry litter (PL) has been increasingly used in United States farms for produce production. In recent leafy greens risk assessment meetings, the potential for contamination by poultry litter was discussed. While improving the soil quality, poultry litter might also provide nutrients and shelter for naturally occurring pathogens. Meanwhile, there are increasing outbreaks of salmonellosis associated with consumption of fresh produce and nuts which result in morbidity and great economic losses.

Purpose: To evaluate whether application of poultry litter on agricultural land would affect the survival of *Salmonella* in soil and migration to plants, when comparing an outbreak strain to an environmental isolate using two levels of humidity.

Methods: *S.* Kentucky (DELMANA environmental isolate) and *S.* Newport (outbreak isolate) were inoculated into 10 g soil (~4 log/g) and mixed thoroughly in a plant peat pot. Then 7.5 g uncontaminated soil or 5 g PL plus 5 g soil was applied onto the contaminated soil and incubated at 22°C for up to 10 days. The soil and PL samples were vortexed with 0.1% peptone water, followed by serial dilutions and plating on XLT-4 agar. The soil surface samples were enriched with lactose broth and Rappaport-Vassiliadis broth at 37 and 42°C for 24 h, respectively, and then transferred to XLD agar, as according to FDA BAM.

Results: At high soil water activity (a_w>0.8) *S.* Kentucky and *S.* Newport migrated to PL quickly, with ~ 6 log/g detected for both strains after day one and maintained at similar concentration until day 10. At low soil water activity (a_w ~0.3–0.4), *S.* Newport migrated to PL more quickly than *S.* Kentucky, with ~ 4 log/g *S.* Newport detected at day five and day 10, while after 10 days *S.* Kentucky in PL was below the detection limit (2 log/g) but positive after enrichment. However, at both water activities, enrichment of surface soil showed no significant difference between soil with PL and control soil for the presence of *Salmonella* ($P < 0.05$).

Significance: Environmental conditions, especially humidity affecting soil moisture, can impact the migration and survival of *Salmonella* isolates in land-applied soil amendments, like PL; thereby, increasing the risk of contaminated crops grown in the fields.

P2-115 Role of Cellulose Production by *Salmonella* on Hydrophobicity and Attachment to Abiotic Surfaces

Raymond Chia, Tom McMeekin, Narelle Fegan and GARY DYKES
Monash University, School of Science, Bandar Sunway, Malaysia

Introduction: Cellulose production is well known to play a role in biofilm development by *Salmonella*. Its role in the hydrophobicity and the initial attachment of this pathogen to abiotic surfaces relevant to the food industry has, however, not been well studied.

Purpose: To establish if cellulose plays a significant role in the hydrophobicity and attachment of *Salmonella* to abiotic surfaces of relevance to the food industry.

Methods: Five *Salmonella* strains belonging to four serovars, namely, *S.* Sofia (n = 2); *S.* Typhimurium (n = 1), *S.* Infantis (n = 1) and *S.* Virchow (n = 1) were used in this study. Deletions were generated in the *bcsA* gene of all strains which resulted in the elimination of cellulose production. Deletion mutants and their associated parent strain were compared for hydrophobicity by the bacterial attachment to hydrocarbon method and for their ability to attach to glass, stainless steel and Teflon®.

Results: No significant differences ($P < 0.05$) in hydrophobicity between deletion mutant and parent strains were observed except for one *S.* Sofia strain in which the deletion mutant displayed a significant ($P < 0.05$) decrease in hydrophobicity as compared to the parent strain. The deletion mutants of two (*S.* Virchow and *S.* Infantis) out of the five strains attached in significantly ($P < 0.05$) lower number to glass as compared to their parent strains. No significant ($P > 0.05$) differences in numbers of cells attaching to stainless steel were observed between any of the deletion mutants and parent strains. The deletion mutant of one strain, the same *S.* Sofia strain that exhibited reduced hydrophobicity in its deletion mutant, attached to Teflon® in significantly ($P < 0.05$) lower numbers as compared to its parent strain.

Significance: This study demonstrated that the role played by cellulose in the hydrophobicity and attachment of *Salmonella* to different abiotic materials is specific to individual serovar/strain and material interactions. A generalized approach targeting cellulose to prevent attachment of *Salmonella* to food processing surfaces is unlikely to be effective.

P2-116 Low Concentration of Ethylenediaminetetraacetic Acid Affects *Listeria monocytogenes* Biofilm Formation by Inhibiting Its Initial Attachment

YUHUA CHANG and Lynne McLandsborough
University of Massachusetts, Food Science, Amherst, MA, USA

Introduction: Biofilms are complex communities of microorganisms attached to surfaces and held together by extracellular polysaccharides. The distribution and survival of the foodborne pathogen *Listeria monocytogenes* in food processing environment is thought to be linked to its ability to attach to many different surfaces and form biofilms.

Purpose: The effect of EDTA on inhibiting biofilm formation in *Listeria monocytogenes* has not been reported yet. The purpose of this study is to elucidate how EDTA affects the biofilm formation of *L. monocytogenes*.

Methods: The biofilm assay was conducted by the 96-well PVC microplate method. Different concentrations of EDTA were added to the modified Welshimer's broth (MWB) and biofilm formation was assessed. To determine at which stage EDTA influences biofilm production, 0.1 mM EDTA was added at different time intervals during growth (0 h, 1 h, 2 h, 3 h, 4 h, 8 h, 20 h, 34 h, and 40 h). The addition Mg^{2+} or Fe^{3+} was added to MWB supplemented with 0.1 mM EDTA, to determine if addition of cations could restore biofilm formation. The surface charge of *Listeria monocytogenes* cells with or without EDTA treatment was measure by zeta potential.

Results: EDTA at concentration of 0.1 mM efficiently inhibited biofilm formation of *L. monocytogenes* without affecting its planktonic growth. Adding EDTA at time zero had the most inhibitory effect against the biofilm formation, while the addition of EDTA after four hours had little or no biofilm inhibitory effects. These data indicate that EDTA functions in the early stage by affecting the initial attachment of *L. monocytogenes* cells to surfaces. The addition of Mg^{2+} or Fe^{3+} did not restore the biofilm formation, suggesting the biofilm inhibition was not due to the depletion of cations from the growth medium. The zeta potential measurement showed that EDTA did not change the surface charge of *L. monocytogenes* cells. Our results indicate that EDTA acts either directly or indirectly to modify the way bacteria cells interact with surfaces, but the mechanism remains unclear.

Significance: To our understanding, this is the first report that low concentration of EDTA inhibits *Listeria* biofilm formation without affecting its planktonic growth. These data suggest a potential use of EDTA to prevent the formation of *L. monocytogenes* biofilms on the food processing surfaces.

P2-117 Prevalence and Characteristics of Methicillin-resistant *Staphylococcus aureus* in Livestock, Humans in Contact with Farm Animals, and Foods of Animal Origin, Switzerland

Helen Huber, Selina Koller, Nicole Giezendanner, Roger Stephan and CLAUDIO ZWEIFEL
University of Zurich, Institute for Food Safety and Hygiene, Vetsuisse Faculty, Zurich, Switzerland

Introduction: Along with hospital- and community-associated MRSA, the topic of livestock-associated (la) MRSA has recently emerged worldwide. Thereby, MRSA of a certain sequence type (ST398) are isolated from healthy livestock, contact persons, and foods of animal origin.

Purpose: The aim of this study was to assess the occurrence of la-MRSA and to further characterize isolated strains.

Methods: Livestock samples comprised nasal swabs from 800 pigs, 300 calves, 400 cattle, and neck skin samples from 100 chicken flocks. Human samples (nasal swabs) originated from 148 pig farmers, 133 veterinarians, and 179 slaughterhouse employees. Moreover, 100 bulk tank milk, 200 raw milk cheese, and 160 minced meat samples were tested. After a two-step enrichment procedure, Oxoid Brilliance MRSA Agar was used for MRSA isolation. Additionally, 142 *S. aureus* strains from bovine mastitis milk were directly plated on the chromogenic agar. Isolates were confirmed as *S. aureus* by 23S rDNA PCR and as MRSA by *mec*A detection. MRSA strains were then characterized by antimicrobial susceptibility testing, MLST, *spa* typing, and SCC*mec* typing.

Results: MRSA strains were isolated from 10 (1.3%) pigs, three (1.0%) calves, one (0.3%) cow, two (1.4%) mastitis milk samples, and four (3.0%) veterinarians. Genotyping of the 20 MRSA strains revealed strains belonging to ST398 (18 strains), ST8, and ST1. Except for one strain from a cow, all MRSA from livestock were of ST398. These strains belonged to two *spa* types (t011, t034) and to SCC*mec* type V. Of the four human strains, three were of ST 398 (*spa* types t011 and t034, SCC*mec* type IV and V) and one of ST8 (*spa* type t064, SCC*mec* type IV).

Significance: The results obtained show that MRSA have entered Swiss farming operations, but to date occur at low numbers. To maintain this favorable situation, further efforts within the field of veterinary public health tasks are required.

P2-118 Effect of Sample Preparation in the Detection and Isolation of *Escherichia coli* O157:H7 from Artificially Contaminated Produce

Karen C. Jinneman, Ken J. Yoshitomi and WILLIS M. FEDIO
New Mexico State University, Las Cruces, NM, USA

Introduction: Escherichia coli O157:H7 has been responsible for a number of foodborne disease outbreaks associated with fresh produce.

Purpose: This study evaluated a composite rinse and a soak procedure to detect and recover *E. coli* O157:H7 from produce.

Methods: One of a five-sample set was spiked with *E. coli* O157:H7 at approximately 0.1 CFU/g or 1.0 CFU/g. All five produce samples in the set were individually rinsed with an equal volume of Butterfield's phosphate buffer, and 25 mL from each were composited and added to 125 ml 2× modified buffered peptone water with pyruvate. Composited samples were enriched for 5 h at 37°C. Acriflavin, Cefsulodin, and Vancomycin were added and incubation continued at 42°C for the remaining 24 h. Enrichments were screened by real-time multiplex PCR (stx_1, stx_2, uidA genes and internal control) and streaked onto selective agar plates (Rainbow and TC-SMAC) for cultural recovery. Napa cabbage, mung bean sprouts, jalapeño peppers, and cantaloupes were tested.

Results: Composite rinsate samples from Napa cabbage were positive for all 6 sample sets at each inoculation level. Jalapeño peppers resulted in positive PCR detection from all 6 samples at the high level inoculum and 1/6 from the low. However, no *E. coli* O157:H7 was recovered from any of the inoculated pepper samples. Bean sprouts and cantaloupes failed to yield positive results by PCR or be culturally confirmed. Individual Jalapeño peppers and cantaloupes were also analyzed by a soak procedure. This did improve detection and recovery of *E. coli* O157:H7 in cantaloupes, with 5/6 positive by PCR screening at the high inoculation level and 6/6 at the low level and cultural confirmation for 4/6 at each inoculation level. However, was unsuccessful for jalapeño peppers, with only 1/6 positive by the PCR screen and confirmed culturally at each inoculation level. This was attributed to reduced survival and growth of the inoculum with this food matrix.

Significance: This study demonstrates the importance of validation for various sample processing techniques and food matrices.

P2-119 Effect of Organic Acids and Quaternary Ammonium Compounds on Survival of *Salmonella* Serovars with SGI1-Mediated Multi-antibiotic Resistance

MARK A. HARRISON, Ana L. Rodriguez and Peter J. Taormina
University of Georgia, Food Science and Technology, Athens, GA, USA

Introduction: It has been speculated that *Salmonella* might acquire adaptive resistance to quaternary ammonium compounds (QAC), and several genes including qacΔE have been implicated in conferring resistance to biocides such as QAC. The qacΔE gene is present in the SGI1 antibiotic resistance gene cluster.

Purpose: MDR and non-MDR *Salmonella* were compared to determine the effectiveness of organic acids after acid adaptation, the effectiveness of QAC, whether organic acid treatments conferred cross-protection against subsequent QAC exposure, and the survival of the organisms in biofilms after QAC treatment.

Methods: Six MDR and two non-MDR *Salmonella* serovars were evaluated when exposed to organic acids and QAC. To determine survival due to acid exposure, acid adapted and non-acid adapted *Salmonella* were exposed to either lactic or acetic acids with survivors enumerated at different times. To determine survival due to QAC exposure, MDR were exposed to three QACs. Samples were taken at different exposure times and survivors enumerated. To investigate whether organic acids conferred cross-protection against further treatment with QAC, strains were pre-adjusted to acid conditions and then treated with QAC. Samples were withdrawn and survivors enumerated. Planktonic and biofilm cells were also treated with QAC and survival rates were determined.

Results: Acid adapted bacteria survived better than non-adapted bacteria when challenged with either acid at pH 3.5. Acid adjustment did not confer cross-protection against further treatments with QAC. There was no significant difference in response to QAC between MDR and non-MDR *Salmonella*. MDR and non-MDR *Salmonella* in biofilms were more resistant to QAC than planktonic cells, but responses to QAC did not vary for MDR or non-MDR cells.

Significance: Treatments with organic acids and QAC were equally effective against MDR and non-MDR *Salmonella* strains. qacΔE was not involved in conferring resistance to QAC since there was little, if any, difference in susceptibility to the QAC treatments between the MDR and non-MDR *Salmonella*.

P2-120 Effect of Planktonic and Immobilized Growth on the Adaptive Acid Tolerance Response of *Listeria monocytogenes* Strains

PANAGIOTIS N. SKANDAMIS, Antonia S. Gounadaki, Ifigenia Geornaras and John N. Sofos
Colorado State University, Animal Sciences, Fort Collins, CO, USA

Introduction: Investigating whether and how the food structure in/on which cells have grown affects their subsequent acid resistance could be useful in determining the ability of *Listeria monocytogenes* to cause illness.

Purpose: This study evaluated the acid resistance of *L. monocytogenes* cells after: (i) short (shock) or long-term (adaptation during growth) exposure to reduced (5.50) or neutral (7.23) pH in a liquid (broth) or on a solid surface (agar), and (ii) growth on the surface of ham and turkey slices or in homogenates of these products.

Methods: Three *L. monocytogenes* strains (serotypes 1/2a, 4b and 1/2b) were individually inoculated (three replicates/two samples each) at: (i) 10^4–10^5 CFU/ml in tryptic soy broth with 0.6% yeast extract (TSBYE) or on tryptic soy agar with 0.6% yeast extract (TSAYE) with or without glucose (TSBYE-G/TSAYE-G and TSBYE+G/TSAYE+G) of pH 7.23 or 5.50 (lactic acid) and incubated at 20°C (up to 96 h), and (ii) 10^2–10^3 CFU/cm^2 on ham and turkey slices (pH 6.39–6.42; formulated with potassium lactate and sodium diacetate) or in their homogenates (1:4 and 1:9; representing viscous [slurry] and liquid residues [purge], respectively) and stored at 10°C (up to 63 days). The acid resistance of each strain was assessed in TSBYE of pH 3.5 (lactic acid) for strains growing in broth or on agar surfaces, and in TSBYE of pH 1.5 (HCl) for strains growing on ham and turkey slices or in their homogenates.

Results: Habituation at pH 5.50 for 3 or 24 h at 20°C increased acid (pH 3.5) resistance of all strains by 100-fold compared to the control (pH 7.23). Maximum resistance for all strains to pH 3.5 was obtained after 96 h at 20°C, in pH 5.50. Cells grown on the surface of TSAYE-G (pH 7.23 or 5.50) showed higher resistance than cells grown in broth (TSBYE-G), whereas the opposite was observed for cells grown on TSAYE+G or in TSBYE+G. *L. monocytogenes* reached 7.5–8.1 log CFU/ml (i.e., 5–6 log increase), depending on the strain, in ham and turkey homogenates within 10 days of storage at 10°C, whereas a 4 log increase was observed only after 51 days on ham and 63 days on turkey slices. Pathogen reductions following exposure to pH 1.5, after 5 and 10 days storage (10°C), were also strain-dependent and ranged from 0.5–1.2, 3.1–6.0 and 4.6–7.6 logs for cells grown on product slices, in 1:4 and 1:9 homogenates, respectively. These reductions were higher in turkey than in ham homogenates, whereas, they decreased 10-fold after storage for up to 63 days.

Significance: The results suggest that *L. monocytogenes* cells growing on food surfaces or in viscous matrices may show higher resistance to lethal acid conditions than cells growing in liquid substrates.

P2-121 Effect of Strain Competition, Meat Microflora, Limited Nutrients and Sequence of Strain Attachment on Biofilm Formation by Two *Escherichia coli* O157:H7 Strains and Subsequent Resistance to a Quaternary Ammonium Compound

PANAGIOTIS N. SKANDAMIS, Antonia S. Gounadaki, Ifigenia Geornaras and John N. Sofos
Colorado State University, Animal Sciences, Fort Collins, CO, USA

Introduction: Investigating the combined effect of surface soil and strain competition on biofilm formation and resistance to sanitizers by pathogens may assist in the design of effective strategies for biofilm control.

Purpose: We evaluated: (i) the biofilm formation ability and resistance to a sanitizer of two *Escherichia coli* O157:H7 strains attached to stainless steel (SS) surfaces conditioned with different nutrients, meat microflora, or pre-existing biofilm of the same species; and (ii) the effect of sequence of strain inoculation and surface-drying on the attachment of the strains.

Methods: Two rifampicin-resistant *E. coli* O157:H7 strains, CI-072 and ISEHGFP (a weak and strong biofilm-former, respectively), were inoculated (10^5 CFU/ml) into tubes with SS coupons (2 × 5 × 0.08 cm) and 20 ml of a non-sterile meat homogenate (MH; 1:10 dilution), or sterile tryptic soy broth (TSB) and incubated at 15°C. We tested (two replicates/three samples each): (i) single strains, (ii) mixed culture of strains at the same level, (iii) CI-072 inoculated first and ISEHGFP inoculated after 3 or 6 days of incubation, and, (iv) each strain inoculated on TSB-soiled SS coupons before or after the coupons were dried (25°C, 2 h). Following incubation, coupons were exposed (5 min) to a commercially-available quaternary ammonium compound (QUAT; 400 ppm). For enumeration, the green fluorescence protein phenotype of strain ISEHGFP was used to differentiate the strains when in co-culture, and rifampicin (100 µg/ml) was used to differentiate both strains from the natural flora.

Results: Counts of attached meat microflora on MH-soiled coupons reached 6.3 log CFU/cm^2 in 15 days, whereas, CI-072 reached 2.4–2.5 and 1.4–1.8 log CFU/cm^2 in single and co-culture with ISEHGFP, respectively. Corresponding counts of ISEHGFP reached 3 log CFU/cm^2 in 3 days, but decreased to 1.2 log CFU/cm^2 by day-15, or when it was inoculated after incubation of MH for 3 or 6 days. In TSB, attached ISEHGFP reached 5.3 log CFU/cm^2 in single or co-culture. No detectable (< 0.3 log CFU/cm^2) survivors were obtained after exposure to QUAT

Poster

for (i) both strains on MH-soiled coupons, and, (ii) ISEHGFP when inoculated 6 days after strain CI-072. On TSB-soiled coupons, survivors after exposure to QUAT increased from 0.5–0.8 to 1.2 (CI-072, single), 4.7 (ISEHGFP, single), and 0.6–1.8 (co-culture) log CFU/cm^2 from day-3 to −11. Both strains yielded the highest biofilm (4.8–5.2 log CFU/cm^2) when attachment was followed by drying of TSB-soiled surfaces.

Significance: These findings may increase our understanding of the role of factors affecting biofilm formation and hence, assist in development of effective sanitation procedures.

P2-122 Influence of Irrigation Method on Persistence of Attenuated *Escherichia coli* O157:H7 in Field-inoculated Lettuce

ANNE-LAURE MOYNE, Michael D. Cahn, Steven T. Koike and Linda J. Harris
University of California-Davis, Food Science and Technology, Davis, CA, USA

Introduction: The level of free moisture is known to influence survival of foodborne pathogens in foods and the environment. Lettuce grown in the Salinas valley is irrigated by furrow, drip and overhead sprinkler methods.

Purpose: Our objective was to evaluate the effect of irrigation (drip and overhead sprinkler) on persistence of attenuated (non-pathogenic) *Escherichia coli* O157:H7 ATCC 700728 inoculated onto lettuce grown under field conditions.

Methods: A split plot design with three replicates was used to evaluate the two main treatments: drip and overhead irrigation, during the three field trials conducted in the Salinas Valley between Spring 2008 and Fall 2009. A rifampicin-resistant attenuated *E. coli* O157:H7 ATCC 700728 was inoculated on 4 week-old lettuce plants at a target level of 7 log CFU/plant. Plants were stomached in 0.1% peptone and directly plated or filtered and plated onto tryptic soy (TS) agar with 50 µg/ml rifampicin; plants were also enriched (100-g or less subsamples) in TS broth with 50 µg/ml rifampicin.

Results: *E. coli* O157:H7 population size declined by 2 to 5 log CFU/plant during the first 2 h to 2 days after inoculation. No significant difference ($P < 0.05$) in counts was observed between plants that were irrigated by drip or overhead sprinkler for all field trials. By day 7 counts were near or below the limit of detection (10 cells per plant). At day 28 from 4 to 28% plants were positive for *E. coli* O157:H7. No difference in percent positive plants was observed between drip and overhead irrigated plants with the following exceptions: significantly greater percent positive overhead irrigated plants were observed on day 28 in Spring 2008 and days 7 and 14 in Spring 2009; the reverse was true for Fall 2009 on day 7.

Significance: Neither drip nor overhead sprinkler irrigation consistently influenced survival of *E. coli* O157:H7 in the field.

P2-123 Antimicrobial Effect of Gamma Irradiation on *Burkholderia thailandensis* (*Burkholderia pseudomallei* Surrogate) under Combinations of pH and NaCl

YOHAN YOON
Korea Atomic Energy Research Institute, Jeongeup, Republic of Korea

Introduction: *Burkholderia pseudomallei* is categorized as a category B pathogen because *B. pseudomallei* is considered as a potential bioterrorism agent with high priority, and the pathogen is also considered as a bioterrorism agent into food supply.

Purpose: This study evaluated antimicrobial effect of gamma irradiation on *Burkholderia thailandensis* (*B. pseudomallei* surrogate) under different levels of NaCl and pH.

Methods: *B. thailandensis* (7 log CFU/ml) in Luria Bertani broth formulated with NaCl (0, 1.5, 2.0, 2.5 and 3%), and pH-adjusted to 4, 5, 6 and 7 was gamma irradiated at 0, 0.1, 0.2, 0.3, 0.4 and 0.5 kGy. Bacterial populations were then enumerated on tryptic soy agar, and the cell count data were also used to calculate D10 values (dose required to reduce 1 log CFU/ml of *B. thailandensis*) using simple linear regression.

Results: Bacterial populations of *B. thailandensis* were significantly decreased ($P < 0.05$) as irradiation dose increased, and no differences ($P \geq 0.05$) in bacterial populations were observed among different levels of NaCl, and pH. D10 values were 0.07 kGy, 0.04 to 0.05 kGy, 0.04 to 0.06 kGy, 0.05 to 0.07 kGy and 0.06 kGy for 0%, 1.5%, 2%, 2.5% and 4% NaCl, regardless of pH level.

Significance: These results suggest that low doses of gamma irradiation should be a useful technology in decreasing the potential bioterrorism bacteria in food supply.

P2-124 Growth of *Escherichia coli* O157:H7 and *Salmonella* spp. in Avocado Pulp and Guacamole at Different Temperatures

Su-sen Chang, CAROL VALENZUELA-MARTINEZ and Harshavardhan Thippareddi
University of Nebraska-Lincoln, Dept. of Food Science and Technology, Lincoln, NE, USA

Introduction: Recent recalls of guacamole highlight the potential risk of avocado pulp and guacamole as vehicles for the transmission of foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*.

Purpose: The growth of *E. coli* O157:H7 and *Salmonella* spp. in avocado pulp, guacamole, and guacamole fortified with lime juice stored at 22°C and 4°C was studied.

Methods: Guacamole was prepared according to a traditional Mexican recipe containing avocado pulp (67.26%), fresh onions (7.43%), salt (0.52%) and water (24.78%). Guacamole with lime juice contained 2% fresh lime juice added to the guacamole formulation. Five strain cocktails of *E. coli* O157:H7 and *Salmonella* spp. were used to inoculate products to an initial concentration of ca. 2.5 log CFU/g, individually.

Results: *E. coli* O157:H7 population increase of > 1 log CFU/g was observed for both and *Salmonella* spp. in all products after 8 h when stored at 22°C. Addition of lime juice to guacamole reduced pH from 6.54 ± 0.04 to 5.30 ± 0.04, but did not inhibit ($P > 0.05$) growth of *E. coli* O157:H7 or *Salmonella* spp. However, storage of avocado pulp, guacamole and guacamole containing lime juice at 4°C prevented microbial growth.

Significance: Results of this study indicate that avocado pulp and guacamole with or without lime juice can support growth of *E. coli* O157:H7 and *Salmonella* spp. at room temperature. It is recommended that avocado pulp or guacamole be refrigerated following preparation or consumed within 8 h if held at room temperature to minimize the risk of foodborne illness.

P2-125 Germination and Outgrowth of *Clostridium perfringens* and *Bacillus cereus* Spores during the Manufacture of Egg White Hydrolysates

Aikansh Singh, Nageswara Rao Korasapati and HARSHAVARDHAN THIPPAREDDI
University of Nebraska, Lincoln, NE, USA

Introduction: Egg white hydrolysates are being used in the health care industry for their angiotensin I-converting enzyme (ACE) inhibitory activity. These egg white hydrolysates provide various health benefits but their manufacturing processes have not been evaluated for microbiological safety.

Poster

Purpose: The objective of this study was to evaluate the potential germination and outgrowth of *Clostridium perfringens* and *Bacillus cereus* spores during manufacture of egg white hydrolysates using trypsin and chymotrypsin.

Methods: Egg white was inoculated with heat activated *C. perfringens* and *B. cereus* spores to obtain ca. 2 to 3 log spores/ml and used for egg white hydrolysates (EWH) preparation. EWH were prepared from the enzymatic hydrolysis of inoculated egg white maintained at optimum pH for enzymes (trypsin and chymotrypsin) and further incubated at 37°C for 24 h. Survivors (vegetative cells and spores) at specific intervals were enumerated with plating on corresponding selective medium.

Results: B. cereus vegetative cell population increased by > 3.5 log/ml during the manufacture of EWH using chymotrypsin, while no increase was observed during trypsin hydrolysis. No increase in *C. perfringens* vegetative and spores counts was observed irrespective of the enzyme used during EWH preparation.

Significance: New manufacturing processes for EWH should be validated for microbial safety to minimize the risk of spore germination and outgrowth.

P2-126 Development and Validation of a Dynamic Predictive Model for the Growth of *Salmonella* spp. in Liquid Whole Egg

AIKANSH SINGH, Nageswara Rao Korasapati, Jeyamkondan Subbiah and Harshavardhan Thippareddi
University of Nebraska, Lincoln, NE, USA

Introduction: Egg and egg products have been associated with salmonellosis outbreaks. Processed egg products can support growth of foodborne pathogens, and temperature abuse of such products can result in potential growth of *Salmonella* spp.

Purpose: Dynamic predictive model for the growth of *Salmonella* spp. in liquid whole egg under varying temperature conditions was developed and validated.

Methods: Liquid whole egg was inoculated with a five serovar cocktail of *Salmonella* spp. to obtain initial population of ca. 2.5 log CFU/mL. *Salmonella* spp. growth data was collected at several isothermal conditions (5 to 47°C). Primary (Baranyi) and secondary (modified Ratkowsky) models were developed. A dynamic model for prediction of *Salmonella* spp. under varying temperature conditions was developed by numerically integrating the primary and secondary model using fourth-order Runga-Kutta method and validated.

Results: The Pseudo-R^2 values of > 0.97 and > 0.99 were obtained for primary and secondary models, indicating a good fit. The RMSE (root mean square error) values for 5 to 15°C and 10 to 40°C sinusoidal temperature profiles were 0.28 and 0.23 log CFU/mL, respectively between predicted and observed values. Predicted values agreed well with observed values in both validation profiles.

Significance: Developed dynamic model can be used to predict the growth of *Salmonella* spp. in liquid whole egg under varying temperature non-isothermal conditions.

P2-127 Effect of Temperature and pH on the Emetic Toxin Production of *Bacillus cereus*

JAI-MOUNG KIM, Jung-Beom Kim, Jae-Ho Choi, Syed M. E. Rahman, Tian Ding, Uranchimeg Purev, Kang-Hyun Choi, Na-Jung Choi and Deoghwan Oh
Kangwon National University, Dept. of Food Science and Biotechnology, Chuncheon Si, Republic of Korea

Introduction: Bacillus cereus can cause diarrheal and emetic syndromes. The diarrheal syndrome is caused by enterotoxins. The emetic syndrome is caused by a small, heat and acid stable circular dodecadepsipeptide known as emetic toxin (cereulide). However, very limited studies have been available on the production of emetic toxin as a function of pH and temperature.

Purpose: The objective of this study was to investigate the effect of pH and temperature on growth and emetic toxin production of *B. cereus*.

Methods: The cocktail of three emetic *B. cereus* strains (F4810/72, JNHE 36 and KCDC 28) was inoculated into tryptic soy broth at pH 5.5, 7.0 and 8.5 (approximately 2.0 to 3.0 log CFU/mL). Each broth was incubated at 5–40°C for 72 h. At appropriate time intervals, each broth was diluted and plated onto TSA. HPLC/MS analysis was performed to detect emetic toxin production at the same time.

Results: No toxin was observed within 72 h below 15°C, but emetic toxin was detected above 20°C. After 12 h incubation, the population of 7.29 log CFU/mL presented emetic toxin production at 40°C, but no toxin was observed at 30°C with the bacterial population of 7.57 log CFU/mL. After 24 h incubation, the population of 7.44 log CFU/mL showed emetic toxin production at 30°C, but no toxin was detected at 25°C at this level of bacterial population. Emetic toxin was detected in all pH ranges after 12 h at 35°C.

Significance: These results suggest that emetic toxin production was more significantly regulated in incubation temperatures than incubation pH and the number of *B. cereus*.

P2-128 Impact of Relative Humidity on the Survival of Desiccated *Salmonella* Cells on Surfaces with Peanut Butter

HAIPING LI, Christina Megalis and Mary Lou Tortorello
Food and Drug Administration, National Center for Food Safety and Technology, Summit-Argo, IL, USA

Introduction: Keeping the environment dry has been a major strategy taken by peanut butter manufacturers to control the presence of pathogens on the processing line. Yet pathogens such as *Salmonella* and *Enterobacter sakazakii* have been documented to survive in dry processing environments. The mechanisms of their survival are not understood.

Purpose: To evaluate the impact of environmental relative humidity (ERH) on the survival of *Salmonella* on surfaces with peanut butter.

Methods: Stationary-phase cells of six *Salmonella* isolates (Typhimurium LT2 and its derivative, Anatum, Enteritidis PT30, Oranienburg, and Tennessee) were inoculated onto paper discs, air-dried and then placed in desiccators with various saturated salt slurries ($MgCl_2$, KCl, and $MgSO_4$) to generate chambers with different humidities. Inoculated discs were stored in the desiccators, and at various intervals for up to 3 weeks, viable *Salmonella* cells were enumerated in a 3-tube MPN method with antibody confirmation. In certain cases, peanut butter (A_w~0.35) was applied to the discs after inoculation; for these experiments strain LT2 carrying a luciferase-producing plasmid was used and cell viability was determined by light emission (Envision, Perkin Elmer) using a microplate MPN method.

Results: At 97% ERH, the viability of desiccation-injured *S.* Typhmurium LT2 cells decreased by 5 log after one day incubation, in contrast to the 2 log reduction when incubated at 33% ERH. The presence of peanut butter resulted in 1-2 log less reduction regardless of the ERH. Strain Tennessee (isolated from a patient in the 2006 peanut butter outbreak) demonstrated the highest resistance to desiccation among all the isolates. Consistent with LT2, Tennessee lost viability faster when stored at higher ERH (wet) than at lower ERH (dry).

Significance: This study found that higher ERH caused the desiccated cells to die more rapidly than lower ERH. Peanut butter residue likely provided a barrier to prevent moisture transfer and created a local environment more critically affecting *Salmonella* survival than the indoor humidity in the facility.

Poster

P2-129 Evaluation of Attachment Capacity of *Listeria monocytogenes* Isolates in Response to Different Growth Conditions Using Multivariate Analysis

WLADIR B. VALDERRAMA and Catherine N. Cutter
Pennsylvania State University, University Park, PA, USA

Introduction: Contamination of food by *Listeria monocytogenes* in food processing environments may be related to its ability/inability to attach to surfaces. Some research suggests that there is a relationship between genetic lineage and incidence of listeriosis outbreaks. However, other research also reveals contradictory results. It is likely that a differential ability to attach to surfaces may be a factor responsible for such contradictions.

Purpose: Forty-five isolates of *L. monocytogenes* were evaluated for attachment using a crystal violet assay in microtiter plates. The isolates were subjected to four extrinsic factors at three different levels: nutrients (tryptic soy broth with yeast extract at 20%, 40%, or 60% concentration); temperature (20, 25, or 30ºC); pH (6, 7, or 8); and water activity (0.96, 0.97 or 0.98), individually and in combination, using a randomized factorial design (34). With data derived from the randomized factorial design, a hierarchical cluster analysis was performed using the average linkage method with a squared Euclidean distance measure.

Methods: The purpose of this study was to compare the attachment capacity of outbreak- and non-outbreak-related isolates of *L. monocytogenes* using multivariate analysis. Various factors were evaluated and groups with similar attachment capacity were uncovered by cluster analysis. It was hypothesized that cluster analyses could demonstrate a relationship between attachment capacity and outbreak occurrence.

Results: Attachment of *L. monocytogenes*, in response to different levels, demonstrated a non-linear function for all factors evaluated. A dendogram was constructed and two main clusters were identified, with a minimum of 95% similarity among them. The cluster analysis suggests that superior attachment capacity may be a common factor among outbreak-related isolates. Also, this information suggests the adaptation of the pathogen to different ecological niches.

Significance: This study is the first to report the use of multivariate analyses to compare attachment capacity of various isolates of *L. monocytogenes*. Additionally, clusters were identified and were based on attachment capacity; a finding that has not been reported previously.

P2-130 Growth and Survival Characteristics of *Arcobacter butzleri* in Broth as a Function of Temperature, pH and NaCl

BO-YEON KIM, Ji-Young Lee and Sang-Do Ha
Chung-Ang University, Food Science and Technology, Ansung-Si, Republic of Korea

Introduction: Arcobacter species was originally a pathogen which causes abortion in pig and cows. Nowadays, *Arcobacter* spp. is known as one or the newly emerging foodborne pathogens. However, it was not performed to investigate the prevalence of *Arcobacter* spp. in Korea.

Purpose: Predictive food microbiology provides a quantitative estimation of microbial growth in foods using mathematical modeling. This study provides a predictive model to describe the effect of temperature, pH and concentration of NaCl on the growth of *Arcobacter butzleri* by response surface methodology (RSM). The model can be used as a reference in controlling *A. butzleri* growth without the need for detection of the organism and may be of use for controlling growth. In this study, the growth and survival characteristics of *A. butzleri* were determined and a predictive model that could be used practically was developed.

Methods: This study was conducted to evaluate the survival characteristics and growth of *A. butzleri* as a function of storage temperature (5 to 40°C), pH value (5 to 9) and NaCl concentration (0 to 8%) with the aim of building a predictive model. The growth curves generated using a Gompertz equation and the relationship of the growth rate to the growth curves was modeled using a quadratic polynomial equation of RSM.

Results: The primary model showed a good fit to the Gompertz equation to obtain growth rates under each condition. The quadratic polynomial model was found to be significant ($P < 0.05$) and predicted values were found to be in good agreement with experimental values (R^2 value of 0.9345). The evaluation of RSM for describing the growth rate of *A. butzleri* used the bias factor (Bf) and the accuracy factor (Af). Both the Bf value (0.839) and the Af value (1.343) were within acceptable ranges. The model was found to be significant and the predicted values were found to be in good agreement with experimental values.

Significance: The model was found to be significant and the predicted values were in agreement with previous studies. The model may be used as a reference in controlling *A. butzleri* growth without the need for detection of the organism, but the model needs validation in a food environment before it can be practically applied. Therefore, it is necessary to develop effective control techniques to reduce the risk of *Arcobacter* spp. in food samples.

P2-131 Growth Characteristics and Development of a Predictive Model for *Bacillus cereus* in Fresh Wet Noodles with Addition of Preservatives

BO-YEON KIM, Ji-Young Lee and Sang-Do Ha
Chung-Ang University, Food Science and Technology, Ansung-Si, Republic of Korea

Introduction: Predictive modeling provides a fast and relatively cost-effective way to obtain reliable first estimates of microbial growth and survival. A predictive model was used to estimate *Bacillus cereus* growth and to determine the shelf life of fresh wet noodles.

Purpose: This study was performed to determine growth characteristics and to predict the growth rate kinetics of *Bacillus cereus* in wet noodles using a response surface methodology (RSM) with a combination of alcohol concentration (0 to 2%) and Vitamin B1 concentration (0 to 2,000 ppm).

Methods: Bacillus cereus F4810/72, which produces an emetic toxin, was used in this study. The noodles were inoculated with *Bacillus cereus* and incubated at 10°C. The growth rate was fitted to the modified Gompertz equation using a nonlinear regression model. The relationship of the growth rate to the growth curves was modeled using an RSM quadratic polynomial equation. The assessment of the RSM for describing the growth of *B. cereus* was evaluated using bias (Bf) and accuracy factors (Af).

Results: The primary model fits well ($R^2 = 0.9505$-0.9991) to a Gompertz equation to obtain specific growth rate (SGR). The quadratic polynomial model was significant ($P < 0.0001$) and the predicted growth rate values were in good agreement with experimental values (R^2 value of 0.9900). A secondary polynomial model was obtained by RSM; it was identified as appropriate based on the bias factor (Bf = 1.006) and the accuracy factor (Af = 1.024).

Significance: Our model may be of application to fresh wet noodles for manufacture of safe products by controlling *B. cereus* growth without the need for detection of the organism.

Poster

P2-132 Survival and Growth of *Clostridium perfringens* on No-Nitrate-or-Nitrite Added (Natural and Organic) Frankfurters, Bacons and Hams

ARMITRA L. JACKSON, Gary Sullivan, Charlwit Kulchaiyawat and Joseph G. Sebranek
Iowa State University, Animal Science, Ames, IA, USA

Introduction: The popularity of preservative-free foods among consumers has stimulated rapid growth of processed meats manufactured without nitrite.

Purpose: The objective of the first phase of this study was to quantify the potential for *Clostridium perfringens* growth in commercial processed meats manufactured without the direct addition of nitrite/nitrate. Subsequently, the objective of the next phase was to identify and test ingredients that might improve product safety without altering the unique natural/organic status of these products.

Methods: Commercial brands of uncured, no-nitrate/nitrite-added frankfurters (10), bacons (9) and hams (7) were challenged with a three-strain inoculation (5 $\log_{10}$ CFU/g) of *Clostridium perfringens*. Reduced inhibition ($P < 0.05$) was observed in seven brands of commercial frankfurters and hams and four brands of commercial bacons when compared to each sodium nitrite-added control. Eight treatments of hams and frankfurters with conventional or natural nitrate/nitrite sources and natural antimicrobials were prepared: (1) uncured control (all typical ingredients except nitrite and nitrate), (2) conventionally cured control (erythorbate, nitrite, lactate/diacetate blend), (3) natural nitrate cure (with starter culture containing *Staphylococcus carnosus*), (4) natural nitrate cure (with culture and natural antimicrobial A containing vinegar, lemon and cherry powder blend), (5) natural nitrate cure (with culture and clean label antimicrobial B containing cultured corn sugar and vinegar blend), (6) natural nitrite cure without additional antimicrobials, (7) natural nitrite cure with natural antimicrobial A and (8) natural nitrite cure with clean label antimicrobial B.

Results: These results indicate that commercial natural/organic cured meats have more potential for pathogen growth than conventionally cured products. Treatments 3, 4, 5 and 8 for hams and 4, 7 and 8 for frankfurters showed no significantly greater ($P < 0.05$) growth by inoculated *Clostridium perfringens* than the control.

Significance: These results suggest that commercial natural/organic cured meats have more potential for pathogen growth than conventionally cured products, but other natural ingredients offer safety improvement.

P2-133 Prevalence and Characterization of *Cronobacter* Recovered from Desiccated Ready-to-Eat Products in Korea

Jeong Hwan Cheon, SUN-YOUNG KIM, Kwang-Young Song, Ji-Yeon Hyeon, Jae-Hoon Lee, Yun-Gyeong Kim and Kun-Ho Seo
Konkuk University, College of Veterinary Medicine, Seoul, Republic of Korea

Introduction: Cronobacter is an opportunistic pathogen which causes serious infections of neonates, and infant formula was identified as one route of transmission of infection in infants. *Cronobacter* (*Enterobacter sakazakii*) was reclassified a new genus consisting of six genomospecies. The main reservoirs for subsequent contamination of foods with *Cronobacter* remain undefined due to the ubiquitous nature of these organisms. For preventing the transmission of infection, it is necessary to identify the primary food sources for *Cronobacter*.

Purpose: Prevalence and characterization of *Cronobacter* recovered from desiccated ready-to-eat products in Korea

Methods: A total of 133 samples were enriched in buffered peptone water (prewarmed at 36°C) and incubated over 18 hours at 36°C. For *Cronobacter* spp. detection, 1 ml of enriched BPW was streaked onto chromogenic selective agar (DFI, Oxoid), and after over 18 hours incubation, presumptive colonies of *Cronobacter* were confirmed by Real-Time PCR, Rapid ID 32E, and various biochemical tests.

Results: Totally nineteen isolates (10.4%) from 133 desiccated ready-to-eat products made in Korea were confirmed as *Cronobacter* strains. Among the 19 *Cronobacter* isolates, 16 strains (83.2%) were confirmed as *C. sakazakii* and 4 isolates (16.8%) were other *Cronobacter* species, such as *C. dublinensis, C.* genosp. 1, and *C. muytjensii*. Molecular subtypes of *Cronobacter* isolates were evaluated using automated repetitive sequence-based PCR system, and the analysis of rep-PCR identified 5 different types among 19 *Cronobacter* strains collected.

Significance: Desiccated ready-to-eat products contaminated with *Cronobacter* spp. could pose a risk to the health of infants and adults. The use of genotype and phenotype analysis for detecting *Cronobacter* could be applied to characterize *Cronobacter* spp. and prevent its transmission into desiccated ready-to-eat products.

P2-134 Filamentous *Salmonella*: Formation, Survival, and Virulence

Robert R. Stackhouse, Nancy G. Faith, Charles W. Kaspar, Charles J. Czuprynski and AMY C. WONG
University of Wisconsin, Food Research Institute, Madison, WI, USA

Introduction: Desiccation and other stressful conditions are frequently encountered by *Salmonella* during pre-harvest, harvest, processing or storage. One response to stress is the formation of filaments that can reach >200 microns in length. When the stress condition is removed, the filaments form septa and divide into multiple cells that would impact estimations of pathogen numbers and risk assessments.

Purpose: To determine formation, persistence, growth, and virulence of stress-induced *Salmonella* filaments.

Methods: Salmonella enterica serovar Enteritidis strain E40 was grown on tryptic soy agar (a_w 0.99) and tryptic soy agar with 7% NaCl (a_w 0.95) plates at 30°C for 4 days to generate control and filamentous cells, respectively. Cells were tested for growth in fresh tryptic soy broth (TSB) and survival in other stresses including low pH (2 and 4), 10 to 20% bile salts, low temperature (4 and 7°C), and heat (45 and 55°C). Cell morphology was viewed after staining with Live/Dead BacLight Bacterial Viability Kit. *Salmonella* cells were assayed for their ability to attach, invade and grow in Caco-2 cells and to infect mice. After 5 days, *Salmonella* in the spleen, liver, cecum, gall bladder, and blood was enumerated.

Results: S. Enteritidis E40 developed filaments when exposed to reduced a_w. In addition, filamentation occurred when the organism was exposed to ultra-violet light. When the filaments were resuspended in fresh TSB at 25 or 37°C, cells formed septa and divided into regular-sized single cells, resulting in a more rapid increase in viable counts than a control population. Control cells and filaments survived all stress conditions tested, although cell numbers decreased. Generally higher decreases in filament viable numbers occurred with the exception of pH 2, where the reverse was observed. Filamentous cells are virulent; they invaded and grew in Caco-2 cells, infected multiple organs in mice, and appeared to persist longer than control cells in the gastrointestinal tract.

Significance: Formation of *Salmonella* filaments is significant to food safety because the detection and estimations of pathogen numbers may be compromised. Information generated on growth, survival, and virulence will help develop informed recommendations on intervention practices and risk assessments.

P2-135 Genotyping of Methicillin-resistant *Staphylococcus aureus* Strains Isolated from Swiss Livestock and Veterinarians

HELEN HUBER, Nicole Giezendanner, Roger Stephan and Claudio Zweifel
University of Zurich, Institute for Food Safety and Hygiene, Vetsuisse Faculty, Zurich, Switzerland

Introduction: Livestock-associated methicillin-resistant *Staphylococcus aureus* (la-MRSA), mostly of sequence type ST398, have emerged among healthy farm animals. Recently, first clinical cases of human infections with MRSA of ST398 have also been reported. Therefore, it is of major importance to investigate prevalence, virulence characteristics, and antimicrobial susceptibility of such MRSA.

Purpose: The aim of this study was to further characterize 20 MRSA strains previously isolated from livestock and humans in contact with farm animals using pulsed-field gel electrophoresis (PFGE) and a DNA microarray chip analysis.

Methods: MRSA strains originated from pigs (10 strains), calves (three strains), cattle (one strain), mastitis milk (two strains), and veterinarians (four strains). Eighteen strains were of ST398, whereas the other two belonged to ST1 and ST8. PFGE analysis was performed after macrorestriction with *Eag*I. The Identibac MRSA bacterial genotyping system, a DNA microarray-based chip analysis, was applied to detect genes encoding antibiotic resistance, enterotoxins, virulence factors of the staphylococcal leukotoxin family, and genes involved in accessory gene regulator.

Results: PFGE analysis yielded seven patterns. Based on a cutoff level of 80% similarity, patterns were grouped into four clusters. The main cluster consisted of 17 MRSA strains of ST398 isolated from pigs (10 strains), calves (three strains), mastitis milk (two strains), and veterinarians (two strains). In 17 of the 20 MRSA strains, genes encoding resistance for beta-lactams(*bla*R, *bla*I, *bla*Z), methicillin (*mec*A), macrolide-lincosamide-streptogramins B (*erm*A, *erm*C), and tetracycline (*tet*M, *tet*K) were detected. None of the 20 strains harbored genes encoding Panton-Valentine leukocidin (PVL; *luk*F-PV, *luk*S-PV), whereas all strains harbored genes encoding hemolysins (*hla, hld, hlg*A) and leukotoxins (*luk*F, *luk*S). Besides, two strains of ST1 (cattle) and ST8 (veterinarian) harbored genes encoding staphylococcal enterotoxins (*ent*A, *ent*B, *ent*H, *ent*K, *ent*Q).

Significance: The results obtained reinforce la-MRSA as a pathogen resistant not only to beta-lactams, but also to macrolide-lincosamide-streptogramins B, and tetracycline. Besides, la-MRSA rarely harbor PVL and staphylococcal enterotoxins.

P2-136 Methicillin-resistant Coagulase-negative Staphylococci Isolated from Livestock, Humans in Contact with Farm Animals, and Foods of Animal Origin, Switzerland

HELEN HUBER, Nicole Giezendanner, Roger Stephan and Claudio Zweifel
University of Zurich, Institute for Food Safety and Hygiene, Vetsuisse Faculty, Zurich, Switzerland

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) are worldwide and of increasing importance in human and veterinary medicine. Methicillin-resistance is encoded by *mec*A. The *mec*A gene is also found in methicillin-resistant coagulase-negative *Staphylococci* (MR-CNS) and can be transmitted between bacterial species.

Purpose: The aim of this study was to assess the occurrence of MR-CNS and to further characterize isolated strains.

Methods: Samples were collected within a Swiss MRSA study. Livestock samples comprised nasal swabs from 716 pigs, 300 calves, 340 cattle, and neck skin samples from 72 chicken flocks. Human nasal swabs originated from 148 pig farmers, 133 veterinarians, and 179 slaughterhouse employees. Moreover, 100 bulk tank milk (BTM) and 111 minced meat samples were tested. After a two-step enrichment procedure, chromogenic agar was used for detection of presumptive MR-CNS isolates. For characterization, 130 isolates were randomly selected. These strains were tested for the presence of *mec*A before species identification was performed by sequencing of the 16S rDNA-fragment. Additionally, the antimicrobial susceptibility of these strains was evaluated by disk diffusion.

Results: In livestock, MR-CNS were detected in 260 (36.3%) pigs, 218 (72.7%) calves, 180 (52.9%) cattle, and 50 (69.4%) chicken flocks. Among humans, 84 (63.2%) veterinarians, 105 (70.9%) pig farmers, and 48 (26.8%) slaughterhouse employees carried MR-CNS. Furthermore, 62 (62.0%) BTM and 36 (32.4%) meat samples tested positive for MR-CNS. More than 60% of the 130 sequenced strains belonged to the species of *S. sciuri*. The vast majority (> 95%) of these strains were resistant to ampicillin, oxacillin, and penicillin, whereas all but two isolates were susceptible to rifampin and vancomycin.

Significance: The results of this study show a high MR-CNS prevalence of 57.8% in livestock, 53.6% in humans, and 47.2% in foods of animal origin in Switzerland. These findings emphasize the importance of investigations on transmission of *mec*A between bacterial species.

P2-137 Dissemination of *Escherichia coli* O157:H7 to the Spinach Phylloplane Via Regurgitation of House Flies (*Musca domestica*)

LAKMINI WASALA, Justin Talley and Jacqueline Fletcher
Oklahoma State University, National Institute for Microbial Forensics and Food and Agricultural Biosecurity, Stillwater, OK, USA

Introduction: House flies have been documented to carry enteric bacterial pathogens and have been associated with food borne disease outbreaks. In 2006, a multistate *E. coli* O157:H7 disease outbreak was reported in United States in association with the consumption of bagged spinach contaminated with this pathogen. However the role of insects in contamination of leafy greens with bacterial human pathogens is poorly understood.

Purpose: The objective of this study was to investigate the potential of house flies to disseminate *E. coli* O157:H7 to the spinach phylloplane via regurgitation after feeding on different *E. coli* O157:H7 contaminated sources.

Methods: Colony raised house flies were exposed to four treatments; autoclaved manure mixed with *E. coli* O157:H7 (EM), *E. coli* O157:H7 lawns grown on LB ampicillin agar (EL), manure mixed with PBS (PM) and LB ampicillin agar plates (LB) for an hour. Exposed flies were caged with spinach plants for five hours. Leaves with regurgitation spots were examined by scanning electron microscope (SEM) using standard sample preparation methods. The presence and absence of rod-shaped bacteria-like organisms on leaf surfaces was observed. Exposed flies were also examined by SEM to determine if bacteria adhered to the labellae (mouthparts) and tarsi (feet).

Results: Leaves exposed to flies exposed to treatments EL and EM had higher numbers of rod shaped bacteria-like organisms compared to PM and LB treatments. Bacteria were observed in a matrix and some were observed in association with stomata. Tarsi and labellae of flies contacted EL and EM treatments were contaminated with rod shaped bacteria while LB and PM treated flies had no bacteria present in these body parts. Comparison of treatments EL and EM with LB and PM strongly suggests that the rod shaped organisms were *E. coli* O157:H7.

Significance: These results suggest that house flies are capable of disseminating *E. coli* O157:H7 to plants via regurgitation under experimental conditions.

Poster

P2-138 Effect of Storage Temperature on the Growth and Survival of Total and Pathogenic *Vibrio parahaemolyticus* in Chesapeake Bay Oysters

MESHACK F. MUDOH, Salina Parveen, Jessica Jones, Jeffrey Krantz, John Bowers, Mark Tamplin, Chanelle White and Angelo DePaola

University of Maryland Eastern Shore, Food Science and Technology, Princess Anne, MD, USA

Introduction: Vibrio parahaemolyticus (Vp) is a naturally occurring halophilic bacterium that can cause gastroenteritis in seafood consumers. Gastroenteritis in the US is usually associated with the ingestion of contaminated oysters. Information is limited on Vp growth and survival in oysters under various storage conditions.

Purpose: This study evaluated the effect of storage temperature on the growth and survival of total and pathogenic Vp in post-harvest shell-stock oysters.

Methods: Oysters harvested from the Chesapeake Bay in the Summer of 2008 were stored at 5, 10, 15, 20, 25 and 30°C for selected time intervals. At each time interval, two replicates of six oysters each were analyzed for total Vp levels by direct plating/DNA probe for the species specific thermolabile hemolysin (*tlh*) gene. Pathogenic Vp levels were determined by MPN-qPCR analysis targeting the thermostable direct hemolysin (*tdh*) and thermostable-related hemolysin (*trh*) genes. The Baranyi D and linear models were fitted to the Vp growth and survival data to estimate the maximum growth rate (GR).

Results: GR estimates of total Vp at 5, 10, 15, 20, 25 and 30°C were -0.0007, -0.0018, 0.038, 0.058, 0.099 and 0.098 log CFU/h, respectively. Assuming a linear model for the initial growth phase, the best estimates of GR of *tdh*- and *trh*-positive Vp at 10, 15, 20, 25 and 30°C were 0.08, 0.14, 0.22, > 0.27, 0.17, and 0.048, 0.17, 0.26, and > 0.25, 0.27 log MPN/h, respectively. No growth of pathogenic Vp was detected at 5°C. The GR of pathogenic Vp was found to be substantially greater than those observed for total Vp.

Significance: Pathogenic Vp may multiply more rapidly than total Vp. The results of this study will assist risk managers and the seafood industry in designing more effective food safety systems.

P2-139 Development of Optimal Methods for Recovery of Murine Norovirus from Surfaces Common in the Preparation of Fresh Produce

Annamalai Suriyanarayanan, BALASUBRAMANYAM PULI, Stephen F. Grove and Alvin Lee

Illinois Institute of Technology, National Center for Food Safety and Technology, Summit-Argo, IL, USA

Introduction: Person-to-person transmission of human norovirus (NoV) is common, and many outbreaks of NoV may be linked to the preparation of food by infected food handlers. Contamination of salad preparation surfaces are of particular concern because these products often do not undergo an intervention step prior to consumption. In order to later investigate virus transfer during salad preparation, methods for inoculation and recovery of murine norovirus (MNV-1) from food preparatory surfaces were optimized.

Purpose: To optimize methods of virus inoculation and recovery in order to obtain reproducible data in future virus transfer trials between surfaces common in salad preparation.

Methods: Fifty microliters (approx. 6-log CFU) of MNV-1 was inoculated onto various surfaces, including poly vinyl cutting board; stainless steel knife; romaine lettuce; poly, vinyl, and latex gloves; and the fingers and palm of human hands. After air drying for approx. 30 min, inoculum was recovered from the surfaces using various recovery tools such as cotton swabs, calcium alginate swabs, composite tissues and sponges in combination with different eluents such as Dulbecco's Modified Eagle's Media (DMEM), 3% beef extract, glycine buffer (50mM glycine+1% beef extract), stripping solution (0.04% K2HPO4, 1.01% Na2HPO4, 0.1% Triton X-100), and Earle's Balance Salt Solution (EBSS).

Results: For the cutting board, the combination of cotton swab and DMEM achieved the highest recovery (30%) of MNV-1. In contrast, the combination of composite tissue with DMEM resulted in highest recovery (49.5%) from knife, which is likely due to the smoother surface of the knife than the cutting board. Stripping solution appeared to be an effective eluent for MNV-1, recovering between 24.2 and 27.1% of inoculated MNV-1 from lettuce pieces, depending on whether the lettuce was vortexed, shaken or stomached. Furthermore, the use of stripping solution in the glove juice method recovered inoculated viruses from hands as well as each of three glove types better than the other eluents tested.

Significance: Optimization of methods for recovering MNV-1 from food preparatory surfaces will serve as a foundation for upcoming cross contamination studies which will in turn lead to the development of a risk assessment model for NoV transfer within the food service setting.

P2-140 Effect of Brine Ingredients on *Escherichia coli* O157:H7 during Storage and Cooking of Moisture-enhanced Vacuum-packaged Beef

NIKOS G. CHORIANOPOULOS, Ifigenia Geornaras, Shivani Gupta, Kyung Yuk Ko, George-John E. Nychas, Keith E. Belk, Gary C. Smith and John N. Sofos

Agricultural University of Athens, Food Science and Technology, Athens, Greece

Introduction: Escherichia coli O157:H7 has become a concern in non-intact beef products that may be intentionally or unintentionally undercooked. Therefore, there is a need for research to generate information to be used in updates of risk assessments.

Purpose: This study evaluated the fate of *E. coli* O157:H7 during vacuum-packaged storage and subsequent cooking, by three cooking methods, of beef steaks moisture-enhanced with different brine solutions.

Methods: Beef knuckles (95% lean) were coarse-ground (0.95 cm), inoculated with an 8-strain composite of rifampicin-resistant *E. coli* O157:H7 (6 log CFU/g) and moisture-enhanced to 110% of initial weight with one of the following treatments: sodium chloride (NaCl, 0.5%) + sodium tripolyphosphate (STP, 0.25%), NaCl + STP + cetylpyridinium chloride (CPC, 0.2%), NaCl + STP + lactic acid (0.3%), or NaCl + STP + sodium metasilicate (SM, 0.2%). Inoculated and treated meat was extruded into polyethylene bags (15.24 cm diameter) and placed at -20°C for 6 h to facilitate cutting of the product into 2.54 cm-thick steaks. Individual steaks were vacuum-packaged and stored at 4°C (up to 28 days) or 12°C (up to 21 days). On days 0, 7, 14, 21 and 28 (4°C samples only), steaks were cooked to a geometric center temperature of 60° C (simulating rare degree of doneness) by pan-broiling, double pan-broiling, or roasting. Cooked and uncooked samples were analyzed for total bacterial (tryptic soy agar with 0.1% pyruvate, TSAP) and E. coli O157:H7 (TSAP with 100 µg/ml rifampicin) populations. Data (two replications, three samples/treatment/replication) were statistically analyzed using the GLIMMIX procedure of SAS.

Results: In uncooked samples, pathogen counts of CPC- and SM-treated steaks stored at 4°C were 1.2 and 0.7 log CFU/g lower ($P < 0.05$), respectively, than those of the control (NaCl + STP), whereas for samples stored at 12°C, no ($P \geq 0.05$) brine treatment effects were observed. Pathogen inactivation during cooking was not affected ($P \geq 0.05$) by the formulation of the brine solution; however, it was influenced ($P < 0.05$) by the cooking method. Thermal inactivation of *E. coli* O157:H7 decreased in order of double panbroiling > roasting > pan-broiling, with respective reductions of 3.6 to 3.9, 2.5 to 2.7 and 1.6 to 1.7 log CFU/g.

Poster

Significance: The findings of this study provide quantitative data on the effect of brine ingredients on thermal inactivation of *E. coli* O157:H7 during cooking of moisture-enhanced beef steaks, and will be useful in risk assessments for non-intact beef products. The data will also be useful for developing cooking recommendations for consumers and the foodservice industry for control of internal pathogen contamination.

P2-141 Fate of *Escherichia coli* O157:H7 in Beef Slaughter Runoff Fluids during Carcass Chilling and Subsequent Decontamination

CATHERINE SIMPSON BEAUCHAMP, Ifigenia Geornaras, Keith E. Belk, Gary C. Smith and John N. Sofos
Colorado State University, Animal Sciences, Fort Collins, CO, USA

Introduction: Inadvertent pulling and tearing the outer fat layer of beef carcasses during mechanical hide removal may create pockets where pathogen contamination and fluids may collect.

Purpose: This study evaluated the fate of *Escherichia coli* O157:H7 in beef carcass runoff fluids during simulated carcass chilling and post-chilling decontamination treatments.

Methods: Fluid was collected as it ran off of beef carcasses exiting a hot water (82°C) wash cabinet at a commercial slaughter facility. The pH of the runoff fluid was left unadjusted (pH 7) or was adjusted to pH 3 or 5 with lactic acid (LA), heated to 25, 45 or 65°C and then inoculated (4 log CFU/ml) with a six-strain rifampicin-resistant *E. coli* O157:H7 composite. Pieces of beef tissue were prepared by dissecting the fat from the lean tissue to create a pocket, wherein which 200 ml of the inoculated runoff fluid was poured. Beef samples (two replications/three samples each) were then stored at 4°C to simulate carcass chilling, and fluid from the pockets was analyzed for bacterial survivors at 0, 12, 24, 36, and 48 h. After 48 h, fluid from the pockets was used to inoculate (1.5 log CFU/cm^2) the surface of fresh beef fat. The inoculated fat was sprayed (25°C, 20 psi, 3 s) with water, LA (5.0%), peroxyacetic acid (PAA, 0.02%) or cetylpyridinium chloride (CPC, 0.5%) to determine decontamination treatment efficacy against cells that survived the chilling process.

Results: Inoculation of *E. coli* O157:H7 into runoff fluid of pH 3 pre-heated to 65°C immediately (0 h) reduced pathogen counts to below the detection limit (0.0 log CFU/ml); however, survivors were detected by enrichment in samples stored for ≥ 24 h at 4°C. Initial pathogen counts in runoff fluids of pH 5 or 7 pre-heated to 65°C were also reduced (by 0.8 to 1.3 log CFU/ml), whereas in corresponding fluids pre-heated to 25 or 45°C, pathogen levels remained unchanged. The initial (0 h) temperature of the runoff fluids did not affect the fate of bacterial survivors during storage; however, growth of natural contamination was suppressed in pH 3 runoff fluid. Post-chilling decontamination treatments were generally more effective against cells derived from pH 7 than pH 3 or 5 runoff fluids, with pathogen reductions ranging from 0.4 to 1.3 and 0.1 to 0.9 log CFU/cm^2, respectively. Treatment efficacy decreased in order of CPC>LA>PAA=water.

Significance: These results indicate that beef processors should implement procedures that avoid creation or cover existing carcass surface defects before decontamination treatments are applied. When encountered at fabrication, employees should also be trained to carefully remove fluid-filled pockets and discard them as inedible.

P2-142 *Pediococcus* spp. NRRL B-2354 (*Enterococcus faecium*) as a Non-pathogenic Surrogate for *Salmonella* Enteritidis PT30 during Moist-air Convection Heating of Almonds

SANGHYUP JEONG, Bradley P. Marks and Elliot T. Ryser
Michigan State University, Biosystems and Agricultural Engineering, East Lansing, MI, USA

Introduction: The final rule for mandatory pasteurization of all California almonds (minimum 4-log reduction of *Salmonella*) was published in the Federal Register in 2007, which has created a significant, industry-wide demand for pasteurization technologies and process validation tools. Using surrogates is a practical method to validate the safety of such processes, since actual pathogens cannot be taken into food processing facilities.

Purpose: The objective of this study was to test *Pediococcus* spp. NRRL B-2354 (*Enterococcus faecium*) as a non-pathogenic surrogate for *Salmonella* Enteritidis PT30 (SE PT30) on the surface of almonds under moist-air impingement cooking condition.

Methods: Raw almonds (~300 g) were surface-inoculated with 22.5 ml of SE PT30 or *Pediococcus* spp. to a level of ~8 log CFU/g and dried for 24 h at 24°C. The inoculated samples were then heated in a laboratory-scale moist-air convection oven (~1.3 m/s gas velocity) based on the following factorial design: 4 dry-bulb temperatures (121, 149, 177, and 204°C), 5 humidities (~0, 30, 50, 70, and 90% Mv[moisture by volume]), and 3 target log reductions (1, 3, and 5 logs) in duplicate experiments. SE PT30 survivors were enumerated on modified TSAYE (supplemented with ferric ammonium and sodium thiosulfate) after 48 h of incubation at 35°C, whereas *Pediococcus* spp. was enumerated on MRS agar after 5 d of incubation at 28°C.

Results: The results indicated that *Pediococcus* spp. was a conservative (~1.4 log lower) surrogate for SE PT30 (at 4.76 log reduction) during moist-air heating ($P < 0.005$), and the standard error of prediction between the surrogate and the pathogen was 0.56 log reduction.

Significance: Based on these findings, *Pediococcus* spp. can be used in place of SE PT30 to validate moist-heat processes, with this surrogate providing greater opportunities for verifying both existing and new moist-air heating processes to meet the mandatory pasteurization requirement.

P2-143 Human Norovirus Transfer to Small Fruits during Handling and Sanitation with a Levulinic Acid Plus Sodium Dodecyl Sulfate

CHRISTOPHER SHARPS and Jennifer Cannon
University of Georgia, Food Science, Athens, GA, USA

Introduction: Human noroviruses (NoVs) are the foremost cause of foodborne illnesses and cause about 30% of outbreaks involving fruits in the U.S. Little is known about the likelihood and rate of virus contamination of hand-picked berries during harvest and NoV is resistant to many hand and surface sanitizers.

Purpose: This study examines the likelihood of NoV contamination of hand-picked berries by ill or healthy workers following restroom use. In addition, a novel levulinic acid plus sodium dodecyl sulfate (SDS) sanitizer is evaluated for its efficacy on hands, stainless steel and small fruits.

Methods: Fomite surfaces (toilet paper and stainless steel) were inoculated with human NoV (10^{12} viruses/ml stool) and fingertip transfer of virus to small fruits was conducted either immediately or after drying. Interventions for hand, surface, and fruit contamination were tested using levulinic acid plus SDS as liquid and foam treatments with murine norovirus (MNV) (10^7 viruses/ml) as a surrogate. Quantification was performed using real-time reverse transcriptase PCR for human NoV and cell culture assay for MNV

Poster

Results: Results indicate that an individual shedding NoV could transfer 6.5 to 7.7 log of virus to small hand-picked berries depending on the moisture content during transfer. A non-ill person could transfer 3.1 or 7.5 logs of virus from a contaminated door handle to hand-picked fruit, under dry or wet conditions, respectively. Sanitation with levulinic acid plus SDS yielded 2-4 log reductions of MNV for hands, door handles and all fruits.

Significance: The data presented here emphasize the importance of rigorous hand sanitation practices by hand harvesters in the field to prevent NoV contamination of foods. The efficacy of a novel sanitizer is also demonstrated on various surfaces, showing great promise for its application as a hand, surface, and produce sanitizer.

P2-144 Research on the Contamination Levels of Norovirus in the Water Used at Food Catering Facilities

MIN GYU CHO, Woon Jong Lee, Min Sik Kim, Jae Hyun Ahn, Young Woo Kwon, Sung Hwi Hwang, Yoon Sook Kang, Jin Sook Kim, Dong Jin Woo, Jeong Woong Park, Sang Ryeol Ryu, Won Sang Choi, Soon Young Paik and Kwang Yup Kim
Chungbuk National University, Food Science and Technology, Cheongju, Republic of Korea

Introduction: Norovirus has become the most common cause of human gastroenteritis in developed countries. Noroviruses are shed in the feces of infected patients at high concentrations and contaminate various environmental waters including surface waters, ground waters and waters in supply systems. Outbreaks of waterborne and foodborne illness caused by noroviruses are increasing in Korea.

Purpose: This study was executed to investigate the contamination levels of norovirus in ground water used at food catering facilities in Korea.

Methods: The ground water samples from 550 schools in Chungbuk, Chungnam, Kangwon, Chonbuk and Chonnam provinces were collected and tested precisely according to the analysis method in Korean Food Code using 1-MDS filtering and RT-PCR. The water temperature, turbidity, pH and residual chlorine concentration were analyzed and the environmental conditions including livestock barns and streams around the ground water sources were investigated.

Results: At the 1st testing period (May–August, 2009), among the 422 tested schools, water samples of 7 places (Chungbuk 3, Chungnam 2, Kangwon 1, Chonbuk 1) were contaminated with noroviruses. The 2nd test (August–November, 2009) was carried out targeting the schools which were negative in norovirus detection at the 1st test. Three hundred ninety-one schools were tested and none of the schools were positive. Due to the very low numbers of positive samples (7 positive samples from the total of 813 samples), there were limitations in statistical analysis to correlate the respective data obtained in positive and negative water samples.

Significance: Through this extensive-scale study, we could find that the positive norovirus detection were significantly influenced more by the levels of management of the water tank than by the physicochemical characteristics of ground water sources. The results collected through this study could be practically used to lower norovirus outbreaks.

P2-145 High-pressure Processing of Minimally Processed Peanut Butter and Peanut Sauce Inoculated with *Salmonella*

J. E. STRATTON, T. Stiles, S. Stephens and D. Smith
University of Nebraska-Lincoln, The Food Processing Center, Lincoln, NE, USA

Introduction: High pressure processing (HPP) is a nonthermal process currently used to inactivate pathogens and reduce spoilage microorganisms in such foods as ready-to-eat meats, oysters, and juices. Recent *Salmonella* outbreaks caused by contaminated peanut butter sickened hundreds of consumers and prompted the recall of thousands of peanut butter-containing products. A post-packaging intervention such as HPP could increase the safety of these products.

Purpose: The goal of this study was to investigate the use of High Pressure Processing to inactivate *Salmonella* inoculated into minimally processed, organic peanut butter and peanut sauce.

Methods: A five-strain cocktail of *Salmonella* that included heat resistant isolates was inoculated at 7–8 log CFU/g into peanut butter and peanut sauce. The peanut sauce had an average water activity of 0.992 while natural peanut butter was near 0.300. These products were then subjected to pressures of 400, 475, 500 and 550 MPa in a Stansted ISO Food Lab System. Holding times ranged from 1 to 10 minutes to determine the necessary conditions for the maximum reduction of *Salmonella*. Samples were plated on TSA plus XLD overlay to determine the total log reduction of *Salmonella*, and on XLD to determine the number of sublethally injured cells.

Results: Inoculated natural peanut butter achieved less than 1 log reductions in *Salmonella* at 600 MPa at hold times ranging from 5 to 30 minutes, indicating that reduced water activity inhibited inactivation by high pressure. A 5.7 log reduction in *Salmonella* was obtained in inoculated peanut sauces at 550 MPa for 4 minutes, 5.26 log at 550 MPa for 5 minutes, 2.97 log at 500 MPa for 4 minutes, 5.19-log at 500 MPa for 5 minutes, 4.94-log at 475 MPa for 4 minutes, 3.04 log at 475 MPa for 5 minutes, 2.29 log at 400 MPa for 5 minutes, and a 2.1 log from 400 MPa at 6 minutes. There was no noticeable difference in color, texture, or oil separation in the peanut butter after processing.

Significance: These results indicate that peanut butter products with higher water activities are suitable for high pressure processing and may lower the risk of future *Salmonella* outbreaks and extensive recalls of peanut butter-containing products.

P2-146 Development of Concentration Methods for Noroviruses in Food Samples Using Histo-bood Group Antigens as Ligands

BLANCA I. ESCUDERO-ABARCA, Helen Rawsthorne and Lee-Ann Jaykus
North Carolina State University, Raleigh, NC, USA

Introduction: Histo-blood group antigens (HGBA) are expressed in human gastrointestinal cells and appear to be receptors for the human noroviruses (HuNoV); similar HBGA-like moities have been identified in oyster tissue. Preliminary work in our laboratory suggests that the H-type 3 (HT3) oligosaccharide is a particularly promising ligand for capture of genogroup II (GII) HuNoV.

Purpose: The purpose of this project was to design effective methods to capture GII HuNoV from food matrices in preparation for detection by reverse transcription quantitative real-time PCR (RT-qPCR).

Methods: Biotinylated synthetic HT3 was bound to streptavidin-coated magnetic beads. Snow Mountain virus (GII.2) from the feces of experimentally infected humans was serially diluted and inoculated into three food matrices, i.e., strawberry, deli-sliced turkey, and oyster diverticula. Sample pretreatment included elution in elevated pH glycine-saline and/or beef extract buffer followed by enzymatic and/or organic solvent extraction, and capture with the HT3-bound beads using a recirculating magnetic capture protocol. RNA was extracted from the beads and detected by RT-qPCR targeting the ORF1/ORF2 junction.

Results: For artificially contaminated strawberries, deli-sliced turkey, and oyster diverticula, consistent detection of viral RNA by RT-qPCR was possible at inoculum levels of 7.5×10^2 genomic copies/25 g sample (or 5 g diverticula); periodic detection was possible at inoculum levels as low as 101 genomic copies. Relatively speaking, oyster diverticula was the most challenging sample matrix, requiring the combined steps of proteinase K treatment and solvent extraction prior to HBGA-mediated virus capture. Based on an RT-qPCR standard curve, virus recovery efficiencies from the combined sample preparation-RNA extraction steps ranged from 15–60%, occasionally higher.

Significance: The approach described here is rapid (4–5 h) and shows promise for application to virus capture from other complex sample matrices.

Poster

P2-147 Thermal Resistance of *Yersinia pseudotuberculosis* and *Yersinia pestis* in Bovine Milk with Different Fat Levels

DRABYENDRA M. NEUPANE, Susanne E. Keller and Stuart Chirtel
NCFST, IIT, Summit-Argo, IL, USA

Introduction: The genus *Yersinia* contains three species which are human pathogens, two of which are foodborne pathogens. Although the third, *Yersinia pestis* is not considered a foodborne pathogen, it has caused illness through food consumption and could be used to intentionally contaminate food. Information on the thermal resistance of both species is limited, especially in different foods which could be a target of deliberate contamination.

Purpose: To determine the thermal resistance of *Y. pestis* and *Y. pseudotuberculosis* in bovine milk with varying fat levels and compare their resistance under similar conditions.

Methods: Y. pseudotuberculosis ATCC 6905 and *Y. pestis* A1122 were grown individually in liquid media, harvested, and resuspended in ultra high temperature pasteurized (UHT) skim milk, whole milk and heavy cream. Samples were sealed into capillary tubes and treated at different temperatures for various time intervals. Survivors were enumerated. Each experiment was run a minimum of 4 times for statistical analysis.

Results: In whole milk, *Y. pseudotuberculosis* had values of D_{52} = 7.25 ± 0.51 min, D_{54} = 2.36 ± 0.55 min, D_{56} = 0.75 ± 0.14 min, with a z-value of 4.05. For *Y. pestis* the values were D_{52} = 6.81 ± 0.28 min, D_{54} = 2.51 ± 0.19 min, D_{56} = 0.89 ± 0.12 min, with a z-value of 4.53 in whole milk. For both strains D- and z-values for skim milk and heavy cream were not significantly different from whole milk (P = 0.83). Despite the apparent similarity, a statistical evaluation indicated *Y. pseudotuberculosis* to be slightly less thermally resistant then *Y. pestis* (P < 0.002).

Significance: Despite its genetic similarity to *Y. pestis*, *Y. pseudotuberculosis* was slightly less heat resistant overall. Both strains showed similar resistance in all substrates confirming that bovine fat plays no role in their thermal resistance. Both species display less thermal resistance then other typical foodborne pathogens such as *Salmonella*.

P2-148 Pulsed-light Inactivation of Foodborne Viruses

JULIE JEAN, Safaa Lamhoujeb and Marie-Natacha Anoman
Universite Laval, Food Science and Nutrition, Quebec, QC, Canada

Introduction: Norovirus (NoV) and hepatitis A virus (HAV) are the most often implicated in outbreaks of viral foodborne diseases and thereby constitute a significant threat to public health. It is therefore necessary to develop methods to reduce their impact. It is well established that ultraviolet light is effective for inactivation of different groups of viruses. However, there is only very few data about the use of pulsed-light technology as an alternative or a complement to conventional method for the inactivation of food-related viruses.

Purpose: This study was conducted to evaluate the inactivation efficacy of pulsed-light against NoV and HAV.

Methods: Viral suspension of murine NoV (a human NoV surrogate) and HAV were exposed to pulses of UV light for various durations and at different distances from the lamp, in presence and in absence of proteins. Similar experiment was conducted after adhesion of viruses on stainless steel and polyvinyl chloride hard surfaces. The inactivation rates, expressed as log reduction, were evaluated for each treatment using the plaque assay.

Results: Our results have shown that the inactivation efficacy of pulsed-light depends on both distance from the lamp and duration. Inactivation of murine NoV and HAV were more important when the viral suspensions were close to the lamp. The highest inactivation of both viral suspensions was obtained after 2 s regardless of distance from the lamp. The presence of proteins reduced the effectiveness of pulsed-light treatment with only 3-log reduction compared to 5-log obtained in the absence of proteins.

Significance: The pulsed-light represents a good alternative for inactivation of foodborne viruses. This inactivation method could easily have applications for disinfection of contaminated water and foods.

P2-149 Fresh Vegetables Contaminated Directly in the Field by Pathogenic Human and Zoonotic Foodborne Viruses

JULIE BRASSARD, Caroline Côté, Marie-Josée Gagné and Mylène Généreux
Agriculture and Agri-Food Canada, Saint-Hyacinthe, QC, Canada

Introduction: Fresh products consumed raw or minimally processed, as fruits and vegetables, provide an ideal route for the transmission of some pathogenic viruses. The epidemiological study made during certain foodborne outbreaks involving fresh products revealed that the contamination origin could concern the beginning of the production line.

Purpose: The purpose of this study was to evaluate the presence of zoonotic and pathogenic human viruses on fresh veggies directly in the field before harvest and also evaluate the impact of irrigation water on the viral contamination.

Methods: Field experiments were conducted in the Lanaudière region of the province of Québec (Canada) in 2009. Plots of broccoli, cauliflower and Chinese cabbage were repeated 4 times in a completely randomized design. Samples were collected before irrigation and 0, 1, 2, 3, 4 and 7 days after the irrigation. Vegetables were tested for the detection of enteric viruses such as Norovirus G1 and G2, human rotavirus, and porcine hepatitis E virus. Irrigation water samples from the water source and at the sprinklers were also analyzed to determine the origin of viral contamination. Feline calicivirus, as a sample process control, was added to every sample prior to the concentration and the extraction procedures and provided an additional quality control over the entire sample process.

Results: Pathogenic human viruses such as Norovirus and rotavirus were detected by RT-PCR in irrigation water and on fresh veggies before and after the irrigation. Norovirus and rotavirus were also detected on vegetables for 7 days after the irrigation. Porcine HEV was only detected in Chinese cabbage samples.

Significance: This study reported that viral contamination of fresh vegetables can occur directly at the farm before harvest and the virological quality of the irrigation water could be a factor in this contamination. Other factors such as soil contamination and agricultural practices should be evaluated in future works.

P2-150 Lack of N-acyl Homoserine Lactone Recognition by *Salmonella* sdiA in the Plant Environment

MARIANNE K. FATICA, Jason T. Noel, Max Teplitski and Keith R. Schneider
University of Florida, Food Science and Human Nutrition, Gainesville, FL, USA

Introduction: Salmonella spp. are not known to produce N-acyl homoserine lactones (AHLs), but the genome of *Salmonella enterica* contains sdiA, which encodes an AHL receptor. The presence of the AHL receptor is hypothesized to play a role in inter-species communication with AHL-producing bacterium. Under *in vitro* conditions, the sdiA-dependent resolution of a srgE recombinase-based (RIVET) reporter was observed in the presence of the AHL signals of *Pectobacterium carotovorum*, a species known to cause soft-rot in plants.

Purpose: The purpose of the research was to study the expression of sdiA and the recognition of AHLs by the sdiA of *S. enterica* serovar Typhimurium in the *in planta* environment of various soft-rot produce.

Methods: The kanamycin-resistant *S. enterica* serovar Typhimurium JSN3216 (containing an sdiA-dependent srgE recombinase-based (RIVET) reporter) and *P. carotovorum* SR38 were co-inoculated onto tomatoes (green and red), bell peppers (green and red), carrots, and green onions. After maintaining the produce in 60–100% relative humidity at 22°C for 7 days, the samples were harvested by removing

Poster

15 mm x 0.5 mm cores at the inoculation sites. Samples were homogenized in PBS and plated onto Xylose-lysine deoxycholate (XLD) agar supplemented with kanamycin. *Salmonella* colonies from each XLD plate were then patched onto LB plates containing kanamycin and tetracycline.

Results: A low incidence of resolution of the reporter, at 4%, was observed in one of the six samples of soft-rotted red tomatoes. There was no resolution of the RIVET reporter in the green tomatoes, bell peppers (green and red), carrots, or green onions. The resolution of the RIVET reporter resolves the tetracycline resistance cassette through the tnpR mechanism in response to sdiA activation.

Significance: The results of the *in planta* studies suggest a lack of sdiA expression within the plant environment, thus preventing the *Salmonella* from recognizing the AHLs of other bacterium.

P2-151 Impact of Sample Weight and Enrichment Ratio on the Isolation of *Campylobacter* spp. from Commercial Retail Broiler Meat

LIN LIU and Omar A. Oyarzabal
University of Colorado, Microbiology, Aurora, CO, USA

Introduction: Twenty-five g of meat enriched in 225 ml of broth is the standard method to isolate *Campylobacter* spp. from retail broiler meat. However, with this method the volume of enrichment needed to test several samples simultaneously becomes a limitation in food microbiology laboratories.

Purpose: This study was conducted to evaluate the efficacy of different meat:broth ratios for the isolation of *Campylobacter* spp. from retail broiler meat.

Methods: The evaluation included 25 g of meat enriched in 100 ml of Bolton broth (1:4 ratio; sub-sample A), 50 g in 200 ml (1:4; B), 100 g in 300 ml (1:3; C), and 150 g in 300 ml (1:2; D). In 29 samples, another sub-sample (E) was evaluated at a 1:9 ratio.

Results: The results from 110 samples showed no differences ($P > 0.05$) in the number of *Campylobacter*-positive samples among sub-samples (A through D), or between A and E. The mixing of the meat resulted in more *Campylobacter*-positive samples than non-mixed samples. By adding A plus B, the number of positive samples increased ($P < 0.05$) in comparison to the numbers from A or B sub-samples alone. The addition of results from sub-sample C plus D yielded only three extra positive samples. Sub-samples C and D were the most contaminated, and the contamination for sub-samples A and B depended more on the original contamination of the meat than the enrichment ratio. No differences were found among positive samples for A, B, C or D based on product type.

Significance: These results suggest that the linear extrapolation of enumeration results may not be appropriate to predict the presence of *Campylobacter* spp., and that a 1:4 enrichment ratio with 25 g of meat is the most practical alternative for the isolation of *Campylobacter* spp. from retail broiler meat.

P2-152 Determining If the Consumer Method for Boiling Shrimp until Floating Effectively Reduces *Listeria* and *Salmonella* Species

GENEVIEVE EDWARDS, Marlene Janes, Miguel Gutierrez, Sarah Kerr and Jenna Young
Louisiana State University, Food Science, Baton Rouge, LA, USA

Introduction: Shrimp must be properly cooked to eliminate the threat of foodborne illness to consumers. The most common method used by consumers to determine if shrimp are thoroughly cooked when boiling, is to wait until the shrimp float to the top of the water and are pink in color.

Purpose: The purpose of this study was to determine whether the current floating and color cooking method is adequate to ensure the elimination of *Listeria monocytogenes*, *Listeria innocua*, *Listeria welshimeri*, *Salmonella* Typhimurium, *Salmonella* Enteriditis, and *Salmonella infantis* in shrimp.

Methods: Shrimp samples were submerged into bacterial suspensions for 30 minutes then allowed to air dry for 1 hour and color parameters were measured using a spectrophotometer. Shrimp samples were separated into three groups; day 0, 1, or 2, and stored at 4°C. The shrimp samples were then treated by placing into boiling water (100°C) on days 0 (inoculation day), 1 and 2. The shrimp were immediately removed from the boiling water once they started floating and color parameters were measured. Bacterial counts were determined by making serial dilutions, spread plating, incubating plates at 37°C for 24 h and calculating log CFU/g.

Results: Initial bacterial counts ranged from 3.0 to 5.4 log CFU/g of shrimp. On day 0, 1, and 2 all bacterial counts were reduced to non-detectable levels for shrimp samples that floated in the boiling water. The redness (a*), yellowness (b*) and lightness (L*) were significantly higher ($P < 0.0001$) in the cooked shrimp compared to uncooked for all days tested. However, the standard deviation for the redness (a*) in the cooked shrimp was large indicating a wide range of pink coloration for all days tested.

Significance: Our results suggest that boiling shrimp until they float will significantly reduce *Listeria* species and *Salmonella* species but color change will not and color variation can occur.

P2-153 Evaluation of the MicroSEQ® *Escherichia coli* O157:H7 Assay: Real-time PCR Detection Method

ERIN S. CROWLEY, Patrick Bird, Marianne Torontali, Kiel Fisher, Katherine Goetz, Joseph M. Benzinger, Eric Questel, James Agin, David Goins, Yanxiang Cao, Robert Tebbs, Priya Balachandran, Lily Wong and Olga Petrauskene
Q Laboratories, Inc., Microbiology R&D, Cincinnati, OH, USA

Poster

Introduction: Escherichia coli O157:H7 is a major foodborne pathogen and one of the main enterohemorrhagic *E. coli* serotypes. The MicroSEQ *Escherichia coli* O157:H7 method uses PCR to amplify unique *Escherichia coli* O157:H7 specific DNA target sequences and TaqMan® probes that detect the amplified sequences. These probes contain a fluorescent dye and a quencher. When no target sequence is present, the fluorescence is quenched. Upon binding to a specific target sequence, the quencher is released and fluorescence can be detected. As target DNA is amplified, the fluorescent signal increases and this increase is detected by the instrument (real-time PCR). Sample DNA is extracted by either the PrepSEQ™ Rapid Spin Sample Preparation Kit which combines centrifugation with a spin column to clarify the sample and concentrate bacteria followed by heat lysis, or the PrepSEQ™ Nucleic Acid Extraction Kit which uses a Proteinase K lysis method followed by DNA purification by magnetic particles.

Purpose: The purpose of this internal evaluation was to evaluate ruggedness and inclusivity/exclusivity of the MicroSEQ method and compare to the USDA-FSIS (meat) ISO 16654 (juice and spinach) reference methods for *Escherichia coli* O157:H7 as part of the AOAC Research Institute™ PTM validation process.

Methods: The method comparison analyzed 3 foods with a 16-h enrichment and a 6-h enrichment and 2 with a 16 h and 8 h using a 25-g test portion. The ground beef and beef trim were also validated as 375-g test portions with 16-h enrichment. Each matrix was spiked with a different strain of *Escherichia coli* O157:H7 at two levels (0.2–2 CFU/25 g and 2–5 CFU/25 g). For each test portion, DNA was extracted by both PrepSEQ™ procedures, analyzed by rtPCR and compared to the ISO 16654 method for spinach, orange juice and unpasteurized apple juice or the USDA-FSIS method for raw ground beef and raw beef trim. The new method was also evaluated for inclusivity/exclusivity and ruggedness parameters.

Results: For this new assay, modified performance parameters in the ruggedness evaluation showed no significant differences. There were no significant differences between the new methods and their corresponding reference method as indicated by McNemar's Chi-square analysis (> 3.84) for all 5 food types at varying incubation times and sample volumes. For inclusivity, all 50 strains of *E. coli* O157:H7 were detected as positive and for 29/30 exclusivity strains were negative and 1 *E. coli* O157 NM was positive.

Significance: This new method is a rapid, reliable alternative to the traditional method of detecting *Escherichia coli* O157:H7 in a variety of foods.

P2-154 Comparison of ISO Method 16140 with USDA and DuPont Qualicon BAX Methods for the Detection of *Listeria monocytogenes* from Naturally Contaminated Environmental Samples

ERROL GROVES and Catherine Donnelly
University of Vermont, Nutrition and Food Sciences, Burlington, VT, USA

Introduction: ISO method 16140 and ALOA Agar are approved for use in the U.S., but are not widely used in this country. We compared the ISO 16140 method with the USDA and BAX methods to show the efficacy of this alternate method for recovery of *Listeria* from naturally contaminated environmental samples.

Purpose: Naturally contaminated environmental sites frequently contain genetically distinct strains *Listeria*. Existing detection and recovery methods vary with respect to sensitivity and inclusivity. The objective of this study was to test the efficacy of tested methods for their ability to identify contaminated sites, and to determine if each method had inclusivity for genetically distinct *Listeria* isolates recovered from tested sites.

Methods: Environmental samples were collected from 120 sites from a dairy facility using environmental sponges. A 10 ml aliquot of Butterfield's buffered phosphate was added to each sponge. All samples were prepared as per the protocols for each method (ISO, USDA, and BAX). BAX positive samples were confirmed by subculture from 24E broth onto the chromagenic media to provide isolated colonies for further tests.

Results: The ISO method recorded the best results with 7 positive samples identified from 120 tested samples, followed by the USDA method with 6 positive samples. The BAX method indicated only 2 positive samples but 3 additional positive samples were isolated from the 24E enrichment broth. No differences in recovery were detected between the ALOA and the CHROMagar. Two distinct *L. monocytogenes* Ribotypes, 19157 and 10144, were detected from the environmental samples with neither Ribotype showing preferential recovery by any method or media.

Significance: The ISO method proved to be superior to the USDA and the BAX methods showing it to be a viable alternative for environmental testing. ALOA and CHROMagar can be interchanged among the methods used with no impact on efficacy of results.

P2-155 RAPD-PCR Determination of *Bacillus cereus* Spore Population Dynamics in Inoculated Pack Studies

JARED STRONG and Ronald Labbe
University of Massachusetts-Amherst, Food Science, Amherst, MA, USA

Introduction: Bacillus cereus is an opportunistic and food borne pathogen. Inoculated pack studies are a proven and accepted method for verification of the effectiveness of kill steps employed under Hazard Analysis Critical Control Point (HACCP) plans. One assumption inherent to inoculated pack studies is that every strain in the mixed spore inoculum germinates and grows at equal rates. Differences in the rate of germination, growth rate, and survival rates of kill steps between *B. cereus* strains have all been shown when grown in isolation.

Purpose: The purpose of this work was to determine the validity of the assumption that mixed spore strains of *B. cereus* germinate and grow at equal rates regardless of toxigenicity and food matrix.

Methods: The Randomly Amplified Polymorphic DNA-PCR (RAPD-PCR) technique for differentiating two classes of *B. cereus* spores was employed to observe the population dynamics of strains grown in mixed-culture, inoculated-pack studies. A mixture of one enterotoxigenic and two emetic spore strains was used. Growth from spore experiments were performed in duplicate in both Nutrient Broth (NB) and reconstituted milk powder (milk).

Results: In NB no significant difference ($P > 0.05$) in relative viable counts among the three spore strains added at time zero was observed during logarithmic and stationary phases of growth. However, a significant difference in cell numbers among inoculated strains was shown in milk at 28 h ($P = 0.004$) and at 48 h ($P = 0.025$). At the 48 h sampling only 17.9% of colonies were of the emetic toxin type as determined by RAPD-PCR rather than the 66% expected.

Significance: These results cannot reject the tested assumption of equal germination, outgrowth and cell division of added spore inocula in NB but does refute the assumption in at least one actual food matrix, milk. This provides support for the importance of selection of spore isolates in inoculated pack studies. In the case of *B. cereus* this includes the importance of the toxigenicity of isolates selected.

P2-156 Infectious Dose in Neonatal Jersey Calves of an *Escherichia coli* O157:H7 Strain from the 2006 Spinach Outbreak

XUNDE LI, Trân H. Nguyen, Edward Atwill, Bruce R. Hoar, Michelle Jay-Russell, Jennifer A. Carabez, Kristine Fernandez, Eduardo J. Vivas, Natalia Martinez and Bret McNabb
University of California-Davis, Western Institute for Food Safety and Security, Davis, CA, USA

Introduction: Fresh produce is increasingly recognized as a growing source of foodborne illness. *E. coli* O157:H7 is one of the major bacteria that contaminate fresh produce. *E. coli* O157:H7 strains from produce, dairy cattle and feral swine during the 2006 spinach outbreak were identical in DNA fingerprinting.

Purpose: The infectious dose of outbreak strains of *E. coli* O157:H7 in food animals are unknown but essential for evaluating the risks of cross transmission between animals and contamination of produce. The objective of the present work was to determine the infectious dose in neonatal Jersey calves of an *E. coli* O157:H7 strain from the 2006 spinach outbreak.

Methods: An *E. coli* O157:H7 strain originally isolated from feral swine during the 2006 spinach outbreak were inoculated to neonatal Jersey calves. Calves were given colostrum after birth and fed with antibiotic free milk replacer during the experiments. Calves at ~ 1 week old were inoculated with 10^4 to 10^{10} CFU of bacteria per animal. Infections in calves were determined by Immuno-Magnetic separation and PCR confirmation of bacteria from feces and intestinal tissue. A portion of inoculated bacteria passed through the gut directly in the first 2 days post inoculation (PI) and shedding of colonized bacteria started at ~ 4 days PI.

Results: Lower doses (10^4 ~ 10^7 CFU per animal) of this *E. coli* O157:H7 strain failed to infect, but higher doses (10^8 ~10^{10} CFU per animal) successfully infected neonatal Jersey calves.

Significance: This specific strain from 2006 spinach outbreaks is dose-dependent infectious to neonatal Jersey calves. Data from the present work demonstrated the potential of *E. coli* O157:H7 transmission from wildlife to food animals and contamination produce in fields.

Poster

P2-157 Comparison of an Automated Most Probable Number Technique to Traditional Plating Methods for Estimating Populations of Total Aerobes, Coliforms and *Escherichia coli* Associated with Freshly Processed Broiler Chickens

J. ERIC LINE and Norman J. Stern
USDA-ARS-PMSRU, Atlanta, GA, USA

Introduction: Traditional microbiological techniques for estimating populations of viable bacteria can be laborious and time consuming. The Most Probable Number (MPN) technique is especially tedious as multiple series of tubes must be inoculated at several different dilutions. Recently, an instrument (TEMPO™) has been developed to automate the MPN technique and reduce the effort required to estimate some bacterial populations.

Purpose: The purpose of our study was to compare the automated MPN technique to traditional microbiological plating methods or Petrifilm™ for estimating the total viable count of aerobic microorganisms (TVC), total coliforms (CC), and *E. coli* (EC) populations on freshly processed broiler chicken carcasses (post-chill whole carcass rinse [WCR] samples) and cumulative drip-line samples from a commercial broiler processing facility.

Methods: Overall, 120 broiler carcasses, 36 pre-chill drip line samples and 40 post-chill drip line samples were collected over 5 separate days (representing 5 individual flocks) and analyzed by the automated MPN and direct agar plating or Petrifilm™ methods.

Results: The TVC correlation coefficient between the automated MPN and traditional methods was found to be very high (0.972) for the pre-chill drip samples which had mean log values of 3.09 and 3.02, respectively. Correlations were, likewise, high between the methods for the pre-chill CC and EC samples with correlation coefficients of 0.812 and 0.880, respectively. The post-chill WCR samples had much lower mean log TVC values of 1.53 and 1.31, respectively. The estimated number of total aerobes was generally greater than the total number of coliforms or *E. coli* recovered for all sample types.

Significance: The automated MPN instrument was easy to utilize and allowed a single operator to perform the three analytical tests in less time than it took 4 trained laboratory technicians to conduct the tests by traditional methods. As a result accurate MPN estimations may be obtained from these sample types with significantly less effort than by traditional methods.

P2-158 Survival of *Salmonella* in Organic and Conventional Broiler Feed at Different Temperatures and Water Activities

Aparna Petkar
Dept. of Food Safety, University of Georgia, Athens, GA, USA

Introduction: Salmonellosis is one of the leading causes of foodborne disease in the United States and around the world. In the poultry industry, transmission of *Salmonella* to the birds has been associated with contaminated poultry feed. Contamination of feed with *Salmonella* can occur during feed processing and storage. Current work in our laboratory showed that the prevalence of *Salmonella* in conventional broiler feed is higher than in organic feed. Previous studies have examined the effect of either temperature or water activity (a_w) on survival of *Salmonella* in conventional poultry feed. However, there is very limited information published on the effect of both a_w and temperature on the survivability of *Salmonella* in conventional versus certified-organic broiler poultry feed.

Purpose: The objective of this experimental study was to compare the survival of *Salmonella* in organic versus conventional broiler feed stored at different temperatures and water activities over a 80-day period.

Methods: Two *Salmonella* inocula (high:10^6 and low: 10^3 CFU/g of feed) were used in the study. Five *Salmonella* serotypes (*S.* Typhimurium, *S.* Enteritidis, *S.* Montevideo, *S.* Heidelberg, and *S.* Senftenberg) were used in the feed inoculation. The effect of temperature and a_w on the feed samples (organic and conventional) at both inocula was assessed using a 3 × 3 factorial experimental design. Three temperatures (11, 25 and 38°C) and three a_w (0.75, 0.55 and 0.43) were used which simulate the different possible feed storage conditions at conventional and organic poultry farms. The concentrations (CFU/g) of *Salmonella* in the feed samples were measured at days 0, 3, 7, 14, 21, 28, 35, 50, 65 and 80. At each sampling point, 10 g portions of inoculated feed were suspended in a 90 ml of LB (Lactose Broth), plated onto XLT4 medium, and after incubation (37°C, 24 h) typical *Salmonella* colonies were enumerated. Enrichment technique was used when direct plating resulted negative.

Results: There was significance difference ($P < 0.05$) in concentrations of *Salmonella* by feed type at the low dose with lower *Salmonella* populations in organic compared to conventional feed over the 80 days of storage for all temperatures and a_w. *Salmonella* populations (CFU/g) in organic feed were significantly lower than in conventional feed at only low inoculum level for temperature and humidity storage conditions: 11°C (0.55 and 0.75) and 25°C (0.55 and 0.75).

Significance: Based on these findings, greater reduction in *Salmonella* populations was observed in low-dose inoculated organic broiler feed at both low and room temperature with moderate to high water activity compared to low-dose inoculated conventional broiler feed.

P2-159 Dispersal of *Escherichia coli* and *Salmonella* from Poultry House Fan Ventilation Systems and Deposition onto Nearby Leafy Greens

Brett D. Smith, Patricia Millner, FAWZY M. HASHEM, Corrie P. Cotton, Lurline E. Marsh and Jessica S. Hagan
University of Maryland Eastern Shore, Princess Anne, MD, USA

Introduction: Transfer of pathogenic bacteria from animal manure has been implicated in several outbreaks of produce contamination. Bacteria-laden manure dust and particulates from various animal production operations, including land application of manure and exhaust air from mechanical ventilation systems in animal housing units, may be transported off-farm in air and deposited onto nearby crops, soil, and surface waters.

Purpose: In this study we examined airborne dispersal/deposition of *Salmonella* and *E. coli* from two different poultry house ventilation systems onto leafy greens at a range of distances downwind from the ventilation exhaust pathways in order to determine the extent to which these ventilation systems represent a food safety risk factor with regard to their proximity to leafy green crops.

Methods: Greenhouse-grown flats of lettuce and spinach were placed at 0, 7.5, 15, and 30 meter downwind from a poultry house with 91.4 cm ventilation fans and 0, 7.5, and 11.1 meter downwind from a poultry house containing high ventilation rate (tunnel) fans. Lettuce and spinach leaf samples along with poultry litter were collected and analyzed at each sampling event (n = 7) for the presence of *E. coli* and *Salmonella*.

Results: Salmonella was never detected in poultry litter or on leaf samples. Litter contained 1.04 × 10^7 CFU *E. coli*/g and was detected on produce leaf surfaces at least 11.1 meters downwind of tunnel fans, whereas it was only detected sporadically at a maximum of 7.5 meters downwind of the 91.4 cm fans.

Significance: Dispersal/deposition of *E. coli* from poultry house ventilation systems to nearby leafy greens varies depending on the type of ventilation system. Further study including periods involving the dust-generating litter clean-out operations are needed to characterize the risk from dispersal/deposition of all bacteria-laden dust sources associated with poultry house operations to nearby leafy greens.

P3-01 Transfer of Internalized *Escherichia coli* O157:H7 from Cut Lettuce during Simulated Dewatering Process and Effect of Sanitizer Use in Preventing Cross-contamination

Manishkumar Patel and TONG-JEN FU
U.S. Food and Drug Administration, National Center for Food Safety and Technology, Summit-Argo, IL, USA

Introduction: Produce grows in natural environment and is susceptible to microbial contamination. *Escherichia coli* O157:H7 can become internalized within growing plants and, if present, could leach out from cut surfaces during postharvest processing. In the fresh-cut processing facilities, produce is subjected to a series of washing steps and excess water is removed by a dewatering process prior to packaging. The risk from the possible spread of contamination during washing has been recognized. Both industry and government have issued guidelines recommending the use of chemical disinfectants in wash water to minimize potential cross contamination. However, little is known about the potential of the release of internalized *E. coli* O157:H7 from cut produce during the dewatering process.

Purpose: This research investigated the release and transfer of internalized *E. coli* O157:H7 from cut lettuce in a simulated centrifugal dewatering process and evaluated the efficacy of sanitizer use in preventing batch cross contamination.

Methods: Romaine lettuce leaves were internally inoculated with *E. coli* O157:H7 by immersing the cut ends in cell suspensions for 1 day to allow uptake. A 2-cm piece of cut lettuce internalized with 5 to 7 log CFU/g of *E. coli* O157:H7 was spun for 1 min in 900 ml sterile DI water in a salad spinner with or without the presence of 100 g of uninoculated lettuce. Collected rinse water and lettuce samples were analyzed for the presence of *E. coli* O157:H7. An *E. coli* O157:H7 strain expressing green fluorescent protein (GFP) was used to allow quantitative analysis of the transfer of the internalized pathogen. The effect of sanitizer use in preventing cross contamination was examined either by subjecting the uninoculated lettuce to soaking in chlorinated water before dewatering or by adding chlorinated water during spinning.

Results: 1.1 to 2.9 log CFU/ml of *E. coli* O157:H7 was found leaching out from the contaminated piece into the rinse water. Using lettuce pieces internally inoculated with the GFP strain (4 to 7 log CFU/g), it was found that the dewatering process led to the release of internalized *E. coli* O157:H7 and resulted in the contamination of the rinse water and uninoculated lettuce at levels as high as 1.8 log CFU/ml and -0.8 log CFU/g, respectively. With the use of 5 ppm chlorine, transfer of the internalized pathogen to the rinse water and co-processed lettuce was observed. However, with the use of 30 ppm chlorine, no cross contamination was observed.

Significance: The results suggest that internalized *E. coli* O157:H7 could leach out and spread during the dewatering process, and the use of sufficient sanitizer could prevent potential batch contamination.

P3-02 Factors Affecting *Salmonella* Cross-contamination during Postharvest Washing of Tomatoes

Shivrajsinh Rana, Brian Parisi, Karl Reineke, Diana Stewart, Joseph Schlesser, Mary Lou Tortorello and TONG-JEN FU
U.S. Food and Drug Administration, National Center for Food Safety and Technology, Summit-Argo, IL, USA

Introduction: Fresh tomatoes have been associated with multiple outbreaks of *Salmonella* infections in the U.S. Postharvest washing has been suggested as a point where contamination could spread. However, the transfer of pathogenic microorganisms from contaminated fruits to wash water and the subsequent uptake by uncontaminated fruits have not been thoroughly investigated. Postharvest washing is also the most practical point of control. The use of sufficient chemical disinfectants in wash water is critical to minimize the potential of cross contamination. Industry and government guidelines have suggested that wash water disinfectants be monitored and have recommended specific performance criteria, yet these standards have not been adequately validated.

Purpose: This study examined *Salmonella* cross contamination during postharvest washing of tomatoes and determined factors that affect the spread of *Salmonella*. The use of the oxidation reduction potential (ORP) as a measure of the antimicrobial efficacy of wash water was evaluated.

Methods: A bench-scale washing system was designed to simulate commercial operations. It consisted of a 15-gal tank equipped with a submersible pump and instrument to measure the chemical and physical properties of the wash water. Field-harvested tomatoes inoculated with 7 or 4 log CFU/tomato of *S.* Typhimurium were added to 40 L sterile tap water along with uninoculated tomatoes. After 5 min, all tomatoes and wash water samples were analyzed for *Salmonella* counts. The same washing operations were repeated at different temperatures and chlorine levels. Industry spent dump tank water was used to provide conditions with higher solid and organic contents.

Results: Uninoculated tomatoes became uniformly contaminated at levels of 3.02 ± 0.51 or 0.10 ± 0.19 log CFU/tomato after washing along with tomatoes inoculated with 7 or 4 log CFU/tomato, respectively. Lower uptake of *Salmonella* was observed when wash water was kept at a higher temperature than that of tomatoes. No transfer of *Salmonella* was observed with 100 or 200 ppm of chlorine. At 5 or 30 ppm, low levels of transfer occurred and the extent of spreading was greater in the industry dump tank water. No linear correlation could be established between ORP and chlorine level at chlorine concentrations > 5 — 20 ppm. At 5 or 30 ppm of chlorine, the ORP readings were frequently higher than the recommended threshold value (650 mV).

Significance: Salmonella could spread during washing operations and the use of sufficient sanitizer could prevent pathogen cross–contamination. Maintaining an ORP value at 650 mV may not guarantee the antimicrobial efficacy of the wash water.

P3-03 Behavior of Internalized *Escherichia coli* O157:H7 in Packaged Fresh Spinach during Postharvest Storage and Distribution Conditions

CHONG MA, Liao Wang, Arlette Shazer, Diana Stewart, Yoon Song and Mary Lou Tortorello
Illinois Institute of Technology, Food Safety and Technology, Chicago, IL, USA

Introduction: Certain conditions have been documented to promote internalization of pathogens in fresh produce, including leafy greens. Many studies of pathogen survival have been performed using cells inoculated onto leaf surfaces, but little information is available on behavior of internalized cells.

Purpose: The objective of this study was to compare the behavior of internalized and surface-inoculated *E. coli* O157:H7 in packaged spinach.

Methods: A green fluorescent protein-encoding strain of *E. coli* O157:H7 was used for inoculation of spinach. For surface contamination, cells were spot-inoculated on leaf surfaces. For internalized contamination, cells were injected by syringe into the ends of leaf stems for uptake into leaf tissue. Micro-perforated and non-perforated packaging films for retail (20% O_2 / 3% CO_2) and low-oxygen (0% O_2 / 15% CO_2) conditions, respectively, were used for control of atmosphere in packages containing 100 g inoculated spinach. Triplicate packages were stored at 5 or 15°C for 14 days, and *E. coli* O157:H7 populations were assessed by fluorescent colony counts of homogenized spinach on BHI+rif agar. The behavior of internalized cells was compared to our previous surface contamination data using DMFit software for calculation of growth kinetics.

Poster

Results: At 15°C in either high- or low-oxygen, greater survivability was shown by surface-inoculated vs internalized cells, as deter-mined by both population level change and growth rate. At 15°C, in high-oxygen, populations increased 0.75 vs 0.02 log CFU/ml with growth rates of 1.02 vs 0.03 log CFU/ml/day, and in low-oxygen they increased 2.1 vs 0.5 log CFU/ml, with growth rates of 0.5 vs 0.33 log CFU/ml/day, for surface vs. internalized cells, respectively. At 5°C, all populations decreased, with the greatest population change (-0.9 log CFU/ml) and rate of decrease (-0.23 log CFU/ ml/ day) in the high-oxygen vs low-oxygen (-0.17 log CFU/ml and -0.16 log CFU/ml/day).

Significance: This study supports our previous work that showed lower risk with cold-storage of packages where high oxygen atmospheres were maintained and provides new evidence that internalized cells present a lower risk, as shown by lower survivability, than cells which contaminate leaf surfaces.

P3-04 Threshold Concentrations of *Escherichia coli* O157:H7 in Soil Required for Internalization into Leafy Greens

CATHY WEBB, Marilyn Erickson, Lindsey Davey, Alison Payton, Li Ma, Ian Flitcroft and Michael Doyle
University of Georgia, Center for Food Safety, Griffin, GA, USA

Introduction: Internalization of *Escherichia coli* O157:H7 (O157) into the edible portions of leafy green plants has been reported under hydroponic and green house/laboratory conditions. The threshold concentration and conditions favorable for the uptake of O157 from the rhizosphere soil and subsequent internal mobilization to leaf tissue of various leafy green plants were determined in this study under growth chamber conditions.

Purpose: To determine: (1) those environmental conditions that would enhance survival of O157 in soil; (2) the threshold concentration of O157 applied to soil via irrigation water that would consistently lead to internalization into the roots; and (3) if pathogen internalized into roots from the soil would be mobilized to leaf tissue.

Methods: Lettuce, spinach, and parsley plants were germinated, transplanted, and grown under growth chamber conditions to different stages of development. Virulent and surrogate strains of O157, transformed with an ampicillin resistant plasmid expressing green fluorescent protein, were cultured in typtic soy broth supplemented with 100 µg/ml ampicillin, harvested, and diluted in sterile distilled water. The diluted inocula were added to the soil, and samples of soil, roots, and leaves were removed at various time points for detection of O157 by plate count enumeration or enrichment culture. Internalized populations were distinguished from total populations by subjecting plant tissue to a surface disinfectant wash of silver nitrate. Two replicates were performed for each experiment.

Results: Saturated moisture conditions increased the duration and survival of virulent and surrogate O157 in soil. Application of inoculum to soil to give a concentration of 6 log CFU/g, led to consistent uptake via the roots under growth chamber conditions. At the same time, internalized populations were detected in the leaves of only a few samples three days post exposure but did not persist to six days post exposure.

Significance: The results of this study indicate that a soil concentration of 6 log CFU/g O157 is necessary to achieve consistent internalization into the roots, but those internalized pathogens are rarely transported from the roots to aerial tissue.

P3-05 Prevalence and Molecular Epidemiology of *Salmonella* in Meat Products

JI-YEON HYEON, Jun-Ho Park, Jeong-Hwan Cheon, Yun-Gyeong Kim, In-Gyun Hwang, Hyo-Sun Kwak and Kun-Ho Seo
Konkuk University, Dept. of Public Health, College of Veterinary Medicine, Seoul, Republic of Korea

Introduction: Salmonellosis is one of the most widespread infectious diseases in the world and is a common cause of gastrointestinal food poisoning. Raw and processed meat products, including poultry, beef, and pork, remain the principal reservoir of *Salmonella*.

Purpose: The objective of this study was to identify and characterize the *Salmonella* isolated from poultry, beef, and pork in a Korean food market. In addition, we evaluated the performance of automated repetitive sequence-based PCR system (DiversiLab™) for subtyping *Salmonella* isolated.

Methods: A total of 120 samples (48 of beef, 48 of pork, 24 of chicken) were examined. Twenty five grams of each sample were enriched in 225 ml of buffered peptone water and incubated for 24 hours at 37°C. And then 0.1 ml of the enriched BPW was added to 9 ml of RV and incubated 24 hours at 42°C followed by streaking onto XLD for *Salmonella* detection. After 24 hours incubation at 37°C, presumptive colonies as *Salmonella* on XLD were confirmed by API 32E and with "O" antisera. In addition, an antibiotic resistance test was performed, and molecular subtypes of *Salmonella* isolates were ascertained using automated repetitive sequence-based PCR system (DiversiLab™, BioMérieux, France).

Results: A total of 13 of 120 (10.8%) *Salmonella* strains were isolated, and 6 of 13 (46%) *Salmonella* strains were identified as serogroup D. On antibiotic resistance test, most of *Salmonella* were resistant to erythromycin and the other antibiotics tested. Automated repetitive sequence-based PCR system for molecular subtypes represented weak differentiation among the same serovar of *Salmonella* isolates, but good differentiation among different serovars (serogroup B and serogroup D).

Significance: Meat products contaminated by *Salmonella* have the possibility to give the serious risk for human health. DiversiLab™ showed potential for differentiating similar serogroups of *Salmonella* and is a possible alternative to phage typing. The DiversiLab™ has a capability that can be added to the microbiology lab toolbox of subtyping methods. It would enhance the response to foodborne outbreaks in regulatory agencies and pathogen contamination in environments including food and drug industries

Poster

P3-06 Food Safety Concerns from Fresh Produce in Local Farmers' Markets and Grocery Stores

SHARON WEATHERSPOON, Ralphenia Pace, Wendell McElhenney, Deidre Tice and Byungjin Min
Tuskegee University, Food and Nutritional Science, Tuskegee, AL, USA

Introduction: Americans are choosing diets high in fresh produce for healthier lives. Ready-to-Eat foods and fast food restaurants are being traded in for "farmer's markets" (FMs) and organic foods in America's pursuit for healthiness. Fresh fruits and vegetables consumption in FMs have increased in the past two decades. FMs regulatory differences might yield food safety concerns compared to grocery stores.

Purpose: The purpose of this study was to evaluate microbial load in fresh produce from local FMs and grocery stores.

Methods: Select fruits and vegetables were obtained from local FMs and grocery stores in Alabama. Each sample was stomached with 0.1% "buffered peptone water" (BPW) and 1mL was inoculated on 3M Petrifilm plates for *E. coli*, coliforms, and total aerobic bacteria. For *Listeria* a swabbing technique was used for sample preparation and inoculated on 3M Petrifilm plates.

Results: The study was performed by completely randomized design. There were significant differences ($P < 0.05$) in total aerobic bacteria counts in fresh produce from FMs (612) and grocery stores (135); coliform bacteria counts for FMs (167) and grocery stores (20); *Listeria* counts for FMs (11) and grocery stores (1). However, there were no significant differences in *E. coli* and *Salmonella*. Also, total aerobic bacteria and coliform bacteria in organic produce were higher than bacterial populations in conventional produce.

Significance: Results suggest that fresh produce from local FM's and grocery stores were exposed to microbial loads. Local FMs and grocery stores need to improve food safety for consumers.

P3-07 Investigation and Evaluation of Current Food Safety Traceability Strategies Employed by the Produce Industry

BENJAMIN CHAPMAN and Michelle Rose
North Carolina State University, Raleigh, NC, USA

Introduction: Recent outbreaks of illnesses caused by *E. coli* O157 and *Salmonella* spp. on produce have caused economic losses to produce growers nationwide. The speed in which a contaminated food product can be traced is critical to public health, consumer trust and limiting economic losses. Implementation of traceability systems and the timely exchange of risk reduction information have been identified as a major gap within the fresh produce industry.

Purpose: The goal of this project was to catalog and assess the effectiveness of current traceability strategies employed by grower/packer/shippers in North Carolina. Collection of this information provides a baseline of practices to build management tools and educational packages from and compare future data against.

Methods: Data was collected using quantitative and qualitative methods in two phases. Phase One included state-wide industry 32 question telephone survey (n = 63). Participants were asked about their traceability practices including product data collection, transfer and information storage. Also focused on was supply chain activities and product movement management. Phase Two was an intense case study of specific sites (n = 11) that included observation, document review and in-depth interviews.

Results: It was found through the phone survey that while some producers have implemented some food traceability strategies there is a patchwork of methods being employed. 46% of respondents reported using a paper-based system that would take up to one week to trace product from a source and downstream to buyers. 16% of respondents reported that they could not effectively conduct a recall.
On-site evaluations confirmed the complexity of information collection and storage systems and highlighted the difficulties in comingling of products and conserving product information through the supply chain.

Significance: Data generated by this project is currently being used to create extension programs for the produce industry, foodservices and consumers. As a result of this project recommendations for future traceability implementation strategies have been created.

P3-08 Fate of *Escherichia coli* O157:H7 and *Salmonella* spp. on Fresh-cut Carrots and Romaine Lettuce Stored at 4 and 23°C

IRENE Y. ZHAO, Michelle D. Danyluk and Donald W. Schaffner
University of California-Davis, Davis, Food Science, Davis, CA, USA

Introduction: Fresh-cut produce is considered to be ready to eat without any further preparation. However, there are a number of standard consumer practices that may increase or reduce the likelihood of pathogen growth or survival in the cut product. A more detailed quantification of the impact of these handling practices is needed.

Purpose: The purpose of this research was to evaluate the impact of storage temperature and time on growth or survival of *E. coli* O157:H7 and *Salmonella* spp. on fresh-cut carrots and romaine lettuce.

Methods: A cocktail of five rifampicin-resistance strains of *E. coli* O157:H7 or *Salmonella* spp. was inoculated onto 20 g of baby or shredded carrots or 10 g of chopped romaine lettuce at a level of 3 log CFU/g. The inoculated carrots were placed in plastic zipper bags and the lettuce was placed in plastic containers. The packages were stored at 4 or 23°C for 0, 4, 8, 24, 48 and 72 h. Levels of inoculated bacteria and aerobic plate count were determined on tryptic soy agar with and without 50 µg/ml rifampicin.

Results: Salmonella spp. and *E. coli* O157:H7 slowly declined on baby carrots by 0.07 and 0.04 log CFU/day during storage of up to 5 days at 4 and 23°C, respectively. For shredded carrots, decreases of 0.1 log CFU/day were observed at 4°C but a 1-log increase for both pathogens was observed over a 24-h period at 23°C. For lettuce samples stored at 23°C, both *E. coli* O157:H7 and *Salmonella* spp. increased by 0.4 and 1.2 log CFU/g in 8 and 24 h, respectively.

Significance: While *E. coli* O157:H7 and *Salmonella* can grow on lettuce and shredded carrots and significant increases were only observed after 8 h.

P3-09 Microbial Quality of Bagged Baby Spinach and Romaine Lettuce – Effects of Top Versus Bottom Sampling

JULIE KASE, Stacey Borenstein and Peter Feng
FDA-CFSAN, ORS-DM, College Park, MD, USA

Introduction: Bagged Ready-to-Eat leafy greens offer consumers a convenient means of increasing produce consumption. However, outbreaks of *Escherichia coli* O157:H7 and *Salmonella* have called into question the safety and the microbial quality of such products.

Purpose: To expand and follow up on findings of high mean total bacteria counts (MTBC) and coliform counts; that there are great variations in counts among lots; that *E. coli* is present and also that there are variations in counts when bags are top- or bottom-sampled.

Methods: Nearly 100 bags of baby spinach and hearts of romaine lettuce from a single brand were purchased at local grocery stores and subjected to both top and bottom sampling. Fifty g of product was blended with 450 mL of buffered peptone water, serially diluted, and plated in duplicate on trypticase soy agar to obtain total bacterial counts. Coliform and *E. coli* levels were estimated with the MPN method using ColiComplete discs.

Results: Total bacteria counts varied widely between lots and in same lot samples. Baby spinach (48 bags from 13 different lots) top sampled -MTBC ranged from (CFU/g) 5.9 to 9.8 log and bottom sampled - MTBC ranged from 5.9 to 10.5 log; with 77% of the lots producing MTBC higher in bottom samples. For hearts of romaine (47 bags from 19 different lots), top samples had MTBC ranging from 4.7 to 9.1 log and bottom samples had MTBC from 5.1 to 9.3 log with 58% showing higher MTBC in bottom samples. But, overall, the average difference between top and bottom samples for both spinach and hearts of romaine was nearly identical (0.57 and 0.55 log CFU/g, respectively). No *E. coli* was detected in any sample and coliform bacterial counts were, with few exceptions, 210 MPN/g or greater irrespective of MTBC.

Significance: In either product, total bacterial counts in individual bottom samples were not consistently or predictably higher with the age of product, but there was also a noted absence of moisture condensation in the bags, suggesting proper product handling and storage. Unlike previous studies, samples contained no generic *E. coli* but had coliforms. However, the levels of coliform bacteria did not correlate with total bacterial counts.

P3-10 Survival and Growth of *Salmonella* Saintpaul in Fresh Mexican Salsa Stored at Room Temperature

ELAINE BLACK, Courtney Kirkland, Michael J. Sadowsky and Francisco Diez-Gonzalez
University of Minnesota, Dept. of Food Science and Nutrition, St. Paul, MN, USA

Introduction: The 2008 large salmonellosis outbreak caused by contaminated jalapeño and serrano peppers raised serious concerns about fresh produce safety. Outbreak investigations indicated that some cases were linked to the consumption of fresh salsa, prepared with raw peppers in Mexican-style restaurants.

Poster

Purpose: The aim of this study was to characterize the survival and growth of *Salmonella* Saintpaul in freshly-made salsa and in its main ingredients stored at room temperature.

Methods: Chopped tomatoes, jalapeño peppers, cilantro, and onions were tested individually or mixed according to 4 different salsa recipes. Ingredients or salsa mixtures were inoculated with 10^3 CFU/g *Salmonella* Saintpaul, Typhimurium, Montevideo, Newport or Enteritidis and stored at room temperature (23°C) for 12 h or 3 days. *Salmonella* was enumerated by spread plating periodically on XLD agar incubated at 37°C for 24 h.

Results: After 3 days at 23°C, the count of *S.* Saintpaul reached 10^9 CFU/g in tomatoes, jalapeño peppers and cilantro. *S.* Saintpaul grew at a slower rate in onions but attained 10^6 CFU/g by day 3. Simple salsa recipes, that included those ingredients with or without lime juice, also supported the growth of *S.* Saintpaul and the final count was approximately 10^8 CFU/g following 3 days. Within 12 h after inoculation there was limited *Salmonella* growth and salsa recipes containing at least 10% lime juice prevented growth at 23°C. The counts of *Salmonella* Typhimurium, Montevideo, Newport and Enteritidis did not noticeably increase in salsas containing 10% lime juice during an incubation of 12 h.

Significance: These findings suggest that *Salmonella* in Mexican-style salsas at room temperature is capable of growing, but its growth occurs largely after first 12 h. Due to the ability of lime juice to minimize growth, this study suggests that careful formulation can reduce the risk of growth of *Salmonella* in fresh unrefrigerated salsa.

P3-11 The Safety and Quality of Washed and Peeled Potatoes of Various Shapes during Storage

KIM JUHUI, Kim SuJin, Sun ShihHui, Kim RuWon and Yoon KiSun
Kyung Hee University, Food and Nutrition, Seoul, Republic of Korea

Introduction: The demand for washed, fresh-cut potato products is significantly increasing in both the retail and foodservice markets. Consumers are placing importance on the quality of fresh-cut potatoes when making purchasing decisions. Therefore, the assurance of safety and quality of fresh-cut potatoes is needed in retail markets.

Purpose: The purpose of this study was to evaluate the safety and quality of fresh-cut potatoes after washing and during refrigerated storage, dependent on the manner of cutting.

Methods: Washed and peeled potatoes obtained from industry were cut into cubes ($2.0 \times 2.0 \times 1.0$ cm^3) or julienned ($0.45 \times 0.45 \times 3.0$ cm^3) with a cutting machine. Twenty-five grams of the cubed or julienned potatoes were packed into vacuum packing pouches and were stored at 4º for 12 days. The microbiological qualities of the cubed or julienned potatoes were measured by the plate counting method. Water activity and peroxidase and polyphenol oxidase activities were measured with a water activity measuring instrument and through absorbance by an ELISA reader, respectively. Color changes were determined using Hunter L-, a-, and b-values at intervals of three days.

Results: There were no significant differences in the total bacterial counts of the potatoes according to the manner of cutting. The initial contamination levels of aerobic microbes for the diced and julienned potatoes were 4.0 log CFU/g and 3.9 log CFU/g, respectively. Water activity and L-values decreased after 12 days, and the enzyme activities of peroxidase and polyphenol oxidase gradually increased up to 6 days of storage and thereafter decreased and browning appeared.

Significance: These data suggest that effective quality control measures are needed for washed and peeled potatoes of various shapes to extend their shelf-life during distribution.

P3-12 Recovery and Fate of *Campylobacter jejuni* in Fresh Spinach Stored at Ideal and Abusive Temperatures

EVELYNE GUEVREMONT and Lisyanne Lamoureux
Food Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Hyacinthe, QC, Canada

Introduction: For a certain number of campylobacteriosis cases, the source of the contamination remains unknown. Recently, fresh vegetables have been associated with a growing number of foodborne outbreaks. The risk of contracting campylobacteriosis through the consumption of leafy vegetables is poorly known. In fact, *Campylobacter jejuni* does not grow below 35°C but is known to survive at lower temperatures.

Purpose: To assess this risk, it is necessary to evaluate with an appropriate technique the survival of the microorganism in vegetables under different temperatures.

Methods: The recovery of known quantities of *C. jejuni* from spinach using three mechanical methods (rinsing, use of a stomacher, and manual rubbing) and three culture media (peptone water, Bolton broth, and NaCl-glycine buffer) was compared. Then, 25 g of fresh spinach were inoculated with 10^6 CFU/g of *C. jejuni*, transferred into plastic bags and stored either at 4°C or 12°C for one, two, or seven days. Bacterial cells were then recovered using the most efficient technique and the enumeration was performed by direct plating. Quantification by real-time PCR was also performed to compare classical and molecular techniques.

Results: The use of a stomacher with the Bolton broth allowed the detection of more than 10^2 CFU/g of *C. jejuni* and was used to evaluate the survival rates of *C. jejuni*. The average D-value for bacteria stored at 12°C was two days compared to five days when stored at 4°C. The average level of bacterial DNA was found to be stable in time as a decrease of approximately 0.2 log CFU/g and 0.4 log CFU/g was observed after seven days of storage at 4°C and 12°C, respectively.

Significance: Significant contamination of spinach can represent a threat as the bacteria were able to survive in the usual storage time. Classical quantitative detection methods are still necessary to properly evaluate bacterial persistence.

P3-13 Impact of Post-inoculation Holding Time and Shred Size on *Escherichia coli* O157:H7 Transfer during Leafy Green Processing

ANNEMARIE L. BUCHHOLZ, Gordon R. Davidson, Paul J. Sirmeyer and Elliot T. Ryser
Michigan State University, Food Science and Human Nutrition, East Lansing, MI, USA

Introduction: Previous work has shown extensive transfer of *Escherichia coli* O157:H7 during production of fresh-cut lettuce when the product was processed 1 h after dip-inoculation. However, both product hold time after inoculation and shred size may be important factors affecting bacterial transfer.

Purpose: The goal of this study was to determine if a longer hold-time between contamination and processing or a smaller shred size would impact *E. coli* O157:H7 transfer during commercial lettuce processing.

Methods: Triplicate batches (22.7 kg) of iceberg lettuce were dip-inoculated to contain a 4-strain cocktail of avirulent, GFP-labeled, ampicillin-resistant *E. coli* O157:H7 at 10^4 CFU/g and then processed either immediately or after 24 h of storage at 4°C. Lettuce was first shredded using an Urschel TransSlicer at the medium blade and belt speed (control - normal shred size) or the highest blade and lowest belt speed to obtain finely shredded lettuce. Thereafter, the lettuce was conveyed to a flume tank, washed and then dried using a shaker table and centrifugal dryer with 19 lettuce (25 g) and 15 water (40 ml) samples collected during processing. After processing, samples were

Poster

collected from product contact surfaces (100 cm^2) on the shredder (n = 14), conveyer (n = 8), flume tank (n = 11), shaker table (n = 9) and centrifuge (n = 8) using Kimwipes®. All sample homogenates prepared in phosphate buffer were quantitatively examined for *E. coli* O157:H7 by membrane filtration or direct plating with trypticase soy agar containing 0.6% (w/v) yeast extract and 100 ppm ampicillin used as the growth medium.

Results: Before processing, control, fine-shred and 24 h-held lettuce contained *E. coli* O157:H7 populations of 4.06, 3.87 and 3.72 log CFU/g, respectively. Overall, a significantly ($P < 0.05$) higher percentage of the inoculum was shed from control (95.81%) and fine-shred lettuce (92.10%) compared to 24 h-held lettuce (55.33%). This same trend was observed after centrifugal drying with 8.13, 12.46 and 42.18% of *E. coli* O157:H7 respectively remaining on control, fine-shred and 24 h-held lettuce. For control, fine-shred and 24 h-held lettuce, only 0.02 to 1.39%, 0.04 to 1.59% and 0.04 to 0.50% of the original inoculum transferred to the equipment surfaces with none of these differences being significant ($P > 0.05$).

Significance: Based on these findings, shred size does not appear to affect the numbers of *E. coli* O157:H7 transferred from contaminated leafy greens during processing. However, extending the time between contamination and processing will decrease the removal and transfer of this pathogen during production of fresh-cut leafy greens.

P3-14 Microflora of Fresh Produce

GUODONG ZHANG, Nhi Vo and Keith A. Lampel
Food and Drug Administration, DM-ORS-CFSAN, College Park, MD, USA

Introduction: Indigenous microflora on fresh produce play an important role in the quality and safety of these foods. Better knowledge of their composition and population level would benefit risk assessment, help develop strategies for produce storage and safety enhancement, and facilitate method development for isolation and detection of foodborne pathogens from produce.

Purpose: To estimate the population composition and level of microflora of twelve commonly consumed produce from different grocery stores.

Methods: Aerobic plate counts of lettuce, spinach, cilantro, parsley, green onion, baby carrots, regular carrots, green bell peppers, jalapeno peppers, serrano peppers, cucumber, and round red tomatoes purchased from three grocery stores were estimated on plate count agar. About 30 colonies from each produce commodity were identified using BIOLOG GEN III Microbial ID System.

Results: Average aerobic plate counts were 6.72 ± 0.73, 8.04 ± 0.31, 7.75 ± 0.63, 7.18 ± 0.67, and 7.26 ± 0.64 log_{10} CFU/g lettuce, spinach, cilantro, parsley and green onion, respectively; 7.20 ± 0.50, 8.56 ± 0.39, 7.75 ± 1.07, 7.48 ± 0.54, 8.44 ± 0.82, 8.22 ± 1.00 and 7.72 ± 0.53 log_{10} CFU/each baby carrot, regular carrot, green bell pepper, jalapeno pepper, serrano pepper, cucumber, and round red tomato, respectively. Twenty three genera, including *Acinetobacter, Arthrobacter, Bacillus, Brevundimonas, Buttiauxella, Chryseobacterium, Curtobacterium, Enterobacter, Exiguobacterium, Flavimonas, Flavobacterium, Microbacterium, Paenibacillus, Pantoea, Pectobacterium, Pseudomonas, Rahnella, Rhizobium, Riemerella, Rothia, Staphylococcus, Stenotrophomonas,* and *Xanthomonas*, were isolated and identified from the vegetables.

Significance: Indigenous microbial cell populations of the vegetables studied were high and very diverse. *Pseudomonas* spp. was isolated from all vegetables. Green onion, jalapeno pepper and baby carrots had a more diversified microflora compared with other vegetables studied.

P3-15 Microbiological Profile of Bell Peppers Produced by Hydroponics in Greenhouses in Mexico

DULCE AVILA-VEGA, Beatriz Alvarez-Mayorga, Ramon Martinez-Peniche, Eduardo F. Escartin and Montserrat Hernandez Iturriaga
Universidad Autonoma de Queretaro, Food Science/Chemistry Faculty, Queretaro, Mexico

Introduction: Greenhouse production of bell pepper is becoming very popular. Greenhouses, as a closed environment, minimize microbial hazards by providing a higher level of protection to the microbial safety of the product. The scenario is further improved by the introduction of hydroponics, which eliminates the use of agricultural soil. However, there is lack of information regarding the microbial content during the production of bell pepper at greenhouses.

Purpose: The present study was performed to obtain information on the bacteriological quality of hydroponically grown bell pepper in a highly technified greenhouse located in Mexico.

Methods: Aerobic plate count (APC), coliforms, molds, and *Escherichia coli* were quantified on harvested bell pepper (279), conveyor belts (71), pepper transportation wagons (10), knives (10), coconut fiber (20), drippers (20), nutrient solution (44), and the greenhouse's air (108); *Listeria monocytogenes* and *Salmonella* spp. were determined for all samples except in nutrient solution.

Results: On bell pepper the populations of APC, coliforms, molds were 6.0, 3.8, and 5.9 log CFU/pepper, respectively. *E. coli*, *L. monocytogenes* or *Salmonella* was not detected. On the surfaces and equipment (conveyor belts, cars, knifes, drippers) the content of APC, coliforms and molds ranged from 3.4 to 6.0, 2.9 to 5.5 and 3.8 to 5.2 log CFU/100 cm^2, respectively. The mean level for APC in nutrient solution was 6.5 log CFU/100 ml, whereas coliforms showed values of 1.5 log CFU/100 ml. *E. coli* was detected in 9 samples (mean value MPN 0.057 /100 ml). The greenhouse's air quality was good, showing values of 1.4 and 2.0 log CFU/100 L for APC and molds, respectively. *L. monocytogenes* was not isolated from any sample whereas *Salmonella* spp. was only detected on the surface of one of the conveyor belts at the packing area.

Significance: Results indicate that the bacteriological quality of the bell pepper sampled in this study was acceptable. Prevention of fecal contamination in the greenhouse environment must be stressed.

P3-16 Analysis of the Microbiological Quality of Fruits and Vegetables Expended in Monterrey Mexico by Rapid Technologies

Mayra A. Gomez, NORMA L. HEREDIA, Santos Garcia and Gabriela Isunza
Universidad A. De Nuevo Leon, San Nicolas, Mexico

Introduction: Mexico is a major exporter of fruit and vegetables, for many countries of the world. Although the microbiological quality of produce of Mexico is considered good, some recent outbreaks urge to monitor their quality.

Purpose: Evaluate the microbiological quality of fruits and vegetables linked to recent international outbreaks (melon, parsley, onion, tomato, jalapeño and serrano peppers) sold in the metropolitan area of Monterrey, Mexico, using new and sensitive technologies.

Methods: A total of 300 samples were collected in supermarkets and popular markets in the metropolitan area of Monterrey Mexico. Total coliforms and aerobic mesophilic microorganisms were analyzed using the TEMPO® automated system. The immunofluorescence miniVidas® system was used for the detection of *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Campylobacter jejuni/coli*. The presence of *Clostridium perfringens* and *Shigella* spp. was analyzed according to the methodology described in the BAM (FDA, USA). The quantification of molds and yeasts was carried out as specified in the Mexican legislation.

Results: According to the Mexican legislation, the maximum levels of total mesophilic microorganisms allowed in raw salad is 1.5 x 10^5 CFU/g. In this work, the samples exhibited moderate levels of contamination, generally found in permissible limits. However, some samples presented very high levels of microorganisms. The mesophilic load varied from less than 10 to > 10^7 CFU/g. In the case of coliforms, fungi and yeasts, most samples presented levels below 10^5 CFU/g, where tomato exhibited the lower levels. *C. perfringens* was isolated from six samples of parsley. *Salmonella* spp., *Campylobacter* spp., and *L. monocytogenes* were found in one sample. However, in all cases these pathogens were found in different samples. *E. coli* O157:H7 or *Shigella* sp. were not detected in any of the samples.

Significance: Most of the samples exhibited moderate levels of contamination, although several exhibited high levels of indicator microorganisms. The presence of pathogenic microorganisms was low. However, it can represent a risk to the consumer if a disinfection treatment is not applied before consumption.

P3-17 Evaluation of Factors Affecting the Recovery of *Listeria monocytogenes, Salmonella* spp. and *Escherichia coli* O157:H7 from Inoculated Avocado Hass

Cristina Orozco-Morales, Porfirio Gutierrez-Gonzalez, Cristina Martinez-Cardenas and OFELIA M. RODRIGUEZ
Universidad de Guadalajara, Farmacobiología, Guadalajara, Mexico

Introduction: Risk of superficial pathogenic bacteria contamination in avocado has been recently established. Information about incidence of pathogenic bacteria on avocado Hass is lacking. Sampling methods for microbiological analysis must be capable of recovering bacteria from rough surface fruits such as avocado.

Purpose: Evaluate factors that can be adjusted to develop a reliable method for the recovery of *Listeria monocytogenes*, *Salmonella* and *Escherichia coli* O157:H7 from inoculated avocado Hass, to be applied during incidence and disinfection studies.

Methods: Avocados were inoculated with a cocktail of *L. monocytogenes* or *Salmonella* and *E. coli* O157:H7 rifampicin-resistant strains. Sampling variables such as inoculum carrier (saline solution or trypticase soy broth), inoculum preparation (non concentrated or concentrated), inoculation technique (dip or spot), time between inoculation and sampling (20 or 30 min), type and volume of sampling diluent used (with or without surfactant and 10 or 100 mL) and sampling technique (weak or strong rubbing, swabbing or excising) were studied. Pathogen counts were carried on trypticase soy agar with rifampicin or lactose-sulfite-phenol red-rifampicin agar.

Results: Major recovery (3.8 to 6.8 log CFU/sample) of *L. monocytogenes* was achieved using concentrated inoculum with trypticase soy broth (TSB) on avocados inoculated by dip or spot, let dry for 20 min, sampled with 10 ml of diluent with or without surfactant and with either rubbing (strong or weak), swabbing or excising sampling techniques. Major recovery of *Salmonella* and *E. coli* O157:H7 was 3.66 to 5.9 and 3.13 to 5.5 CFU/sample respectively. In case of *Salmonella* spp. and *E. coli* O157:H7 inoculum preparation did not affect recovery. *Salmonella* recovery was not affected by drying time, but weak rubbing was more effective, whereas *E. coli* recovery was not affected by strong or weak rubbing.

Significance: Factors affecting recovery of bacteria from avocado surface are specific from each type of pathogen.

P3-18 Recovery and Survival of Murine Norovirus on Inoculated Lettuce and Tomato Salad Mix

TZU-YUN CHANG and Carol Shieh
IIT/National Center for Food Safety and Technology, Summit-Argo, IL, USA

Introduction: Ready-to-Eat foods such as salad mixes may be contaminated with noroviruses if prepared under unsanitary conditions, improper handling, or by ill workers. To study norovirus contamination during vegetable salad preparation, we have used murine norovirus (MNV) as a surrogate for human norovirus.

Purpose: Research objectives are to (1) develop protocols to efficiently recover noroviruses from contaminated salad mix, and (2) examine viral survival during inoculation and elution process.

Methods: Various eluents with different pH were evaluated for the efficiency of eluting MNV from different produce (lettuce leaves, whole and sliced tomatoes, as well as salad mixes from both ingredients). The protocol to elute MNV from salad mix was optimized. MNV w as quantified by qRT-PCR assay and plaque assay using Raw 264.7 cells grown on 6-well microplates.

Results: In evaluating various eluents with pH of 7.5, 7.8, 8.5, and 9 for the recovery of MNV from sliced tomatoes, the pH 8.5 eluent showed the highest recoveries by infectivity; however, all CT values by qRT-PCR were similar among eluents compared. Beef extract (BE, 3%, pH 8) eluted greater amount of viruses from whole tomatoes than minimum essential media (MEM, containing 3% serum, pH 7.5), shown similarly by both infectivity and qRT-PCR. Additionally, we observed that MNV was susceptible to drying process of 20 min after the inoculation of 15 µl virus stock on tomato, plastic bag and centrifuge tube surfaces, determined by infectivity. A protocol was finalized for recovering noroviruses from lettuce-based salad mixes containing whole or sliced tomatoes (10-g lettuce and 5-g tomato). Average recovery from inoculated salad mix was approximately 37% by infectivity; however, greater recoveries were observed frequently when qRT-PCR was utilized.

Significance: The developed virus elution protocol will be used to study norovirus cross-contamination during vegetable salad preparation. MNV recoveries derived by both infectivity and molecular assays will facilitate our projection of human norovirus recovery from contaminated produce, in which no infectious assay (other than human-feeding) is readily available for human norovirus detection.

P3-19 Isolation and Detection of *Listeria monocytogenes, Escherichia coli* O157:H7, Non-O157 Shiga Toxin-producing *E. coli* and *Salmonella* from Produce Fields

LAURA K. STRAWN, Esther D. Fortes, Elizabeth A. Bihn, Randy W. Worobo and Martin Wiedmann
Cornell University, Food Science, Ithaca, NY, USA

Introduction: A number of recent produce outbreaks have been traced back to the primary production environment. However, prevalence, persistence, and transmission of foodborne pathogens on produce farms are relatively unknown.

Purpose: To address this need, a field study was conducted in New York State to determine prevalence, persistence, and diversity of *Listeria monocytogenes, E. coli* O157:H7, non-O157 shiga toxin-producing *E. coli* and *Salmonella* on produce farms. These four pathogens combined account for approximately 1,100 out of the 1,800 deaths associated with known foodborne pathogens in the US.

Poster

Methods: A total of 4 area drag swabs, 5 pooled soil samples, all farm water sources and any fecal material located on site were collected June to December 2009 from five produce farms ranging from 10 to 1,600 acres. Samples were processed based on specific protocols for each pathogen consisting of a nonselective pre-enrichment, selective enrichment and plating onto selective and differential media. Up to ten presumptive positive colonies were selected and confirmed with PCR and sequence analysis.

Results: *E. coli* O157:H7, non-O157 shiga toxin-producing *E. coli* and *Salmonella* were recovered infrequently from produce fields as evidenced by 0.79%, 2.36% and 0.79% positive samples, respectively. *L. monocytogenes* was recovered from 17.3% of samples indicating a high *L. monocytogenes* prevalence. On one farm, multiple samples tested positive for the same *L. monocytogenes* lineage I subtype throughout the 6 months, indicating persistence of *L. monocytogenes* in the produce farm environment. Samples with the highest percentage of *L. monocytogenes* positives were from fecal material (64.3%) and water sources (18.2%). The same *L. monocytogenes* lineage I subtype was recovered in both fecal material and water sources on one farm.

Significance: These data demonstrate the potential for foodborne pathogens to be present in the primary production environment and are an initial estimate of persistence and prevalence of select human foodborne pathogens in produce farm environments.

P3-20 Isolation of *Listeria monocytogenes, Salmonella, Escherichia coli* O157:H7 and STEC from Pristine Environments in New York State

TRAVIS CHAPIN, Rachel Pfuntner, Esther Fortes, Kendra Nightingale and Martin Wiedmann
Cornell University, Food Science, Ithaca, NY, USA

Introduction: While considerable prevalence and molecular subtype data exist for foodborne pathogens isolated from human clinical cases, and food and farm environments, limited data exist for isolates obtained from natural, non-food associated environments.

Purpose: Therefore, the purposes of this study were to assess the prevalence of *Listeria monocytogenes*, *Salmonella*, *E. coli* O157:H7, and non-O157 Shiga toxin-producing *E. coli* (STEC) in non-food associated environments, specifically in pristine environments, and to collect isolates for subsequent subtype characterization.

Methods: Five samples each of wildlife feces, soil, and water as well as one drag swab were collected per area for three areas within five pristine environments across New York State during each spring, summer and fall of 2009. Soil, as well as water samples, were pooled by area. Each sample was non-selectively enriched for (i) *Listeria* and (ii) *E. coli* and *Salmonella*; samples were subsequently selectively enriched and plated on selective and differential media to isolate and detect *L. monocytogenes, Salmonella, E. coli* O157 and non-O157 STEC. Presumptive positives were confirmed by PCR. A total of 360 samples, including 225 wildlife fecal samples and 45 each of soil, water, and drag swab samples were microbiologically analyzed to isolate and detect the four target pathogens.

Results: Of the total samples, none were positive for *E. coli* O157:H7, 0.3% (1/360) were positive for STEC, 1.1% (4/360) were positive for *Salmonella*, and 10.3% (37/360) were positive for *L. monocytogenes*. Of 225 fecal samples, 16.4% (37) were positive for *L. monocytogenes*.

Significance: These data demonstrate the potential for the four target pathogens to exist in natural, non-food associated environments and is an initial estimate of *Salmonella, E. coli* O157:H7, and STEC prevalence in pristine environments. Additionally, *Listeria, Salmonella,* and *E. coli* subtypes collected from pristine environments will be available to populate the publicly accessible Pathogen Tracker database.

P3-21 Microbiological Analysis for Application of GAP System to APC of Hydroponics Paprika

Kyeongyeol Kim, ROK-WON R. HEO, Adelard B. A. Mtenga, Won-Bo W. Shim and Duck-Hwa D. Chung
Gyeongsang National University, Division of Applied Life Science, Jinju, Republic of Korea

Introduction: Foodborne outbreaks causing risk to human health has been frequently reported due to the increased consumption of fresh agricultural products contaminated with bacteria.

Purpose: The objective of this study was to identify risk factors which may contribute to cross-contamination of foodborne pathogens in agricultural products processing center(APC) to establish the GAP system of paprika.

Methods: The samples for microbiological assessment were collected from sort processing steps (entrance, roller, scale, sorter shelf, packing shelf and box), plant (paprika), personal hygiene (worker's hand, clothes and gloves) and airborne bacteria at APC for paprika located in Jinju. The samples were used to analyze sanitary indications [total plate count(TPC), coliform and *Escherichia coli*], foodborne pathogens (*Escherichia coli* O157, *Staphylococcus aureus, Bacillus cereus*, *Salmonella* spp. and *Listeria monocytogenes*) and fungi. PCR and API kit were used for the confirmation of *E. coli* and foodborne pathogens.

Results: Contamination levels of TPC and coliform in the samples were 1.2-5.4 and 1.0-2.8 log CFU/hand or 100 cm^2. Especially, worker's hand and glove as an indicator of personal hygiene showed the highest contamination levels for TPC(5.4 and 4.2 log CFU/ hand or 100 cm^2) and coliform (1.6-2.8 log CFU/hand or 100 cm^2). Moreover, *S. aureus* was also determined in the range of 1.4-1.6 log CFU/hand or 100 cm^2 in hand and gloves, and *B. cereus* was only detected in hand. Fungi was detected at levels of 1.7-3.3 log CFU/100 cm^2 or g or hand. TPC (1.2 log CFU/plate), *B. cereus* (0.1 log CFU/plate) and fungi (1.0 log CFU/plate) from the inside of APC were determinated by airborne bacteria sampling. However, *E. coli* and foodborne pathogens(*E. coli* O157, *Salmonella* spp., *L. monocytogenes*) were not detected in the all samples.

Significance: These results suggest that total safety system, GAP, including the management of microorganism is needed to ensure the safety of paprika from food pathogens.

P3-22 Microbiological Safety Assessment of the Soybean Postharvest Facility for the Application of GAP System

KYEONGYEOL KIM, Rok-Won R. Heo, Won-Bo W. Shim, Duck-Hwa D. Chung and Adelard B. A. Mtenga
Gyeongsang National University, Division of Applied Life Science, Jinju, Republic of Korea

Introduction: Soybean is an important economic crop in the world as a major source of protein, polyunsaturated fats and other nutrients. However, it has the potential to contaminate with microorganisms such as pathogens, fungi, due to improper postharvest management.

Purpose: Objectives of this study were to investigate microbial risk factors in postharvest facility of soybean and to provide the information of microbial contamination.

Methods: Samples for microbial analysis were collected from processing steps (entrance, selection, dry, storage), personal hygiene (hand, glove, cloth), tools (tent, sack, plastic box) and airborne bacteria (storage, dryer) at postharvest facility of soybean located in Korea. The samples were assessed for sanitary indications [aerobic plate count (APC), coliform, *Escherichia coli*], foodborne pathogens (*E. coli* O157, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella*) and fungi. Especially, *E. coli* and pathogens were confirmed with PCR and API kits.

Results: As a result, APCs and coliform bacterial populations in the all samples were detected at levels of 3.0~5.9 and 1.5~2.9 log CFU/ hand, 100 cm^2. Especially, dryer's floor, worker hand, cloth and sack showed the highest contamination for APC (5.0~ 5.9 log CFU/ hand, 100 cm^2) and coliform (2.2 ~ 2.9 log CFU/ hand, 100 cm^2). In case of pathogens, *B. cereus* was determined in the range of 1.1~2.0 logs CFU/ hand, 100 cm^2 in almost all the sample, and *S. aureus* (1.2 log CFU/ hand) was only detected in hand. Moreover, airborne bacteria in storage and dryer were detected with APC (2.4 and 1.7 log CFU/plate), *B. cereus* (0.2 and 0.1 log CFU/plate), fungi (2.1 and 2.4 log CFU/plate). While *E. coli*, *E. coli* O157, *L. monocytogenes* and *Salmonella* were not detected anywhere.

Significance: In conclusion, postharvest facility should apply GAP system based on HACCP system to minimize the microbial risk, and it is necessary to periodic monitoring for microorganism contamination and education for the safe production of soybean.

P3-23 Influence of Constituents of Water, Soil or Manure on Adherence of *Escherichia coli* O157:H7 to Plant Tissue

SEUNGWOOK SEO and Karl R. Matthews
Rutgers University, Food Science, New Brunswick, NJ, USA

Introduction: Many foodborne outbreaks caused by *Escherichia coli* O157:H7 have been associated with consumption of fresh produce. Potential contamination sources include untreated manure, irrigation water, runoff water from livestock facilities, and human and animal feces. The exposure of *E. coli* O157:H7 to nutrient conditions may influence their ability to attach to plant tissues.

Purpose: The objective of this study was to determine the influence of constituents of water, soil, or manure on adherence of *E. coli* O157:H7 to plant tissue.

Methods: E. coli O157:H7 inoculated into water, soil, or manure was evaluated on day 0, 3, 7, 14, and 21 post-exposure for adherence to plant tissue. Lettuce and spinach tissue (3 × 3 cm) were immersed for 3 min at room temperature in bacterial inoculums (10^7 CFU *E. coli* O157:H7/ml) that were collected at pre-determined days post-exposure from water, soil, or manure. Following two rinses in sterile distilled water, the number of bacteria remaining associated with the plant tissue was determined by plating on TSA/amp.

Results: A slightly greater number of *E. coli* O157:H7 cells exposed to manure attached to lettuce tissue compared to cells exposed to water or soil. Populations of adhered cells to lettuce tissue for manure, water, and soil were 5.22, 4.84, and 4.99 log CFU/g, respectively. The highest numbers of adhered cells (5.95 log CFU/g) on lettuce tissue were observed on the 7th day of post-exposure to manure. There were no significant differences in *E. coli* O157:H7 population adhered to spinach tissue following exposure to water, soil, or manure. For each medium adherence to spinach tissue was greater for cells collected on days 7 and 14 post-exposure.

Significance: This study suggests that adherence of *E. coli* O157:H7 to plant tissue was influenced by exposure of the pathogen to water, soil, or manure. Understanding physiological or genetic changes that may occur could lead to methods that enhance the microbial safety of produce.

P3-24 The Effect of Varying Carbon-to-Nitrogen (C:N) Ratios on Pathogen Elimination in Unturned Dairy Manure-based Compost Heaps

MARION W. SHEPHERD, Jinkyung Kim, Marilyn C. Erickson, Michael P. Doyle and Xiuping Jiang
Clemson University, Dept. of Biological Sciences, Clemson, SC, USA

Introduction: Escherichia coli O157:H7 and *Salmonella* spp., commonly found in dairy manure, have been linked to foodborne disease outbreaks related to fresh produce consumption. Used as soil amendments, the microbial safety of manure-based composts needs to be ensured.

Purpose: The purpose of this study was to determine how pathogen inactivation during dairy-manure composting is influenced by the initial C:N ratios of compost mixtures.

Methods: In two two-month trials, the survival of *E. coli* O157:H7 B6914 and *Salmonella* Typhimurium 8243 was monitored when composting unturned dairy manure-based heaps targeted at initial C:N ratios of < 20, 25 and > 30:1, under field conditions. Compost samples, on the surface and interior locations of the heaps, were analyzed at selected intervals to determine populations of *E. coli* O157:H7, *Salmonella* and *Enterobacteriaceae*.

Results: In the winter trial, internal compost temperatures did not reach 55°C in any of the heaps. Neither *E. coli* O157:H7 nor *Salmonella* were detected through enrichment after day 14 inside the 25 and >30:1 heaps, whereas in the < 20:1 heap *Salmonella* and *E. coli* O157:H7 were non-detectable after 21 and 30 days, respectively. In the summer trial, temperatures at the bottom of the 25 and > 30:1 heaps reached at least 55°C for 6 and 9 days, respectively, within the first two weeks of composting. *E. coli* O157:H7 was not detected inside the > 30:1 heap after day 7, and was last detected in the < 20 and 25:1 heaps on day 21. *Salmonella* was not detected in the < 20, 25 and > 30:1 heaps after days 14, 3 and 1 of composting, respectively. Importantly, in both trials, *E. coli* O157:H7 and *Salmonella* were detected after 60 days at the surface of all heaps.

Significance: The data suggests that the higher initial C:N ratios of the compost, the more rapid pathogen inactivation occurs in unturned heaps under field conditions.

P3-25 Persistence of *Escherichia coli* on Strawberries after Irrigation: Field Experiment

MYLÈNE GÉNÉREUX and Caroline Côté
IRDA, St-Hyacinthe, QC, Canada

Introduction: Surface water used for crop irrigation can be contaminated by potential human pathogenic microorganisms. Product safety is influenced by many parameters including crop, irrigation method, water source as well as the delay between irrigation and harvest. Because of the possible short irrigation-harvest delays and the tendency to consume them raw, strawberries could represent an increased risk of microbial contamination.

Purpose: The purpose of this study was to determine the persistence of *Escherichia coli* on strawberries using two irrigation methods (spray and drip) and two different mulch types (straw and plastic).

Methods: Field experiment was conducted in the province of Quebec (Canada) in 2008 and 2009. A split-plot design was established including 4 repetitions with the irrigation method as the whole plot factor and the mulch as the subplot factor. Strawberries were sampled in all plots before irrigation as well as 1 h, 4 h and 1 day after irrigation. Samples were also taken 2 and 6 days after irrigation in some plots. Water samples were taken during irrigation and a composite sample of 10 strawberries was taken in each plot. *E. coli* populations in water were determined using the mTEC modified membrane filtration method whereas cut strawberry samples were processed using both direct plating on Petrifilms count plates and enrichment with the Colilert medium. A generalized linear mixed model using GLIMMIX SAS procedure and contrasts were performed to evaluate the impact of mulch and irrigation method on *E. coli* prevalence.

Results: *E. coli* populations in irrigation water ranged from 573 to 1,853 CFU/100 mL but bacteria were not detected in any sample of strawberries using the Petrifilms method. However, some samples were positive using the enrichment procedure. Statistical analysis showed a significant impact of mulch on *E. coli* prevalence ($P = 0.0392$). *E. coli* prevalence was 5.75% and 1.69% for straw and plastic mulches, respectively.

Significance: This study highlights the limited persistence of *E. coli* on strawberry crops irrigated with water containing up to 1,800 CFU/100 mL. Fruit pH and morphology as well as dessication and UV exposition could be parameters explaining these results.

P3-26 Assessment of Irrigation Water Quality and Vegetative Filter Strip Soil Quality Using Indicator Organisms

SEAN BECKMAN and Karen Killinger
Washington State University, School of Food Science, Pullman, WA, USA

Introduction: With recent produce outbreaks being linked to contaminated water supplies, understanding agricultural management practices to enhance water quality is important.

Purpose: A two-year study was conducted to assess water quality of an irrigation ditch and soil microbial levels in a vegetative filter strip near a cattle feedlot for water quality improvement.

Methods: Fourteen water sampling sites were selected along an irrigation ditch. A vegetative filter strip near a cattle feedlot and adjacent to two water collection sites was examined for soil quality. Two transects (TE and TM) were sampled at 3 locations at two depths (surface and 10 cm). Sampling periods (13 total) occurred between April-November over 2 years. Water and soil samples were quantified for indicator organisms using a five tube most probable technique. Washington Department of Ecology (WADOE) utilizes a water quality standard for secondary contact recreation water of 2.3 log CFU/100 ml fecal coliforms and the Leafy Greens Marketing Agreement recommends <2.37 log MPN/100 ml generic *E. coli* for foliar irrigation application and <2.76 log MPN/100 ml for non-foliar application.

Results: A seasonal trend in fecal coliforms was observed in Year 2 for water sampling sites, with an increase in April (2.7 average log MPN/100 mL) through July (4.6 average log MPN/100 mL) and a slight decrease in August (4.3 average log MPN/100 mL); however, in Year 1, variation between sites was observed. For fecal coliforms, six sites in Year 1 and eleven sites in Year 2 failed to meet the WADOE standard. In Year 1, sites met the LGMA foliar application standard 12.5-100% of the sampling periods and 50-100% for non-foliar application; in Year 2, sites met the LGMA standards more often, 40-100% for foliar application and 60-100% non-foliar application. In Year 2, 78.6% of the sites failed to meet the WADOE fecal coliform standard, yet 40-100% of the sites met the LGMA generic *E. coli* standard. In both sampling years, indicator organisms were higher in TE than TM. Fecal coliforms in TE ranged from 1.2-4.8 log MPN/100 g while TM ranged from 0.3-2.3 log MPN/100 g. Generic *E. coli* at TE ranged from 0.3-1.5 log MPN/100 g and for TM ranged from 0.3-0.86 log MPN/100 g. Within each transect, ranges of fecal coliforms and generic *E. coli* were lower in Year 2 compared to Year 1.

Significance: The type of indicator organism selected and standard utilized has significant ramifications on evaluation of bacterial water quality. Soil indicator organism levels within a vegetative filter strip can vary greatly depending on sampling location.

P3-27 Bacterial Water Quality Assessment of an Irrigation Water Delivery and Drainage System in the Lower Yakima Valley of Washington

KATHERINE WARREN, Elaine S. Brouillard and Karen M. Killinger
Washington State University, Food Science, Pullman, WA, USA

Introduction: Irrigation delivery and drainage systems are an important component of produce production and represent a potential source of foodborne pathogens.

Purpose: A canal and drainage system was selected based on historical concerns for a targeted bacterial water quality evaluation.

Methods: Sixteen sites along an irrigation canal, five drains, and a central drain leading to a river were sampled over two irrigation seasons (May-September) with 12 total sampling periods. Fecal coliforms and generic *E. coli* were examined using a five-tube most probable number technique. Washington Department of Ecology (WADOE) utilizes a water quality standard for secondary contact recreation water of 2.3 log CFU/100 ml fecal coliforms and the Leafy Greens Marketing agreement (LGMA) recommends <2.37 log CFU/100 ml generic *E. coli* for foliar irrigation application and <2.76 log CFU/100 ml for non-foliar application.

Results: For the canal sites (sites 5, and 16), fecal coliforms ranged between 0.3-4.2 log CFU/100 ml and generic *E. coli* ranged between 0.3-3.9 log CFU/100 ml. For the central drain locations (sites 1, 3, 6) fecal coliform ranges were higher (3.1-5.5 log CFU/100 ml) compared to canal sites, whereas generic *E. coli* ranges were similar (0.3-3.5 log CFU/100 ml) for canal and central drain sites. Both canal sites met the WADOE fecal coliform standard 25% of the sampling periods, whereas none of the central drain sites met the standard. For the canal sites, site 5 met the LGMA standard for foliar and non-foliar application for all sampling dates, while 83% of site 16 samples met the foliar application standard and 92% met the non-foliar application standard. For the central drain locations, 50% of site 1 samples (closest to the river) met the LGMA standard for foliar application and 58% were acceptable for non-foliar application. Interestingly, 66% and 92% of site 3 samples met the foliar standard and non-foliar standards, whereas the drain site further upstream, site 6, only met the LGMA standards for both foliar and non-foliar application 50% of the sampling periods.

Significance: Canal water samples were not always acceptable in water quality based on WADOE and LGMA standards, and the quality of water observed in the central drain was significantly lower, emphasizing the potential for pathogen contamination of irrigated crops in the agricultural area.

P3-28 Agronomic Practices That Alter Spinach Leaf Morphology, Surface Topography and Exudates, Increase *Escherichia coli* O157:H7 Associated Risks

EDUARDO GUTIÉRREZ-RODRÍGUEZ, Judy Jernstedt, Heiner Lieth and Trevor Suslow
University of California Davis, Plant Science, Davis, CA, USA

Introduction: While pathogenic bacteria on produce have been comprehensively studied, many questions remain unresolved, including the impacts of various agronomic practices and environmental conditions on the phyllosphere as a habitat for human pathogens. We previously showed that N fertilization in spinach affects cuticle properties, leaf intercellular spaces, and nutrient availability thereby influencing plant:*E.coli* O157:H7 interactions.

Purpose: The objective of our study was to further describe how N fertilization, light quality and leaf phenology influence growth and spatial distribution of *E. coli* O157:H7.

Poster

Methods: Greenhouse and field spinach were cultivated with various N treatments; light intensity was manipulated under greenhouse conditions. Preharvest inoculations were made by spraying two attenuated strains of *E. coli* O157:H7 at log 0.3 and 0.56 CFU/m^2. Postharvest inoculations were by immersion using these isolates or strain PTVS 081(clinical isolate; lettuce outbreak) at log 2 CFU/ml. Recovery from individual leaves was performed with and without disinfection with 1% $AgNO_3$. Detection of *E. coli* O157:H7 below the limit of plating detection was by Assurance GDS-O157™.

Results: Nitrogen fertilization altered the morphology and topography of spinach. Reduced light also modified these attributes, producing thinner leaves with reduced leaf toughness when compared to normal light conditions; enhancing pathogen colonization by 1.3 CFU/54.4 cm^2 ($P < 0.05$). Plants from the high N treatment, were on average internalized by the pathogen 4-fold ($P < 0.05$) more than those from the low N treatment. Stomata in all N treatments were variably colonized by *E. coli* O157:H7; however hydathodes were consistently colonized. Higher residual pathogen populations were recovered in plants from all N treatments and low light quality after disinfection, when compared to normal light conditions (>50% vs. 23.6% ($P < 0.05$) respectively).

Significance: Our findings indicate that excessive N fertilization and reduced light quality in spinach, leads to conditions that modify the morphology and chemical characteristics of leaves, increasing growth and internalization of *E. coli* O157:H7.

P3-29 Survival of *Salmonella* on Spinach Leaves Treated with Contaminated Irrigation Water

JITU PATEL and Katherine Darlington
ARS, US Dept of Agriculture, Beltsville, MD, USA

Introduction: Fresh produce may be contaminated with *Salmonella* during on-farm contact with contaminated water. Transmission of *Salmonella* from contaminated irrigation water to spinach plants in growth chamber settings requires further evaluation.

Purpose: The purpose of this study was to evaluate the efficacy of the California Leafy Green Marketing Agreement (LGMA) establishing water quality criteria for irrigation water applied to spinach plants.

Methods: A green fluorescent protein-labeled *Salmonella* Typhimurium was cultivated in water extracts of dairy manure solids. Spinach cultivar "Whale" was grown in pasteurized organic soil in a BL-2 growth chamber (14 h day 21°C, 10 h night 16°C, 50% RH). The individual three-week old spinach plants were spray-inoculated every two weeks with ~ 5 ml water containing 130 (low) or 13,000 (high) *Salmonella* per 100 ml. Plants were also irrigated with potable water every week to maintain plant turgor. Four replicates of each plant shoot were analyzed on day one and weekly for up to six weeks for *Salmonella* populations by direct plating on XLT4 agar and MPN (enrichment in BPW followed by selective enrichment in tetrathionate broth and subsequent spot plating on XLT4 agar).

Results: Salmonella were undetectable (detection limit < 1.1 MPN/g) on spinach leaves after one day or after six weeks when irrigation water was contaminated at low or high levels. A single irrigation event with very high levels of *Salmonella* contaminated water (8 log CFU/100 ml) resulted in survival of ~4.5 log *Salmonella* MPN/plant after one day. *Salmonella* persisted on spinach leaves for at least two weeks when plants were irrigated at a very high level.

Significance: Results may aid in understanding the role of irrigation water in *Salmonella* contamination on spinach leaves. The persistence of *Salmonella* should be studied at the field levels to determine the efficacy of California LGMA Irrigation water standards.

P3-30 Examination of Pathogen Presence and Indicator Organism Levels in Soil and Lettuce in an Organic Farming System Field Experiment

CALEB JAMES, Achyut Adhikari, Andy Bary, Craig Cogger and Karen Killinger
Washington State University, Food Science, Pullman, WA, USA

Introduction: Produce foodborne outbreaks have emphasized the need to understand sources of pathogen introduction in farming systems.

Purpose: A two-year study examined pathogen presence and indicator organism levels in soil and lettuce crops in an organic farming system field experiment.

Methods: Soils were sampled on 14 dates from vegetable plots receiving mixed-feedstock compost, including dairy manure and broiler litter, or broiler litter compost (4 plots each), and from the pasture phase of a grazed pasture-vegetable rotation (8 plots) that had not received recent soil amendments. Lettuce was sampled twice annually at harvest; 4 crop plots (2 mixed feedstock, 2 broiler litter) were examined (30 lettuce heads per plot). Soil and lettuce were examined for fecal coliforms, generic *Escherichia coli* (*E. coli*) and the presence of *E. coli* O157:H7 and *Salmonella*.

Results: For soil samples, fecal coliforms averaged 2.8 log MPN/g over all dates and plots, whereas generic *E. coli* levels in pasture plots (0.9-1.3 log MPN/g) were higher than in crop plots (0.5-0.6 log MPN/g). Pasture plots yielded more pathogen positive soil samples (13%) compared to crop plots (7% mixed feedstock, 5% broiler litter). In the soil, pathogens were detected at mean fecal coliform and generic *E. coli* levels of 2.67 and 0.89 log MPN/g, respectively. For lettuce samples in Year 1, fecal coliforms ranged between 5.17-6.2 log MPN/g for both harvest dates, but in Year 2, fecal coliforms were higher in Harvest 2. For generic *E. coli* levels in lettuce in Year 1-Harvest 1, one mixed feedstock plot had elevated generic *E. coli* (5.2 log MPN/g) compared to the other 3 plots (1.3-2.1 log MPN/g); in the second harvest, 3 plots exhibited generic *E. coli* levels greater than 4.6 log MPN/g. In Year 2, a smaller increase in generic *E. coli* was observed between harvests. In Year 1, *E. coli* O157:H7 was detected in 4.5% of lettuce during each harvest (in mixed feedstock and broiler litter plots in Harvest 1; only mixed feedstock plots in Harvest 2). In Year 2, *E. coli* O157:H7 was observed once (1.1%) on lettuce from a mixed feedstock plot. In lettuce, pathogens were detected at mean fecal coliform and generic *E. coli* levels of 5.39 and 3.46 log MPN/g, respectively.

Significance: Pathogens were detected in soil samples with lower average indicator organism levels (2.67 log MPN/g fecal coliforms and 0.89 log MPN/g generic *E. coli*) than in pathogen positive lettuce samples (5.39 log MPN/g fecal coliforms and 3.46 log MPN/g generic *E. coli*). Even though pathogen presence in the crop soil plots was low (5-7%), *E. coli* O157:H7 positive lettuce samples were observed.

Poster

P3-31 *Salmonella* Enteritidis Cross-contamination onto Mango by Means of Contaminated Knives

ANA L. PENTEADO and Maria F. Castro
Embrapa-CTAA, Food Microbiology, Rio de Janeiro, Brazil

Introduction: The possible transfer of *Salmonella Enteritidis* (SE) onto cut mango (*Mangifera indica* Linn.) due to contaminated knives may occur depending on the level of contamination of this utensil, but this hypothesis has not yet been demonstrated.

Purpose: To determine the cross contamination of SE onto mango by using a contaminated knife with different levels of inocula.

Methods: Fruits without any defects (peel ruptures, bruised areas) were used in the experiment. Mangos were aseptically surface disinfected with an alcoholic solution of iodine (BAM-FDA) and left 20 minutes in an air flow cabinet. Knives were previously inoculated on both sides with a final bacterial population of 10^6, 10^4 and 10^3 CFU in 100 µl of peptone water (0,1%), and left to dry inside the air cabinet. These contaminated knives were used to cut the mango. The cut pieces were placed on HE and XLD plates, with the cut side facing the agar surface, moved back and forth and then discarded. The occurrence of SE on each mango piece, following incubation at 35°C/24 h, was then determined by counting the colonies on HE and XLD. Bacterial colonies were confirmed by culture on TSI and LIA, and then biochemically

confirmed using API 20E (bioMérieux). Three repetitions for each essay were carried out. After cutting the mango, each knife was washed in 100ml peptone water, and 1ml of this water was pour plated in TSA. The plates were incubated at 35°C for 24 h followed by counting, with the results being expressed in CFU/g.

Results: Detection of SE in cut mango was observed for knives inoculated at 10^6 and 10^4 CFU/ml with this microorganism. Knife level contamination of 10^3 did not show any growth of SE on the cut fruit. Analysis of the washed knives did not show any colonies of SE.

Significance: The study indicates that the inside of a mango could be contaminated with *S.* Enteritidis during slicing by using a contaminated knife. Precautions should be taken in handling mango to minimize such contamination from the surface to the interior of the foods by using a cutting knife. Since SE can survive on the mango pulp even at lower temperatures, good manufacturing practices should be applied for manipulation of this fruit.

P3-32 *Salmonella* Transfer Potential during Hand Harvesting of Tomatoes

PARDEEPINDER K. BRAR and Michelle D. Danyluk
University of Florida, Food Science and Human Nutrition, Gainesville, FL, USA

Introduction: Tomatoes have been linked to numerous outbreaks of *Salmonella*, however the route of *Salmonella* contamination often remains unknown.

Purpose: The objective of this study is to determine *Salmonella* transfer coefficients (TCs) between gloves and mature green, round, staked tomatoes during harvesting. Three *Salmonella* transfer scenarios were studied: a) from gloves to tomatoes; b) from tomatoes to gloves and c) from glove to ten subsequently touched tomatoes.

Methods: A five-strain cocktail of Rifampicin resistant *Salmonella* (6 log CFU/surface) was spot inoculated onto gloves or tomatoes. Two types of gloves (single-use and reusable) were evaluated. Uninoculated tomatoes or gloves were touched, for no longer than 5 s, to the inoculated gloves or tomatoes immediately and following a 1-h drying period. Following stomaching (gloves) or rubbing (tomatoes), samples were enumerated on non-selective and selective agar supplemented with Rifampicin. Ten tomatoes were subsequently touched to an inoculated glove, to simulate commercial picking, and enumerated as described above. TCs were calculated by dividing the population of *Salmonella* on the touched surface (CFU/surface) by the inoculated surface (CFU/surface).

Results: When tomatoes were touched to gloves immediately or following 1-h drying, TCs are 0.31 ± 0.15 and 0.29 ± 0.16 for single-use gloves and 0.24 ± 0.06 and 0.44 ± 0.15 for reusable gloves, respectively. When gloves were touched to inoculated tomatoes immediately or following 1 h drying, TCs of 0.32 ± 0.17 and 0.35 ± 0.20 were calculated for single-use gloves and 0.19 ± 0.05 and 0.36 ± 0.23 for reusable gloves, respectively. *Salmonella* was isolated from all 10 tomatoes subsequently touched with both glove types, immediately and after 1 h of inoculum drying. In all scenarios, TCs were less than 0.01 for tomatoes 6-10.

Significance: *Salmonella* has the potential to transfer between contaminated gloves or tomatoes during harvest and cross-contaminate subsequently picked tomatoes.

P3-33 Effect of Laser Labeling and *Pectobacterium carotovorum* Subsp. *carotovorum* on the Survival of *Salmonella* spp. on Tomato Surfaces

LORETTA M. FRIEDRICH, Lenin O. Villeda, Keith R. Schneider, Edgardo J. Etxeberria and Michelle D. Danyluk
University of Florida, CREC, Lake Alfred, FL, USA

Introduction: Laser labeling produce is an alternative to adhesive labels using low energy carbon dioxide laser-beam generated pinhole depressions to etch information onto produce. It is not known if this process influences food safety risks.

Purpose: The objectives of this study were to evaluate the impact of laser labeling and different post harvest treatments on the fate of *Salmonella* on tomatoes; and examine the effects of co-inoculation with *Pectobacterium carotovorum* subsp. *Carotovorum* (soft rot organism) on the fate of *Salmonella* on laser-etched tomato surfaces.

Methods: Mature green, round, tomatoes were laser labeled using an energy level of 0.678 W per character at 35 µs with a duty cycle range of 25%, spot inoculated with a five strain cocktail of Rifampicin-resistant *Salmonella* and/or one strain of Nalidixic Acid-resistant *P. carotovorum* and swabbed with commercial wax. Ten different sequences of laser label, inoculum, and wax application were evaluated. Samples were stored for up to 28 days at 4, 12, or 25°C. Bacteria were enumerated following a rub-shake-rub of tomatoes on tryptic soy agar supplemented with Rifampicin for recovery of *Salmonella* or Nalidixic Acid for recovery of *P. carotovorum*. The effect of each scenario on bacterial populations was determined using ANOVA.

Results: There were no significant differences ($P < 0.05$) between populations of *Salmonella* on laser labeled or unlabeled tomatoes stored at 4, 12, or 25°C. *Salmonella* held at 4°C decreased ca. 2 log CFU/tomato over 28 days, while those held at 12 and 25°C for 28 and 14 days, respectively, remained stable (ca. 6.5 log CFU/tomato). The addition of *P. carotovorum* at higher, lower, or equal population densities' to *Salmonella* did not significantly alter the behavior of *Salmonella* on tomatoes stored at 25°C, regardless of laser labeling.

Significance: Laser labeling tomatoes does not adversely affect the tomato surface with respect to pathogen survival, even in the presence of soft-rot pathogens and offers industry a permanent, safe alternative to traditional adhesive labeling.

P3-34 Disinfection of *Salmonella* spp. on Tomato Surface by Pulsed Ultraviolet Light and Selected Sanitizers

Leonard Williams and OLASUNMBO AJAYI
Alabama A&M University, Food and Animal Sciences, Normal, AL, USA

Introduction: Sanitizers are currently being used to reduce microbial loads on fresh produce in the industry as well as at homes. However, sanitizers do not completely eradicate microorganisms in every treatment. Some bacteria survive the usage of sanitizers because of improper dosage, washing procedure or other factors. Therefore, alternative non-thermal methods for inactivating microbes on the surface produce need to be explored.

Purpose: The objectives of this study were to examine the efficacy of selected sanitizers, when used alone or in combination with PUV, on the inactivation of *Salmonella* spp. on the surface of whole tomatoes.

Methods: The efficacy of three sanitizers (10 mL/L peracetic acid, 10 mL/L hydrogen peroxide and 20 mL/L commercial disinfectant) for inactivating *Salmonella* spp. inoculated onto the surface of whole tomatoes stored up to 8 days was investigated when they were used alone or in combination with pulsed ultraviolet light (PUV).

Results: 10 mL/L peracetic acid alone resulted in an average 3 $\log_{10}$ reductions throughout the 8-day storage. Ten mL/L hydrogen peroxide and 20 mL/L commercial disinfectant alone yielded 1.41 and 1.29 $\log_{10}$ reductions, respectively, on day 0, but from day 2 to day 8 of storage their disinfecting effect dwindled, with no significant difference detected from control (water rinse) on day 8 ($P \leq 0.05$). The 60s PUV treatments alone achieved less than 2 $\log_{10}$ reductions, but when it was combined with 10 mL/L hydrogen peroxide, the bacterial reduction significantly increased to over 4 $\log_{10}$ during the 8-day storage ($P \leq 0.05$). Similarly, PUV also enabled 20 mL/L commercial disinfectant to have a dwindled bactericidal effect throughout the 8-day storage period.

Significance: Results from this study showed that sanitizers combined with PUV radiation could generate a significant and lasting inactivation of *Salmonella* spp. on the surface of tomatoes.

P3-35 Thermal Inactivation of *Escherichia coli* O157 in Fresh Compost by Simulating Field Composting Process

RANDHIR SINGH, Jinkyung Kim, Xiuping Jiang and Feng Luo
Clemson University, Microbiology, Clemson, SC, USA

Introduction: Thermophilic phase during composting is critical for inactivation/killing of pathogens in animal wastes. However, the persistence and survival of pathogens during composting has been reported.

Purpose: To study thermal inactivation of low nutrient-adapted (LN) *Escherichia coli* O157:H7 in fresh dairy compost by simulating field composting process.

Methods: A three-strain mixture of *E. coli* O157:H7 grown in 1:10 tryptose soya broth (TSB) for low nutrient adaptation was inoculated into the fresh dairy compost (ca. 10^7 CFU/g) with 40 and 50% moisture. The culture grown in full strength of TSB served as control. Compost packed in pouches was placed in an environmental chamber (ca. 70% humidity) programmed to ramp from room temperature to selected composting temperatures (50, 55 and 60°C) in 2 days to simulate the early composting phase. The surviving population was analyzed by direct plating or enrichment at predetermined time.

Results: During 2 days of come-up time at early stage of composting, the population of *E. coli* O157 declined gradually. Afterward, *E. coli* O157:H7 survived for 72, 48 and 24 h in compost with 40% moisture, and 72, 24 and 24 h with 50% moisture at 50, 55 and 60°C, respectively, for both control and LN-adapted cultures. Overall, *E. coli* O157:H7 was inactivated faster in the compost with 50% moisture than the compost with 40% at 55 and 60°C. One day before the end of the 2-day come-up period, control *E. coli* O157 survived better ($P < 0.05$) in compost with 40% and 50% moisture than the LN adapted culture at 50 and 60°C. There was a difference ($P < 0.05$) in thermal resistance at 60°C between control and LN-adapted cultures for compost with 40 and 50% moisture but not at 50 or 55°C.

Significance: Our results suggest that slow come-up time can extend the survival of pathogen during composting. Additionally, both the physiological stage of cultures and the compost moisture level affect the rate of pathogen inactivation as well.

P3-36 The Effect of Wash Water Quality on the Decontamination Efficacy of Spinach under Commercial Processing Conditions

Maria J. Barrera and KEITH WARRINER
University of Guelph, Guelph, ON, Canada

Introduction: Leafy green vegetables have been implicated in at least 35 major foodborne illness outbreaks over the last decade. In large scale outbreaks, the source of contamination is invariably introduced at the primary production stage making post-harvest washing a significant intervention in the fresh produce chain. However, under commercial conditions it is possible for wash water to accumulate contamination and result in dissemination to subsequent product batches.

Purpose: The following study was directed towards identifying parameters that impact on the efficacy to decontaminate spinach and the potential for cross-contamination in the course of washing in two commercial processing facilities.

Methods: Wash water was sampled, in addition to pre- and post-wash leafy greens, at different time points during the processing period (n = 6 sampling points). The pH, conductivity, temperature, Oxidation Reduction Potential (ORP), heterotrophic plate count (HPC) and *E. coli*/coliform count of the wash water was determined. The temperature, in addition to aerobic colony count (ACC) and *E. coli*/coliform counts of pre- and post wash spinach samples were quantified. The log count reduction was calculated and correlated to the various measured parameters.

Results: Spinach processed in Facility A had a significantly higher bacterial loading (3.1 — 6.8 log CFU/g) at pre-wash, in addition to greater HPC and *E. coli*/coliform counts associated with the wash water compared to Facility B. However, there was no significant difference observed in the LCR achieved between both facilities (Facility A -0.2 to 1.24 log CFU/g; Facility B -0.26 to 1.49 log CFU/g) although higher residual counts on spinach processed in Facility A was observed. The ACC loading and conductivity (solids content) of the water positively correlated to the LCR. The LCR was negatively correlated to spinach temperature at pre-wash (high produce temperatures leading to lower LCR). *E. coli* were sporadically recovered from spinach processed in Facility A. Evidence was obtained to support the hypothesis that *E. coli* present on pre-wash spinach contaminated wash water and acted to disseminate the bacterium to proceeding produce batches.

Significance: In conclusion, it was identified that washing of leafy greens was more effective when produce/water temperature was low, ensuring low microbial loading on incoming material and applying an ORP based system to maintain hypochlorite levels in water. In addition, wash water should be changed in the course of processing to minimize opportunities for cross-contamination.

P3-37 Improving Pathogen Reduction by Chlorine Wash Prior to Cutting in Fresh-cut Processing

XIANGWU NOU and Yaguang Luo
USDA-ARS, BARC-EMFSL, Beltsville, MD, USA

Introduction: Currently, most fresh-cut processing facilities in the United States use chlorinated water or other sanitizer solutions for microbial reduction after lettuce is cut. Freshly-cut lettuce releases significant amounts of organic matter that negatively impacts the effectiveness of chlorine or other sanitizers for microbial reduction.

Purpose: The objective of this study is to evaluate whether shifting the primary chlorine wash intervention to pre- cutting stage can significantly improve microbial reduction efficacy compared to traditional post-cutting sanitizer washing.

Methods: Romaine lettuce was inoculated with *Escherichia coli* O157:H7 strains and subjected to sanitizing washes in chlorinated water prior to or after cutting. Survival and transference of *E. coli* O157:H7 strains on lettuce shreds were determined by selective plating and MPN procedures.

Results: Chlorine wash of cut lettuce reduced *E. coli* O157:H7 count by approximately 1.5 log units. Reversal of the cutting-washing sequence resulted in an addition one log unit reduction of *E. coli* O157:H7 counts. High level of *E. coli* O157:H7 transference was observed when inoculated and non-inoculated lettuce leaves were co-processed following the traditional cutting and washing sequence. This cross contamination was significantly reduced when chlorine wash was performed prior to cutting.

Significance: Sanitizer wash performed at the whole-leaf stage significantly enhanced the efficacy of chlorine for pathogen reduction and cross-contamination prevention.

P3-38 Use of Caprylic Acid and Monocaprylin as Novel Antimicrobials for Reducing *Escherichia coli* O157:H7 and *Salmonella* spp. on Alfalfa Seed

Su-sen Chang, MAURICIO A. REDONDO-SOLANO and Harshavardhan Thippareddi
University of Nebraska-Lincoln, Dept. of Food Science and Technology, Lincoln, NE, USA

Introduction: Raw alfalfa seeds have been implicated in *Escherichia coli* O157:H7 and *Salmonella* spp. outbreaks worldwide. Continuous issues with alfalfa seeds necessitate the need for novel, effective antimicrobials to be investigated.

Purpose: The potential use of caprylic acid (CA) and monocaprylin (MC) for reducing *E. coli* O157:H7 and *Salmonella* spp. on alfalfa seeds was evaluated.

Methods: Three concentrations of CA and MC (25, 50, and 75 mM) were used. Planktonic and alfalfa surface inoculated *E. coli* O157:H7 and *Salmonella* spp. were treated for up to 12 h and 90 min, respectively, and survival populations of each pathogen was enumerated using tryptic soy agar (TSA). Non-inoculated seeds were soaked for up to 120 min to evaluate the effect of CA and MC on seed germination.

Results: For planktonic cells, the efficacy of the treatments were: 75 MC>50 MC>25 MC>75 CA>50 CA>25 CA. Both *E. coli* O157:H7 and *Salmonella* spp. were reduced to below the detection limit (0.6 log CFU/ml) within 10 min exposure to 75 MC. When applied to inoculated alfalfa seeds, maximum reductions of 1.56 ± 0.25 log CFU/g and 2.56 ± 0.17 log CFU/g for *E. coli* O157:H7 and *Salmonella* spp., respectively, were achieved when treated with 75 MC for 90 min. Germination rates of CA or MC treated seeds ranged from 84%—99%. No significant differences ($P > 0.05$) between germination rates of CA or MC soaked seeds and water soaked seeds (control) were observed up to a soaking time of 90 min.

Significance: This study indicates that soaking of sprouting seeds in solutions containing 75 mM MC for 90 min can reduce *E. coli* O157:H7 and *Salmonella* spp. on alfalfa seeds without compromising seed germination.

P3-39 Inactivation of Peroxyacetic Acid-injured *Escherichia coli* O157:H7 Cells on Baby Spinach Using Low-energy X-ray Irradiation

SCOTT R. MOOSEKIAN and Elliot T. Ryser
Michigan State University, Food Science and Human Nutrition, East Lansing, MI, USA

Introduction: In response to recent *Escherichia coli* O157:H7 outbreaks involving leafy greens, the FDA approved the use of irradiation at doses up to 4 kGy for baby spinach and iceberg lettuce. While considered a safe process, the efficacy of irradiation against bacterial pathogens on commercially sanitized leafy greens is poorly understood.

Purpose: This study examined the potential for peroxyacetic acid-injured cells of *E. coli* O157:H7 to develop enhanced resistance to X-ray irradiation on baby spinach.

Methods: Three *E. coli* O157:H7 strains - K3995 (2006 spinach outbreak), K4830 (2006 lettuce outbreak A), and K4492 (2006 lettuce outbreak B) were grown in trypticase soy broth containing 0.6% (w/v) yeast extract (TSB-YE), combined in equal volumes, pelleted by centrifugation, resuspended in phosphate buffer solution (PBS) to ~9.68 log CFU/ml and exposed to 5.1 mg/L peroxyacetic acid-based sanitizer (PABS) (Tsunami 100, Ecolab, St. Paul, MN) to obtain 89 to 95% injury. The injured cocktail was then pelleted by centrifugation and resuspended in PBS to obtain a population of 7.3 to 8.9 log CFU/ml. Prior to inoculation, round-cut (5.07 cm^2) baby spinach leaves were pre-irradiated at a dose of 0.167 kGy to reduce background microflora. Three replicates of 15 leaves each were irradiated on each side for combined doses of 0.012, 0.028, 0.043, 0.054 and 0.063 kGy using a prototype low-energy X-ray irradiator (Rayfresh Foods, Ann Arbor, MI). The irradiation dose was confirmed using radiochromic film dosimeters. Numbers of *E. coli* O157:H7 in homogenized samples were determined by plating appropriate dilutions on Sorbitol MacConkey Agar (SMAC) and SMAC overlayed with TSA-YE for recovery of healthy and healthy plus sublethally injured cells, respectively.

Results: When the samples were irradiated 30 minutes after inoculation, percent injury significantly decreased ($P < 0.05$) from 89 to 95 to 44 to 90%. D_{10}-values for the PABS-injured and non-injured *E. coli* O157:H7 cocktail on baby spinach were 0.0188 and 0.0264 kGy, respectively, with prior exposure to PABS significantly ($P < 0.05$) enhancing the susceptibility of *E. coli* O157:H7 to X-ray irradiation. Regardless of the irradiation dose, no appreciable injury was seen in the healthy control with percent injury remaining unchanged ($P < 0.05$) for the PABS-injured cells after irradiation.

Significance: These results suggest that this novel X-ray technology can be used without hesitation for processing leafy greens since PABS-injured cells of *E. coli* O157:H7 did not develop any increased resistance to irradiation.

P3-40 Plant Extracts as an Alternative for *Salmonella* Decontamination of Cilantro

NYDIA A. ORUE, Norma L. Heredia and Santos Garcia
Universidad A. De Nuevo Leon, San Nicolas, Mexico

Introduction: Cilantro has been identified as a vehicle of transmission of *Salmonella* and other pathogens. Several outbreaks associated with consumption of contaminated cilantro have been documented. Since this product is regularly consumed, alternative strategies for decontamination of cilantro are required.

Purpose: The objective of this study was to determine the efficacy of plant extracts as alternatives of disinfection of cilantro artificially contaminated with *Salmonella* Typhimurium.

Methods: Extracts from plants (20 g) using ethanol (100 ml) as the extraction solvent were prepared. A micromethod was used to determine the minimal bactericidal concentration (MBC). Plant extracts exhibiting the lowest MBC were selected for further studies. Leaves of cilantro (60 g) were washed with sterile water and dried for 2 h, after that, the leaves were submerged in 60 ml of a suspension of *S.* Typhimurium (adjusted to 10^8 CFU/ml) for 10 min. The samples were then kept at room temperature for 2 h and finally stored at 4°C for 22 h. After this, cilantro leaves were submerged in a container and subjected to different treatments: 200 ppm chlorine, 200 ppm Citrol® or selected plant extracts (at their MBC concentration) for 20 min. Sterile distilled water was used as control. After these treatments, cilantro leaves were stored at 4°C, and bacterial counts were determined by plate count at 0, 1, 3, 5 and 7 days using XLD agar.

Results: Two extracts of edible plants exhibiting MBC for *S.* Typhimurium of 8.16 mg/ml and 6.42 mg/ml were selected for the analysis. These extracts were as effective as chlorine or Citrol® to reduce 2 log the population of *Salmonella* in the cilantro leaves.

Significance: The two extracts from edible plants could represent a good natural and edible alternative to reduce the risk of *Salmonella* contamination of cilantro.

P3-41 Inactivation of *Salmonella* on Roma Tomatoes by High-concentration-short-time Chlorine Dioxide Gas Treatments

VALENTINA TRINETTA, Mark T. Morgan and Richard H. Linton
Purdue University, Food Science Dept., West Lafayette, IN, USA

Introduction: *Salmonella* outbreaks have been recently linked to the consumption of tomatoes. It is necessary to develop technologies that: a) reduce the risk of contamination of both pathogenic and spoilage organisms, b) increase product shelf-life, c) keep fresh fruit attributes and d) do not compromise consumers' health. Chlorine dioxide gas is an innovative technology for microbial inactivation and microbial risk reduction.

Purpose: Evaluate effects of high-concentration-short-time chlorine dioxide gas treatments on *Salmonella*-inoculated Roma tomatoes and determine optimal treatment conditions for microbial inactivation.

Methods: Effects of ClO_2 concentration, $XClO_2$, (2, 5, 8, 10 mg/l) and exposure time, Xtime, (10, 30, 60, 120 and 180 s) on inoculated *Salmonella* tomatoes were studied. Tomatoes were spot inoculated with a cocktail of *Salmonella enterica* strains (serotype Montevideo, Javiana and Baildon). At the end of each treatment, surviving *Salmonella* population was determined. After data analysis, three conditions were selected for further study, based on highest microbial reduction achieved. Fresh tomatoes were treated at these conditions and stored at room temperature for 28 days. Microbial spoilage population, change in color and chlorine dioxide residuals were evaluated.

Results: Data analysis by Multiple Linear Regression showed that both factors and interaction are important for *Salmonella* inactivation on Roma tomatoes ($P < 0.001$); the linear model, Remaining population$_{Salmonella}$ = 6.2333 − 0.2486β_{ClO2}-0.0077 β_{time} − 0.0005 β_{time}*β_{ClO2}. Log reduction = -0.016 × ClO_2 -0.012 × time + 5.94, was highly significant ($P < 0.001$ and $R^2 = 0.92$). Contour plots were generated and the adequacy of the model was confirmed. Results showed that high log reduction was obtained at the following conditions: 8 mg/l ClO_2 for 60 s, 3-log reduction; 10 mg/l ClO_2 for 120 s, 4-log reduction; 10 mg/l for 180 s, 5-log reduction. These treatments significantly ($P < 0.05$) reduced the initial microflora population, while fruit color (for all the storage period) and residual contents (by day 1) were not significantly different ($P < 0.05$), as compared to the control.

Significance: Results suggests the potential for high-concentration-short-time chlorine dioxide gas treatments as an effective on-line pathogen inactivation technology for large-scale produce packing operations.

P3-42 Inhibition of *Escherichia coli* O157:H7 and *Clostridium sporogenes* in Spinach Packaged in Modified Atmospheres after Treatment with Chlorine and Lactic Acid Bacteria

ALISON L. BROWN, Enusha Karunasena, Chance Brooks, Alejandro Echeverry and Mindy Brashears
Texas Tech University, Animal and Food Science, Lubbock, TX, USA

Introduction: Implementation of modified atmosphere packaging (MAP) into retail produce has lagged due to potential changes in product quality and the concern over anaerobic pathogens. *Clostridium botulinum* is found ubiquitously throughout the environment and can easily contaminate spinach through soil, water or fertilizer. Additionally, *Escherichia coli* O157:H7 can contaminate spinach through identical routes and has recently been responsible for multiple spinach outbreaks across the United States.

Purpose: To determine if MAP, chlorine, and lactic acid bacteria (LAB) combined as hurdle technology inhibits pathogens in fresh spinach.

Methods: Spinach was co-inoculated with *E. coli* O157:H7 and *Clostridium sporogenes*, a surrogate for *C. botulinum*, and treated either with a water rinse or a hurdle intervention that included water, chlorine, and LAB rinses at a level of 1.46×10^8. Spinach from each treatment was then packaged in air (traditional overwrap), oxygen (80% O_2, 20% CO_2) or nitrogen (80% N_2, 20% CO_2) and stored in a retail display case for 9 days at 4-7°C. Spinach was sampled for pathogen inhibition and LAB survivability on day 0, 1, 3, 6, and 9.

Results: The hurdle intervention demonstrated greater inhibition of both *E. coli* O157:H7 and *C. sporogenes* when compared to the control by reducing numbers by 1.43 and 1.10 logs, respectively ($P < 0.05$). The nitrogen atmosphere was outperformed by the air and oxygen atmospheres in the reduction of *E. coli* O157:H7 ($P < 0.05$). All three atmospheres did not affect *C. sporogenes* growth.

Significance: The steady survival of LAB in the hurdle intervention and reduction of pathogen growth demonstrates that implementation into the produce industry for the control of pathogens may improve the safety of the product.

P3-43 Naturally Occurring Bacteriocinogenic Lactic Acid Bacteria as Bioprotective Cultures to Enhance the Safety of Sprouts

GUOPING FENG, John J. Churey and Randy W. Worobo
Cornell University, Food Science and Technology, Geneva, NY, USA

Introduction: Alfalfa sprouts are a well known food vehicle for human pathogens and have been responsible for numerous foodborne illness outbreaks. A biological control strategy using food-grade protective cultures may be promising in preventing pathogens from rapidly growing to high levels during sprouting. Unlike single-step treatments with chemical sanitizers such as calcium hypochlorite which may allow survival of pathogens trapped inside of the seeds and thus rapid growth during subsequent sprouting process, protective cultures provide a sustainable antimicrobial effect during the whole process of sprouting by acid production, bacteriocin production, and competitive colonization for growth.

Purpose: The purpose of the study was to investigate the effect of naturally occurring lactic acid bacteria on growth of sprout-associated pathogens during sprouting.

Methods: Enterococcus mundtii CUGF08 and *Lactococcus lactis* AA4 are two bacteriocinogenic lactic acid bacteria (LAB) isolated from sprouts. *E. mundtii* CUGF08 produces mundticin L while *L. lactis* AA4 produces nisin Z. These two lactic acid bacteria were inoculated, singly or in combination, to alfalfa seeds which were pre-inoculated with different levels of sprout-associated pathogens *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli* O157:H7. A five strain cocktail for each pathogen was used for pre-inoculation of the seeds. The growth curves of *S. enterica, L. monocytogenes* and *E. coli* O157:H7 as well as *E. mundtii* CUGF08 and *L. lactis* AA4 during sprouting of alfalfa seeds were monitored by plating on selective media. Experiments were performed in triplicate.

Results: Both protective cultures significantly ($P < 0.05$) controlled the growth of all pathogens tested. In general, *L. lactis* AA4, singly or in combination with *E. mundtii* CUGF08, showed significantly higher efficacy in controlling the growth of pathogens than single inoculation with *E. mundtii* CUGF08.

Significance: The study shows that bioprotective cultures are an alternative strategy, or an additional hurdle in combination with other decontamination methods to enhance the safety of sprouts.

P3-44 Use of High Hydrostatic Pressure to Eliminate *Escherichia coli* O157:H7 and *Salmonella* on Scallions (Green Onions)

HUDAA S. NEETOO, Sanaz Nekoozadeh, Zheng Jiang and Haiqiang Chen
University of Delaware, Animal and Food Sciences, Newark, DE, USA

Introduction: In recent years, the consumption of various minimally-processed fresh produce, including scallions, has increased substantially. Unfortunately, scallions have been listed as one of five commodity groups that together, make up over 75% of produce-related foodborne illnesses. This commodity has, thus, caused heightened concern and garnered scientific attention over its microbiological safety.

Purpose: The objective of this study was therefore to evaluate the potential of using high hydrostatic pressure (HHP) technology for the decontamination of scallions against *E. coli* O157:H7 and *Salmonella*.

Methods: Scallions inoculated with a cocktail of five nalidixic-acid resistant mutant strains of *E. coli* O157:H7 and *Salmonella* (ca. 5 log CFU/g) were subjected to pressures ranging from 250-400 MPa for 2 min at 20°C in a dry, pre-wet or pre-soaked state. In addition, inoculated samples were also treated at 450-550 MPa for 2 min at 20, 30 and 40°C in a pre-wet or pre-soaked state.

Results: The decontamination efficacy of HHP for either pathogen, increased in the order of soaked > wet > dry states of scallions at all pressure levels. Soaking of scallions prior to pressurization (450 MPa for 2 min at 20°C) significantly ($P < 0.05$) reduced populations of

Poster

E. coli O157:H7 (5.0 log CFU/g) and *Salmonella* (5.5 log CFU/g). The pressure-sensitivity of the enteric pathogens was also higher at elevated treatment temperatures. Processing of scallions in the pre-wet state at 450 MPa for 2 min at 40°C or in the pre-soaked state at a reduced pressure level of 400 MPa for 2 min at 40°C were found to be adequate in completely decontaminating the vegetable.

Significance: Results from this study thus highlight the promising applications of a nonthermal technology such as HHP to alleviate the risks of *E. coli* O157:H7 and *Salmonella* infections associated with consumption of scallions.

P3-45 Comparative Effectiveness of Common Commercial Levels of Chlorine and Chlorine Dioxide to Prevent *Salmonella* spp. Cross-contamination on Inoculated Fresh Cut Red Chard

ALEJANDRO TOMÁS-CALLEJAS, Francisco López-Gálvez, Adrian Sbodio, Francisco Artés, Francisco Artés-Hernández and Trevor Suslow

Technical University of Cartagena, Postharvest and Refrigeration Group, Dept. of Food Technology, Cartagena, Spain

Introduction: *Salmonella* has increasingly been identified on leafy greens in surveillance testing and in outbreaks with fresh and fresh-cut product. Raw material can arrive contaminated and sub-optimal processing steps can spread pathogens within and among lots.

Purpose: To evaluate pathogen removal and cross-contamination potential during the washing-disinfection, rinsing, and de-watering steps in a model system.

Methods: Typical industry rates of 20 ppm hypochlorite (at pH 7.0) and 3 ppm chlorine dioxide were used with baby Red chard (*Beta vulgaris* var. cicla), inoculated with *Salmonella* spp. Chard was obtained directly from an organic field in Salinas Valley, California. Leaves were dipped in a phosphate buffer with log 5.2 CFU/ml for 1 min, de-watered, and held 18 h at 15°C and RH > 95%. Leaves were marked for differentiation from non-inoculated leaves. Non-inoculated leaves were mixed with inoculated leaves (about 5% of total weight) and processed as a unit. After the wash-disinfection process, inoculated and non-inoculated leaves were analyzed separately. Enumeration was by direct plating on selective agar. Below the limit of quantitative detection BAX®-*Salmonella* was used for detection. Recovery from prewash, wash, rinse and de-watering was by filtration of 10 ml using the Neogen ISO-GRID™ system and placement of membranes on selective/differential agar.

Results: *Salmonella* on inoculated leaves, pre-wash, was 4.27 ± 0.10 log CFU/g. During the washing-disinfection step no transfer of *Salmonella* to the process water or non-inoculated leaves was detected. However, adhering water removed from inoculated leaves at de-watering had an applied *Salmonella* population of 55 CFU/10 ml and 139 CFU/10 ml for chlorine and chlorine dioxide treatments, respectively. *Salmonella* was not detected by direct plating of leaves but, after selective enrichment, all the samples were positive by PCR-BAX® for *Salmonella*.

Significance: Selected levels of chlorine and chlorine dioxide substantially prevented cross-contamination by *Salmonella* among co-mingled Red chard leaves but neither fully disinfected the pathogen from inoculated leaves.

P3-46 Electrostatically Sprayed Lactic Acid Bacteria as a Pre-harvest Intervention Strategy for Reduction of *Escherichia coli* O157:H7 on Spinach

ANGELA M. LAURY, Alejandro Echeverry, Manuel Alvarado, Sara E. Gragg, Alison Brown, Claudia A. Narvaez, Praveena Sunkara and Mindy M. Brashears

Texas Tech University, Dept. of Animal and Food Science, Lubbock, TX, USA

Introduction: Concerns of contamination of *E.coli* O157:H7 onto spinach plants during the growing cycle still remains a major concern in the food industry. Lactic acid bacteria (LAB) are known to have antagonist properties that are capable of reducing *E. coli* O157:H7 in multiple environments including the plant system.

Purpose: The objective of this study was to evaluate the effectiveness of electrostatically sprayed LAB on the reduction of *E. coli* O157:H7 in spinach plants during the growing cycle.

Methods: Spinach plants were grown in a BSL-2 growth chamber to mimic fall in central California conditions. Spinach plants were water-inoculated with *E. coli* O157:H7 at either planting or week 1, 2, 3, or 4 post-planting at 10^3 CFU and received a treatment of electrostatically sprayed LAB either at planting or week 1, 2, 3, or 4 post-planting at 10^9 CFU. Control plants received only *E. coli* O157:H7 on one of these periods for a total of 30 plants. On harvest day, samples of the leaves, soil, and entire plant were collected for each treatment combination and *E. coli* O157:H7 and LAB were enumerated on SD-39 and MRS, respectively. The experiment was replicated four times and data were analyzed using a SAS program.

Results: If *Escherichia coli* O157:H7 contaminates the spinach plant during planting and LAB is applied during the third and forth week of the growing period, the *E. coli* O157:H7 numbers are significantly lower than the control plants by as much as 1.76 log CFU/ML ($P < 0.10$). If *E. coli* O157:H7 contaminates the spinach plant during weeks one through four of the growing cycle and LAB are applied electrostatically anytime between planting and the fourth week of growing, then the *E. coli* O157:H7 levels on the plant are significantly lower at harvest when compared to the control plants by as much as 2 log CFU/ml ($P < 0.10$).

Significance: Electrostatically sprayed lactic acid bacteria is a potential intervention method that has the ability to reduce low levels of *E. coli* O157:H7 by as much as 2 log CFU during the growing cycle of spinach plants; that can be applied to other commodities.

P3-47 The Impact of Almond Moisture on the Survival of *Salmonella* Enteritidis PT30 after Exposure to Hot Oil

HARBIR KAUR and Linda J. Harris

University of California-Davis, Food Science and Technology, Davis, CA, USA

Introduction: Oil roasting experiments have been done to evaluate the survival of *Salmonella* on fresh inoculated almonds and impact of storage period on the oil roasting process of almonds. However, the impact of moisture on the heat resistance of *Salmonella* on almonds has not been evaluated.

Purpose: The objective of this study was to determine the heat resistance of *Salmonella* Enteritidis PT30 on almonds equilibrated to moisture levels from 4 to 9%.

Methods: Whole Mission and Nonpareil almonds inoculated with *Salmonella* Enteritidis PT30 (9.3 log CFU/g) were dried at 23°C for 3 days to moisture and water activity levels comparable to uninoculated almonds (4-5% moisture, 0.40-0.50 a_w). After drying, *Salmonella*-inoculated almonds were held at 23°C in the presence of saturated KI, NaCl, KCl or K_2SO_4 salts for 3 days. Almonds (10 g) were heated in 121°C hot oil for 1 min, drained for 10 s, transferred to 20 ml of cold (4°C) tryptic soy broth, and stomached for 2 min. Appropriate dilutions were plated onto tryptic soy agar and bismuth sulfite agar and incubated at 37°C for 24 and 48 h.

Poster

Results: For almonds stored under ambient (30% RH) or saturated KI (68% RH), NaCl (75% RH), KCl (80% RH), or K_2SO_4 (90% RH), moisture and water activity levels were 5.1, 6.1, 6.5, 7.1, or 8.6% and 0.48, 0.60, 0.63, 0.70, or 0.77, respectively. Corresponding reductions in the populations of *Salmonella* on the surface of these almonds were 2.2, 3.5, 4.0, 4.7, or 5.1 log CFU/g, respectively, after the hot oil treatment. No significant difference ($P > 0.05$) was observed in the reduction of *Salmonella* between Mission and Nonpareil varieties when moisture levels were the same.

Significance: Relative humidity rapidly impacts the percent moisture and water activity of almonds and can significantly impact the efficacy of a thermal treatment. Moisture levels should be monitored in thermal validation studies.

P3-48 The Impact of Dehydration on Microbial Levels of In-shell Walnuts

TYANN BLESSINGTON, Elizabeth J. Mitcham and Linda J. Harris
University of California-Davis, Food Science and Technology, Davis, CA, USA

Introduction: After harvest and wet removal of their outer hull, walnuts are dried in large bins with low-temperature (≤ 43°C) forced air to ~ 8% moisture prior to storage.

Purpose: To determine the impact of commercial and laboratory-simulated dehydration on the natural microbiota of freshly-harvested walnuts and on inoculated *Citrobacter youngae* and *Salmonella*.

Methods: Aerobic plate count, molds/yeast, *Enterobacteriaceae*, and *Escherichia coli* levels were enumerated on in-shell walnuts collected from three commercial facilities before and after dehydration (24 h). Walnuts inoculated at 7 to 8 log CFU/nut with rifampicin-resistant *Citrobacter youngae* (non-pathogenic surrogate for *Salmonella*) were dried for 24 or 48 h in a pilot-scale dehydrator set at 43°C. Walnuts were also inoculated with *Salmonella* Enteritidis PT30 at 8 and 4 log CFU/nut and dried in a laboratory oven at 43°C for 24 or 48 h. Microbial populations were enumerated by shaking walnuts in 0.1% peptone and plating onto appropriate selective media or using the Colilert Quanti-Tray/2000 MPN test (*E. coli*). Transfer of *C. youngae* from inoculated to uninoculated walnuts during dehydration was determined by enrichment in tryptic soy broth with rifampicin and plating onto tryptic soy agar with rifampicin.

Results: Aerobic plate count, molds/yeast, and *Enterobacteriaceae* levels decreased by up to 1.2 log CFU/nut after commercial dehydration; little to no reduction was observed in levels of *E. coli*. *C. youngae* declined by 1 to 3 log CFU/nut after pilot scale dehydration; declines varied by as much as 2.5 log CFU/nut in adjacent nuts. *C. youngae* was not isolated from any of 72 uninoculated walnuts. *Salmonella* was reduced by 1 log CFU/nut after drying for 48 h at 43°C.

Significance: Microbial reductions during commercial or simulated walnut dehydration were highly variable making this step an unlikely food safety control.

P3-49 Behavior of *Salmonella* in High-water Activity Pecan Nutmeats, Inedible Nut Components, and Orchard Soil

LARRY R. BEUCHAT and David A. Mann
University of Georgia, Center for Food Safety, Griffin, GA, USA

Introduction: Outbreaks of salmonellosis associated with consumption of almonds have raised interest in determining the survival and growth characteristics of *Salmonella* on other tree nuts. Pecans are exposed to high-moisture environments before harvest and during processing, yet the behavior of *Salmonella* in high-a_w pecans has not been described.

Purpose: We undertook a study with objectives to determine 1) survival and growth characteristics of *Salmonella* in high-a_w pecan nutmeats, inshell pecans, and inedible nut components; and 2) the behavior of *Salmonella* upon suspending in pecan shuck extract and inoculating pecan orchard soil saturated with extract.

Methods: Pecan nutmeats (halves, pieces, and granules) at a_w 0.96 - 0.99, inshell pecans (kernel a_w, 0.94), and water-saturated shucks (hulls), shells, and middle septum tissue (a_w 0.99) were inoculated with a five-serotype mixture of *Salmonella* and stored at 4, 21, 30, and 37°C. Samples were analyzed for the presence (by enrichment) and populations of *Salmonella* for up to 48 h, 8 days, and 6 days, respectively. Inoculated aqueous shuck extract and soil saturated with extract were held at 21 and 37°C; extract and soil were analyzed for up to 48 h and 49 days, respectively.

Results: *Salmonella* did not grow on nutmeats stored at 4°C but growth did occur at 21, 30, and 37°C. Increases of 1.77 - 5.87 log CFU/g occurred within 48 h at 37°C. The order in which nutmeats supported growth was granules > pieces > halves. *Salmonella* did not grow in inshell pecans or septum tissue but did grow on shucks and shells. Shuck extract was toxic to *Salmonella* in planktonic suspension and in saturated soil.

Significance: The ability of *Salmonella* to grow in high-a_w pecan nutmeats and inedible nut components emphasizes the importance of limiting the time of exposure of pecans to water in pre- and post-harvest environments.

P3-50 Comparing Multiple Methods for Validating Dry Air Pasteurization of Almonds

BRADLEY P. MARKS, Subrahmanya K. Kasibhatla and Michael K. James
Michigan State University, Biosystems and Agricultural Engineering, East Lansing, MI, USA

Introduction: *Salmonella* linked to low water activity food products remains a pressing concern for the industry. However, validating surface pasteurization methods for low water activity products is non-trivial, given the challenges associated with monitoring the critical surface parameters, which control the rate of pathogen inactivation.

Purpose: The objective was to quantify the variability and uncertainty associated with five different methods of thermal process validation for surface pasteurization of almonds (three based on an inactivation model applied to different time-temperature measurement methods and two based on biological testing with the target pathogen, *Salmonella* Enteritidis PT30, and an accepted surrogate, *Enterococcus faecium*).

Methods: Almonds were inoculated with *Salmonella* Enteritidis PT30 or *Pediococcus* spp. (*Enterococcus faecium*, NRRL, B-2354) to ~10^8 CFU/g, per previously published methods. The almonds (~500 g) were placed in a shallow bed on a mesh tray and then heated in a pilot-scale air impingement oven on a continuously moving conveyor. Process variables were jet velocity (~0.6 or 2.7 m/s), air temperature (121, 177 or 232°C), and target lethality (3 or 5 log), with the full factorial design conducted in triplicate. During heating, almond surface temperature was measured real-time three ways: (1) a thin-wire thermocouple affixed just below the surface of three almonds, (2) three thermocouple-instrumented aluminum almonds, and (3) a non-contact, infrared temperature sensor mounted inside the oven above the product on the belt. A previously published thermal inactivation model for *S.* Enteritidis PT30 on almonds was used to predict lethality based on the three measurement methods, and results were compared to the experimental results for the pathogen and surrogate.

Poster

Results: Repeatability and accuracy (against actual *Salmonella* lethality) varied ($P < 0.05$) among the validation methods. For example, for the 177°C results, the repeatability of the lethality outcomes (log reductions) were 0.8, 1.9, 1.1, 0.4 and 0.5 log for the *S.* Enteritidis PT30, *E. faecium*, aluminum almond, surface probe and infrared methods, respectively. The mean lethality error (deviation from the *Salmonella* outcome) was -0.4, -0.3, -3.9 and 1.6 log for the *E. faecium*, aluminum almond, surface probe, and infrared methods, respectively.

Significance: The method used to validate surface pasteurization methods is critical in determining process efficacy. The results of this study will help improve process design and operation by ensuring appropriate levels of certainty in evaluating product safety.

P3-51 Assessment of Risk of Salmonellosis from Consumption of Pistachios

ROBERT MIKSCH and Mansour Samadpour
IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA

Introduction: The FDA has recently released a proposed “Guidance for Industry: Measures to Address the Risk for Contamination by *Salmonella* Species in Food Containing a Pistachio-Derived Product as an Ingredient” which recommends use of a validated intervention process capable of delivering a 5-log reduction. This risk assessment study provides evidence that the proposed high performance thresholds may not be applicable in a low-risk product such as pistachio.

Purpose: The aim of this study was to assess the risk of salmonellosis from the consumption of pistachios using the Monte Carlo approach and to assess strategies to mitigate these risks.

Methods: Starting conditions were based on prevalence rates observed during hold and release testing of incoming pre-processing in-shell and kernel materials, and population levels of *Salmonella* enumerated in test lots for which FDA-BAM confirmed positive results were obtained. Strategies to reduce this risk were assessed including the use of microbial interventions to lower *Salmonella* load, and using pre-process raw material testing to reduce the prevalence of *Salmonella*.

Results: The assessment found that untreated, untested, pre-processing in-shell pistachios would be regarded as a moderate risk category food item on a number of cases per annum or per billion servings basis based on the definitions by the US-FDA and USDA. Pistachio kernels would be considered a high risk item on a per billion servings basis only. Use of a single or a combination of multiple validated interventions with reductive capacities of 1 log or more was found to be sufficient to produce low risk category pistachio products according to FDA/USDA definitions which are based on median (half above, half below) values. The assessment further showed that a reductive capacity of 3 log would be sufficient to ensure that at 95% probability, less than 1 illness per year would occur. Pre-process raw material testing to identify contaminated material and removing it from the processing stream was also found to be highly effective in reducing the prevalence of *Salmonella*.

Significance: This risk assessment found that, while control is clearly needed, the public health goal of producing low risk pistachio products can be met by using less stringent thresholds of performance.

P3-52 *Campylobacter* Prevalence Estimates for Swabs and Neck Flaps of Commercial Turkey Carcasses

IRENE V. WESLEY and Fernando Rivera
National Animal Disease Center, Food Safety and Enteric Disease, Ames, IA, USA

Introduction: Campylobacter is the major cause of bacterial gastroenteritis with consumption of poultry a major risk factor.

Purpose: The purpose of this study was to compare the prevalence of *Campylobacter* on three turkey carcass sites: external carcass swabs (~100 cm^2), internal thoracic cavity (300 cm^2) and excised neck skin samples (100 cm^2), which has been considered as a potential sampling site.

Methods: Samples were taken on nine occasions at a commercial abattoir by a single investigator after the final spray wash and immediately prior to carcasses entering the chiller. All samples were taken during the first shift of the week. Methods for isolation and speciation of *Campylobacter* have been previously described by the authors.

Results: Campylobacter prevalence estimates were obtained at nine sampling dates for external carcass swabs (102/275, 37.1%), internal cavity swabs (71/210, 33.8%) and neck skins (29/227, 12.78%). Although no statistically significant differences were found between either the internal cavity or external carcass swabs, neck skins yielded a significantly lower prevalence estimate ($P < 0.05$). No statistically significant differences in *Campylobacter* prevalence were found between either swabbing or homogenizing (pulsifying) the neck flap ($P > 0.05$). No differences were seen in *Campylobacter* titers when a pure culture was pulsified for up to 3 minutes indicating minimal damage to *Campylobacter* during sample processing. Because our *Campylobacter* prevalence estimates were lower than the 1997 Young Turkey Baseline post-chill estimates (~90%), ceca were also sampled as an indicator of on-farm prevalence. In the current study, the overall *Campylobacter* prevalence for ceca was 94.2% (113/120) which indicates that interventions have not significantly reduced the *Campylobacter* on-farm prevalence. In 2004 we estimated the *Campylobacter* prevalence on turkey carcasses both pre- (116/150, 77.3%) and post- (78/150, 52%) chiller at this same slaughterhouse using similar swabbing and culture enrichment protocols. HACCP strategies may explain the significant differences between the earlier 2004 (77.3%) *Campylobacter* estimates and our 2009 carcass data (37.1%).

Significance: A higher *Campylobacter* prevalence was seen when swabs of either the external surface or the internal cavity were compared with neck skin samples. This indicates that while easily accessible, neck skin samples may underestimate the *Campylobacter* prevalence. The significantly lower prevalence estimates obtained for carcass swabs when compared with ceca indicate the efficacy of post slaughter HACCP interventions.

P3-53 Survivability of *Campylobacter* spp. in Chicken Juice on Surfaces, Determined Using a Range of Sampling and Enrichment Techniques

Jeremy W. Chenu, Anthony Pavic and JULIAN M. COX
University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Introduction: Thermophilic campylobacters, such as *C. jejuni* and *C. coli*, account for the majority of human foodborne bacterial gastroenteritis worldwide. Poultry processing residues such as chicken juice are important sources of *Campylobacter* on surfaces and could therefore play an important role in cross contamination. The improvement of surface sampling techniques (e.g., 3M Enviroswab, 3M Quickswab and tampons) and sampling and enrichment media, such as 3M Tecra Campylobacter Enrichment broth (TCEB), may provide new information on the survivability of *Campylobacter* on surfaces.

Purpose: To determine the persistence of *C. jejuni* and *C. coli* in chicken juice on stainless steel and polyethylene surfaces using various sampling implements and enrichment media.

Methods: Filter-sterilized chicken juice was inoculated with 10^4 CFU/ml of *C. jejuni* or *C. coli*. A 200 µl aliquot of the cell suspension was inoculated onto 25cm^2 portions of stainless steel and polypropylene. Each surface was sampled in triplicate every 30 min for up to 3 h, with the 3M Enviroswab, 3M Quickswab, a tampon or a pre-moistened cotton swab. The first three swabs were immersed in TCEB; the cotton swab was immersed in Preston Enrichment Broth, as a reference method. *Campylobacter* was enumerated on Skirrow and Preston agars (1ml across 3 plates) and the remainder of the TCEB enrichments (with delayed addition of antimicrobial agents) used for qualitative detection with an ELISA and selective plating.

Results: Recovery of *C. jejuni* and *C. coli* was 1 log higher using the tampon and 3M Enviroswab than with the 3M Quickswab or the cotton swab. During the 3 h holding period, the inoculum dried on the surface and the *Campylobacter* population declined, though the bacterium could still be detected after enrichment.

Significance: *C. jejuni* and *C. coli* in chicken juice can survive on commercially relevant food contact surfaces even when the juice has dried. Choice of sampling implement is important when analyzing surfaces for significant pathogens. Swabs with a larger surface area and which are more absorptive are better at recovering cells from surfaces.

P3-54 Distribution of *Salmonella* in Young Chicken Carcass Rinse Samples at Post-chill and Re-hang Locations in the Nationwide Microbiological Baseline Survey

JAMES ROGERS, Harry Marks, Parmesh K. Saini, Priscilla Levine and Uday Dessai
USDA-FSIS-OPHS, MD, Washington, D.C., USA

Introduction: The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS), conducts national microbiological baseline surveys to evaluate and compare microbiological trends over time. Reported are the *Salmonella* results of the latest baseline survey for young chicken carcasses conducted during the 12-month period of July 2007 to June 2008. Unlike in the past surveys, analyses were performed on samples collected from carcasses at the Re-Hang location in addition to those collected at the Post-Chill location. An important feature of the latest baseline survey was the identification of serotypes for each positive sample.

Purpose: The purpose of this article is to present summary results of the distribution of *Salmonella* serotypes found at the Re-Hang and Post-Chill locations during the 2007/2008 broiler baseline study and explore its potential impact in risk assessments, risk-based sampling programs, and regulatory policy decisions.

Methods: Carcass rinse samples were collected and analyzed using methods in the FSIS Microbiology Laboratory Guidebook (MLG). The numbers of samples scheduled for establishments depended on the establishment-specific volumes determined during 2006.

Results: Using a 400 ml carcass rinse, the estimate for weighted volume carcass-specific prevalence (not the national prevalence estimate for chickens) of *Salmonella* was 7.5% at Post-Chill and 45.5% at Re-Hang. The non-volume adjusted data published on FSIS website indicates 8.15% positive Salmonella at Post- Chill and 45.8% at Re-Hang. Greater than 50% of the *Salmonella*, serotypes identified from positive samples were from those that have previously been linked to human illnesses. Of these, the most commonly occurring serotype isolated from Post-Chill samples was *S.* Typhimurium, consisting of 22% of all positive results; at Re-Hang the most common serotypes were *S.* Heidelberg (13.6%) and *S.* Typhimurium (13.0%). *S.* Enteritidis (SE) was detected in approximately 3% and 6% of the positive samples, respectively, at Re-Hang and Post-Chill. However, at Post-Chill there was an exceptionally large percentage (27%) of SE for samples collected from the larger establishments.

Significance: Multiple factors could be contributing to the differences and frequencies of serotypes found, which suggests that a multi-faceted approach toward control of *Salmonella* is needed.

P3-55 Examination of Chlorine, Peroxyacetic Acid, and Lactic Acid Antimicrobial Rinses in Small-scale Poultry Slaughter Operations

TERESA WHISLER, Andy Bary, Craig Cogger and Karen Killinger
Washington State University, Food Science, Pullman, WA, USA

Introduction: Many cases of foodborne illness are linked to contaminated poultry. During poultry slaughter, chlorine is commonly used to reduce microbial contamination; however, organic, small-scale poultry processors seek organic compounds such as peroxyacetic acid and lactic acid as alternatives.

Purpose: Chlorine, lactic acid, and peroxyacetic acid were investigated as antimicrobial rinses in small-scale poultry slaughter operations.

Methods: Two replications were conducted; one under small-scale poultry slaughter conditions and one in a mobile poultry slaughter operation. In each replication, twenty carcasses were individually dipped for three minutes in one of three treatment rinses (50-100ppm chlorine, 2% lactic acid, or 80ppm peroxyacetic acid). Untreated carcasses (water-sprayed) were used as controls and examined for the presence of *Salmonella* using standard methods. After treatment, each carcass was placed in a sterile bag and massaged for two minutes in buffered peptone water. Serial dilutions of the rinse solution were plated on tryptic soy agar for aerobic plate counts (APC) and incubated for 48 hours at 35°C. For total coliform (TC) enumeration, dilutions were plated on tryptic soy agar, incubated at room temperature for 2 hours to allow for injured cell recovery followed by a double strength VRBA overlay. TC plates were incubated 24 hours at 35°C. Colonies were enumerated, logarithmically transformed and analyzed.

Results: Salmonella were detected on 2.5% of untreated carcasses (1/40 carcasses). APC and TC for untreated carcasses were 4.4 log CFU/carcass and 3.7 log CFU/carcass, respectively. Lactic acid produced a significant 2 log ($P < 0.01$) reduction in APC and TC (2.4 log and 1.5 log CFU/carcass, respectively) compared to untreated carcasses. Significant ($P < 0.01$) 0.95 and 0.70 reductions were also observed in APC and TC for carcasses treated with peroxyacetic acid (3.5 log and 3.1 log CFU/carcass, respectively). Chlorine resulted in small, but significant 0.5 and 0.3 log reductions in APC and TC (3.9 and 3.4 log CFU/carcass). Lactic acid also produced significantly greater reductions in APC and TC compared to both peroxyacetic acid and chlorine. Peroxyacetic acid produced significantly greater reductions in APC and TC compared to chlorine.

Significance: In this study, 2% lactic acid was more effective than 80 ppm peroxyacetic acid or 50-100 ppm chlorine as an antimicrobial rinse for small-scale poultry slaughter operations.

P3-56 Mapping the Distribution of *Salmonella* on the Chicken Carcass

Geoffrey K. Rutto, Jacquelyn B. Ludwig, THOMAS P. OSCAR and Salina Parveen
U.S. Dept. of Agriculture, Agricultural Research Service, Princess Anne, MD, USA

Introduction: Knowledge of the distribution of *Salmonella* on the chicken carcass will improve efforts to assess and manage this risk to public health. Sampling methods such as rinsing or swabbing that do not recover all *Salmonella* on the chicken carcass are not adequate for mapping studies. A method such as whole carcass incubation that recovers all *Salmonella* on the chicken carcass is needed for mapping studies.

Purpose: The objective of this study was to map the distribution of *Salmonella* on the chicken carcass for the purpose of improving poultry inspection and chicken safety.

Methods: Young chickens (n = 70) in the Cornish game hen class were obtained at retail. The carcasses were aseptically sectioned into 12 parts. *Salmonella* were isolated from whole part incubations using standard culture methods. Isolates were characterized for serotype and antibiotic resistance and by pulsed field gel electrophoresis (PFGE).

Results: Salmonella incidence was 22% (181/840) for parts and 57% (40/70) for carcasses. Fisher's Exact test indicated differences ($P < 0.05$) in *Salmonella* incidence among parts. The number of *Salmonella* isolates per carcass ranged from 0 (n = 30) to 1 (n = 7) to 2 to 6 (n = 21) to 7 to 12 (n = 12). Within contaminated carcasses (n = 40), there were 37 different patterns of contamination among parts. Most isolates were serotype Typhimurium (94%) and most (97%) were resistant to multiple antibiotics. More than one serotype, antibiotic resistance profile and PFGE profile where observed on 12%, 33% and 100%, respectively, of carcasses with two or more contaminated parts.

Significance: Results indicate that *Salmonella* contamination is randomly distributed on the chicken carcass and that multiple subtypes of *Salmonella* are commonly present on carcasses with two or more contaminated parts. Thus, whole carcass incubation and analysis of multiple isolates per carcass are needed to properly assess and manage this risk to public health.

P3-57 Thermal Inactivation Kinetics of a Three-strain Composite of *Salmonella* Enteritidis and Oranienberg in Commercially-acquired Liquid Whole Egg and 10% Salted Liquid Egg Yolk

JOSHUA B. GURTLER, Harry M. Marks, Deana R. Jones and Rebecca B. Rivera
USDA-ARS, Wyndmoor, PA, USA

Introduction: Liquid egg pasteurization requirements are based on time/temperature combinations in the Code of Federal Regul-ations, Title 9, Ch. III, Sec. 590.570 from data acquired prior to 1970. These guidelines are being reevaluated in light of recent risk assessments, which take into account changes in industrial practices.

Purpose: The goal of this study was to determine the inactivation kinetics of thermally-resistant *Salmonella* in commercially-acquired liquid whole egg (LWE) and 10% salted liquid egg yolk (LEY).

Methods: Heat-resistant *Salmonella* Enteritidis and Oranienburg were grown in tryptic soy broth at 37°C, concentrated by centrifugation, and resuspended in peptone water. Each inoculum was added to liquid egg products and mixed, resulting in final populations of ca. 8.4 log CFU/ml. Inoculated egg was injected into sterile glass capillary tubes, flame-sealed and heated in a water bath at 56, 58, 60, 62 or 64°C for LWE and 62.2, 63.3, 65, 67 or 69°C for salted LEY. Contents were surface plated, incubated at 37°C for 24 h, and colonies were enumerated.

Results: Survival curves were not log-linear (log levels versus time). After initial periods, for respective products, survival curves became linear. Asymptotic decimal reduction values were calculated from survivor curves with a minimum inactivation of 5 log CFU/ml at each temperature. The asymptotic thermal D-values for LWE were 5.77, 1.64, 0.41, 0.11 and 0.047 min at 56, 58, 60, 62 and 64°C, respectively. Asymptotic D-values for 10% salted LEY were 7.35, 4.42, 2.33, 1.15 and 0.46 min at 62.2, 63.3, 65, 67 and 69°C, respectively. Thermal Z-values were 3.72 and 5.80°C for LWE and 10% salted LEY, respectively. A model that predicts lethality for given times and temperatures was developed.

Significance: This model will assist the USDA, FSIS in issuing pasteurization performance standards and provide industry guidance for designing pasteurization processes that will ensure safe product.

P3-58 Propyl Paraben Sensitizes Heat-resistant *Salmonella* Enteritidis and Oranienburg to Thermal Pasteurization in Liquid Egg Albumen

JOSHUA B. GURTLER
USDA-ARS, Wyndmoor, PA, USA

Introduction: The USDA, Food Safety and Inspection Service requires that liquid egg albumen (or egg white, LEW) undergo pasteurization at 56.7°C for 3.5 min prior to distribution. This process is known change the physical properties of LEW. The use of antimicrobials to sensitize salmonellae to pasteurization would be useful in reducing thermal treatment times and preserve sensitive LEW proteins. Propyl paraben (PrPa) is a non-toxic antimicrobial, occurs in nature, and has been given GRAS status by the FDA when added at levels up to 1,000 ppm.

Purpose: The goal of this study was to determine the effects of propyl paraben on the thermal sensitivity of three heat-resistant strains of *Salmonella* in LEW.

Methods: The pH of LEW was adjusted to pH 7.8 or 8.9 and PrPa was added to LEW at concentrations of 0, 125, 250, 500 and 1,000 ppm. A composite of heat-resistant *Salmonella* was washed and added to LEW at ca. 8 log CFU/ml. Inoculated LEW was held for two hours at 22°C, heated at 56.7°C for up to 25 min and analyzed for *Salmonella*.

Results: When pasteurized under USDA guidelines (3.5 min at 56.7°C) without the addition of PrPa, 1.16 and 4.32 log CFU/ml *Salmonella* were inactivated at pH 7.8 and 8.9, respectively. The D56.7 values for *Salmonella*, based on the presence of 0, 125, 250, 500 and 1,000 ppm of PrPa, were 3.03, 1.05, 0.64, 0.26 and 0.13 min at pH 7.8, and 0.81, 1.02, 0.64, 0.21 and 0.09 at pH 8.9. Times necessary to achieve a 6-log reduction at pH 7.8 with 0, 125, 250, 500 and 1,000 ppm of PrPa were 18.18, 6.30, 3.84, 1.56 and 0.78 min, respectively, while at pH 8.9, times of 4.86, 6.12, 3.84, 1.26 and 0.54 min were required, respectively.

Significance: Propyl paraben may be a useful antimicrobial for sensitizing *Salmonella* to thermal pasteurization in liquid egg albumen.

P3-59 Thermal, Biochemical, Serological and Fatty Acid Methyl Ester Analysis of *Salmonella* spp. Isolated from Pasteurized Egg Products

JOSHUA B. GURTLER, William C. Cray and Arthur Hinton
USDA-ARS, Wyndmoor, PA, USA

Introduction: The Egg Products Inspection Act of 1970 requires that egg products in the U.S. must be pasteurized prior to release into commerce. The USDA Food Safety and Inspection Service (FSIS) is responsible for regulating egg products. Salmonellae are infrequently isolated from pasteurized egg products by food manufacturers or the FSIS and may be present as a result of either pasteurization-resistant bacteria or post-processing contamination.

Poster

Purpose: The goal of this study was to evaluate twenty strains of *Salmonella* for variations in thermal resistance, biochemistry, serology, and fatty acid methyl ester (FAME) profiles.

Methods: Seventeen strains of *Salmonella* isolated from pasteurized egg products and three heat-resistant strains used in ongoing USDA-ARS-FSIS egg pasteurization studies were compared for thermal resistance in liquid whole egg (LWE) at 60°C, enzymatic profiles with the BBL Crystal Identification System Autoreader, serotyped at the USDA, APHIS, National Veterinary Service Laboratory in Ames, Iowa, and FAME analysis was conducted by gas chromatography and MIDI Sherlock software.

Results: The D60 values in LWE ranged from 0.30 to 0.50 min, and seven of twenty strains survived pasteurization at 60°C for at least 3.5 min. Isolates serotyped as Heidelberg, Mbandaka, Cerro, Thompson, 4,12:i:-, and Enteritidis (8 isolates). Although some strains exhibited atypical enzymatic activity (e.g., reduction of adonitol, hydrolosis of proline nitroanilide or p-n-p-beta-glucuronide, and nonreduction of melibiose) no differences in biochemical reactions could be correlated with similarity in thermal resistance. FAME analysis revealed that fatty acid profiles may be associated with heat resistance.

Significance: This data represents the first steps in determining whether *Salmonella* contamination in pasteurized egg products may be the result of either thermally-resistant isolates or post-processing contamination. *Salmonella* isolates are currently being evaluated for PFGE profiles, antimicrobial resistance and more detailed biochemical differences.

P3-60 Thermal Destruction of *Listeria monocytogenes* in Liquid Egg

CSABA NÉMETH, László Friedrich, Csaba Balla and Csilla Mohácsi-Farkas
Corvunus University of Budapest, Dept. of Refrigeration and Livestock Products Technology, Budapest, Hungary

Introduction: Nowadays food manufacturing plants prefer "ready-to-use" liquid egg products to eggs. However, the shelf life of pasteurized liquid egg products is relatively short, there is a need to elaborate new technologies — like the long term (6-24 h) heat treatment of liquid egg products at 53-55°C.

Purpose: The aim of our work was to develop a model for a new long-term heat treatment under laboratory conditions to reduce viable count of *Listeria monocytogenes* (NCAIM B1371) by this procedure.

Methods: Liquid egg samples (white, yolk, whole) were inoculated with *Listeria monocytogenes* to reach log 6 CFU/ml, and incubated at 53 and 55°C. Viable cell count was examined in every 30 min during 6 h, and after 24 h of heat treatment.

Results: Surviving cell count of *Listeria monocytogenes* during long term incubation at 53°C and 55°C was significantly different ($P < 0.05$) in the various liquid egg products. Reduction of viable cells was the fastest in the egg white and slowest in the yolk in each case. Our measurements have shown that treatment at low temperatures for long time reduced the cell count of *Listeria monocytogenes* below the detection level in all samples irrespectively of the treatment temperature, the previous life of bacteria.

Significance: The long term heat treatment at 53-55°C can be used to reduce *Listeria monocytogene*s counts to ensure the microbiological safety of liquid egg products.

P3-61 Frequency of *Listeria monocytogenes* on the Eggshell of Commercial Chicken Eggs Using a Combined Method of Enrichment and Nested-PCR

GERARDO GUZMAN-GOMEZ, Miguel A. Ayala-Valdovinos, Elisa Cabrera-Díaz, Julia A. Perez-Montaño and Sandra L. Ruiz-Quezada
Universidad de Guadalajara, Producción Animal, Zapopan, Mexico

Introduction: Chicken eggs have not been identified as vehicles of *Listeria monocytogenes* outbreaks. However, this pathogen is associated with cooked/ready-to-eat foods as a post-processing contaminant and cross contamination from eggshells may occur. Studies on *Listeria monocytogenes* frequency on eggs are scarce and only one report has shown isolation of this pathogen from unpasteurized eggs. The low numbers of *Listeria monocytogenes* on eggs makes difficult its detection in order to conduct frequency studies. A combined method of selective enrichment followed by Nested-PCR shows more sensibility than bacteriological methods and is a good alternative for the investigation of *Listeria monocytogenes* on eggshells.

Purpose: To investigate the frequency of *Listeria monocytogenes* on eggshells using a selective enrichment procedure followed by Nested-PCR.

Methods: Six hundred and fifty chicken eggs representing 5 commercial brands (130 eggs per brand) were purchased from three grocery stores from June to September (summer season). Ten eggs of each brand were combined in order to obtain 65 pooled samples. The detection method consisted of enrichment in trypticase soy broth supplemented with 0.6% yeast extract and selective agents (acriflavin, nalidixic acid and cycloheximide) for 48 h, followed by Nested-PCR.

Results: From the 65 pooled samples analyzed, 3 tested positive for *Listeria monocytogenes* (4.6%). The isolation frequency of *Listeria monocytogenes* was higher for brand E (3.1%), followed by brand C (1.5%). Brands A, B and D tested negative for this pathogen.

Significance: The isolation frequency of *Listeria monocytogenes* on eggshells (4.6%) was higher to that reported in USA (0%), Trinidad and Tobago (0%), and Japan (0.6%), where a bacteriological method was used. The combination of selective enrichment followed by Nested-PCR is useful for *Listeria monocytogenes* detection in foods where the expected number of cells is low.

P3-62 Effect of a Native Microflora on the Growth of *Listeria monocytogenes* in Cooked Ham

CHENG-AN HWANG and Shiowshuh Sheen
USDA-ARS-ERRC, Wyndmoor, PA, USA

Introduction: Refrigerated ready-to-eat meat products contaminated with *Listeria monocytogenes* have been linked to outbreaks of foodborne illnesses. The *L. monocytogenes* contamination was mainly caused by improper processing and/or cross contamination.

Purpose: This study examined the growth characteristics of *L. monocytogenes* as affected by native microflora in cooked ham at refrigerated and abusive temperatures.

Methods: A five-strain mixture of *L. monocytogenes* and a native microflora, consisting of *Brochothrix* spp., isolated from cooked ham were inoculated alone (monocultured) or co-inoculated (co-cultured) onto cooked ham slices. Ham slices were vacuum-packed and stored at 4, 6, 8, 10, and 12°C. The growth characteristics, lag phase duration (LPD, h), growth rate (GR, $\log_{10}$ CFU/h), and maximum population density (MPD, $\log_{10}$ CFU/g), of *L. monocytogenes* and the native microflora during storage were determined.

Results: At 4-12°C, the LPDs of co-cultured *L. monocytogenes* (62-32 h) were not significantly different ($P > 0.05$) from those of monocultured *L. monocytogenes* (58-38 h) in ham, indicating the LPDs of *L. monocytogenes* were not influenced by the presence of the native microflora. At 4-8°C, the GRs of co-cultured *L. monocytogenes* (0.0114-0.0130 $\log_{10}$ CFU/h) were significantly lower ($P < 0.05$) than

those of monocultured *L. monocytogenes* (0.0132-0.0145 log_{10} CFU/h), indicating the GRs of *L. monocytogenes* at 4-8°C were reduced by the presence of the native microflora. The GR-suppression effect was not observed in ham stored at 10 and 12°C. The MPDs of *L. monocytogenes* at 4-8°C were also reduced by the native microflora.

Significance: Findings from this study add to our understanding of the survival and growth of *L. monocytogenes* in RTE meats, and can be used to design processing and handling conditions to reduce *L. monocytogenes* hazards in these products.

P3-63 Survival and Death of *Listeria monocytogenes* on Cooked Bacon at Three Storage Temperatures

PETER J. TAORMINA and Warren J. Dorsa
John Morrell Food Group, Cincinnati, OH, USA

Introduction: Industrially-produced, cooked, ready-to-eat bacon has a large and growing market in food retail and foodservice settings. The process of cooking bacon has been demonstrated to be sufficiently destructive to the Gram-positive, psychrotrophic, foodborne pathogen *Listeria monocytogenes*. However, cooked bacon product is exposed to the environment post-lethality, and the risk of recontamination and subsequent growth by *L. monocytogenes* must be addressed.

Purpose: This study was undertaken to measure the survival and death of *L. monocytogenes* from a high-level inoculum and to determine how long a low-level inoculum would remain detectable in various cooked bacon products as affected by storage temperature.

Methods: Survival of *L. monocytogenes* on cooked bacon cubes (a_w 0.910 ± 0.080), strips (a_w 0.726 ± 0.054), and bits (a_w 0.620 ± 0.038) was determined during a 25 week storage period at -20, 4.4, and 22°C. Samples inoculated at ca. 5.5-log CFU/g were analyzed over time by direct plating on modified Oxford medium (MOX), while selective enrichment and subsequent enzyme linked fluorescent antibody (ELFA) detection were used to asses survival on samples inoculated at ca. 1-log CFU/g. The Baranyi model was fitted to the inactivation curves using the DMFit program.

Results: At -20°C, a decline of about 1-log CFU/g on all cooked bacon types at the high inoculation level was evident by 14 weeks, although most low inoculation level samples remained positive by the ELFA detection method for 25 weeks. Some strips and bits samples were negative for the pathogen within 3 weeks at 4.4 and 22°C, and > 1.5 log CFU/g reductions occurred on strips and bits by 8 weeks at either 4.4 or 22°C. Rate parameter estimates indicated that population declines occurred fastest on strips and bits at 22°C.

Significance: This study demonstrates that cooked bacon does not support the growth of *L. monocytogenes* and that the pathogen gradually dies off during prolonged storage. Rates of death appear to be influenced by a_w of cooked bacon as well as temperature.

P3-64 Effect of Different Marination Application Systems on the Growth of *Listeria monocytogenes* in Ready-to-Eat Pork Loin

KARINA G. MARTINO, Olamide Oyetunji, Mark Harrison and Alex M. Stelzleni
University of Georgia, Food Science and Technology, Athens, GA, USA

Introduction: Listeria monocytogenes (LM) is a recognized potential hazard for post-processing contamination of Ready-to-Eat meat products (RTE). Incorporation of salts and other ingredients in the marinade before cooking are becoming very important. The effects of these ingredients and the marination process on the effectiveness of antimicrobial after cooking are not yet well understood by the meat industry.

Purpose: The goal of this study was to evaluate the effect of post-injection tumbling and tumble duration (0, 15, 30, 45 and 60 min) on growth of LM in RTE marinated pork loin using potassium lactate as an antimicrobial.

Methods: The marinade used had 1.5% NaCl, 0.45% sodium tripolyphosphate, and 1 or 2% potassium lactate. Samples were injected with the marinade and then tumbled. After tumbling, samples were inoculated with LM with an initial concentration of 3 log cm^2, and then, they were stored at 4°C for a total of 56 days. Samples were taken during that period to monitor LM growth.

Results: Without antimicrobial, regardless of tumbling duration, a 2 log CFU/cm^2 increase was observed in 7 days. At 1% potassium lactate, without tumbling, a 2 log CFU/cm^2 increase was observed in 28 days, while with tumbling, 2 log increase took 35 days. At 2% potassium lactate, less than 2 log increase in LM growth was observed during the entire 56 day storage period. Tumbling duration was a significant factor on LM growth only at 1% potassium lactate ($P < 0.05$). After 35 days of storage, only the samples tumbled for 45 min had less than 2 log CFU/cm^2 increase of LM, the other tumbling times had more than 2 log CFU/cm^2 increase. Presence of potassium lactate significantly ($P < 0.05$) reduced LM growth. Within the 56 day storage, a maximum of 5.04 log CFU/cm^2 increase was observed on loins without potassium lactate. At 1% and 2% concentrations, a maximum of 4.43 log CFU/cm^2 and 2.19 log CFU/cm^2 respectively were observed.

Significance: When designing and optimizing marination systems, the interaction of variables such as tumble duration and marination ingredients, could significantly contribute to the inhibition of LM on marinated pork loin. Furthermore, delivery systems could have an impact on the efficacy of antimicrobials used in the marinade, and its interaction with the muscle. The information provided in this study could help processors design a model for their marination process.

P3-65 *Salmonella* and Pathogenic *Escherichia coli* Prevalence and Generic *Escherichia coli* Quantitative Baselines in Raw Pork and Beef at Retail Outlets in Mexico

ANSEN R. POND, Mark Miller, Alejandro Echeverry, Guy Loneragan, Maria Rubio, Adrian Chavez, Nelson Huerta, John Rupnow, Todd Brashears and Mindy Brashears
Texas Tech University, Animal and Food Science, Lubbock, TX, USA

Introduction: Food safety is a high concern in developing countries. In Mexico, the number one cause of death for children ages 1-4 is foodborne illness.

Purpose: To determine the prevalence of *Salmonella* and *E. coli* O157:H7 and to quantify generic *E. coli* and APC in fresh beef and pork products from retail outlets in major cities in Mexico.

Methods: Through microbiological analysis, the prevalence was determined for *Salmonella* and *E. coli* O157:H7 in Mexican whole beef, whole pork, ground beef and ground pork products. These samples were collected at four types of retail channels (supermarkets, wet markets, street vendors and butcher shops) in the most populated cities of Mexico: Mexico City, Guadalajara and Monterrey. Each city was divided into four zones (North, East, South and West), from each part up to 3 of the retail channels was included in the study. Sample swabs were shipped to Texas Tech University under cold conditions for microbiological analyses within a day after collection. *Salmonella* and *E. coli* O157:H7 presence were confirmed using the DuPont Qualicon BAX® system. APC and generic *E. coli* were enumerated using Petrifilm®.

Results: No *E. coli* O157:H7 was found in any of the samples. Overall *Salmonella* results for this study were 13.836% positive for Guadalajara, 1.084% positive for Monterrey and 10.209% positive for Mexico City. When comparing vendors, supermarkets had the lowest number of positive results ranging from 1.198% positive in Mexico City to 1.98% positive in Guadalajara. The range for wet markets, butcher

shops and street vendors were 8.434% - 40.625% positive, 0.592% — 26.922% positive and 13.636% positive (samples only taken in Mexico City) respectively. For all cities, APC numbers ranged from 9.077-9.386 log CFU/g in ground product and from 6.731-7.395 log CFU/cm in whole products. Coliforms ranged from 0.602-7.298 log CFU/g.

Significance: Determining the Mexican baseline will help to understand where improvements are needed in food sanitation.

P3-66 Effect of Phosphate and Meat (Pork) Types on the Germination and Outgrowth of *Clostridium perfringens* Spores during Abusive Chilling

AIKANSH SINGH, Nageswara Rao Korasapati, Vijay K. Juneja and Harshavardhan Thippareddi
University of Nebraska, Lincoln, NE, USA

Introduction: Spores of *Clostridium perfringens* can germinate, outgrow and multiply during abusive cooling of cooked, Ready-to-Eat meat products. Inorganic phosphates are extensively used in meat and poultry products and impart several functional properties, including antimicrobial properties.

Purpose: The effect of phosphate type, source and their mixtures on the germination and outgrowth of *C. perfringens* in pale, soft, exudative (PSE), Normal and dark, firm, dry (DFD) pork during abusive exponential chilling profiles was evaluated.

Methods: Two different phosphates, tetrasodium pyrophosphate (TSPP) and sodium acid pyrophosphate (SAPP; from two different sources, SAPP1 and SAPP2) were used. The ground pork loins representing each meat type and three phosphate blends (SAPP1 + SAPP2, TSPP + SAPP1 or TSPP + SAPP2) were added (0.3% total, equal proportions of 0.15% each type) along with salt (1.0%). The pork was then mixed with a three-strain *C. perfringens* spore cocktail of ca. 2.5 log spores/g. The inoculated product was heat shocked for 20 min at 75°C and chilled exponentially from 54.4 to 4°C in 6.5, 9, 12, 15, 18 or 21 h.

Results: C. perfringens population increases of 5.95, 4.73 and 5.95 log CFU/g meat in normal, PSE and DFD pork, respectively, were observed following 21 h chilling profile in control samples. In DFD meat, all the three phosphate combinations failed to minimize the germination and outgrowth of *C. perfringens* spores to < 1 log CFU/g beyond 6.5 h of chilling. The combination of SAPP1 + SAPP2 (0.3%) was effective in restricting *C. perfringens* population increase to < 1 log CFU/g during 12 and 21 h chilling profiles in normal and PSE pork, respectively. The types of phosphate and the meat type affect ($P \leq 0.05$) the germination and outgrowth of *C. perfringens* spores in cooked pork during abusive chilling profiles.

Significance: This study concluded that different Pork type, phosphate type and blends affect the potential germination and outgrowth of *C. perfringens* spores during cooling of cooked, Ready-to-Eat pork.

P3-67 Analysis of ALLRTE and RTE001 Sampling Results for *Listeria monocytogenes*, Calendar Years 2005–2008

Kristina E. Barlow, Evelyne Mbandi, Tim B. Mohr, STEPHEN W. MAMBER and Peter Evans
USDA-FSIS, Washington, D.C., USA

Introduction: The Food Safety and Inspection Service (FSIS) ALLRTE and RTE001 sampling programs are used to detect *Listeria monocytogenes* (Lm) contamination of Ready-to-Eat (RTE) meat and poultry products such as deli meats and hot dogs in production environments. ALLRTE is a random sampling program for all types of RTE products, while RTE001 is a risk-based program for post-lethality exposed RTE products.

Purpose: To analyze ALLRTE and RTE001 sampling data for Lm collected over a 4-year period.

Methods: The Agency analyzed results of Lm testing of meat and poultry product samples collected under the ALLRTE and RTE001 sampling programs for calendar years 2005-2008. Samples were tested in accordance with FSIS's Microbiology Laboratory Guidebook.

Results: These analyses involved data for 11,822 ALLRTE samples collected from 2,556 establishments and 33,278 RTE001 samples collected from 1,989 establishments. The percentages of Lm-positive samples averaged 0.52% for ALLRTE (range, 0.41 to 0.64%), and 0.50% for RTE001 (range, 0.39% to 0.71%). Annual percentages of establishments with Lm-positive samples ranged from 0.85 to 1.17% for ALLRTE and from 2.28 to 3.23% for RTE001. Overall, 3.9% of 2674 establishments had at least 1 Lm-positive sample. The 230 positive samples (62 ALLRTE, 168 RTE001) were from diverse RTE product types including deli meats, hot dogs, sausages, salads/spreads and multicomponent products such as burritos. About 30% of the positives were from sliced/diced/shredded products. There were about 130 PFGE patterns from the 230 Lm isolates. All but 8 of the Lm-positive samples were obtained from establishments with HACCP sizes of Small (141) or Very Small (81). Almost half of the positive samples were obtained from establishments employing *Listeria* control Alternative 3 (sanitation only/highest risk).

Significance: This detailed ALLRTE and RTE001 data analysis will help guide changes in policies, regulations, inspection procedures and enforcement actions relevant to the prevention of Lm contamination in RTE products and production environments.

P3-68 Prevalence of *Salmonella* Fecal Shedding and Environmental Contamination in Dual Purpose Cattle Farms under Tropical Conditions

CLAUDIA NARVAEZ BRAVO, Yrimar Fuenmayor, Argenis Rodas-González, Gabriela Carruyo, Armando Hoet and Thomas Wittum
Texas Tech University, Animal and Food Sciences, Lubbock, TX, USA

Introduction: Salmonellosis is an important animal health and public health concern worldwide. The development of suitable intervention strategies to control *Salmonella* populations at the farm level requires reliable data on the prevalence and potential sources of the pathogen

Purpose: To evaluate the prevalence of *Salmonella* fecal shedding in cattle and calves across six dual purpose cattle farms. In addition, the distribution of this pathogen on environmental samples as potential contributors to the exposure and dissemination of *Salmonella* was investigated.

Methods: Fecal samples (n = 3964) were collected directly from healthy dual purpose cows and calves at 6 farms located in the eastern region of Zulia state, Venezuela. Additionally, environmental samples (n = 333) were taken from those farms that consisted of flies (FL), potable water (PW), soil (S), commercial feed (CF), chicken litter used in the animal diet (CL), ponds/lagoons (P), water trough (WT), and molasses (Mo). Also milk (M) and milker's hands (MH) were sampled. *Salmonella* detection was carried out using standard microbiological enrichment and culture methods, and confirmed with somatic antigen using standard biochemical reactions and polyvalent O antiserum.

Results: The overall prevalence of *Salmonella* at the farms was 10.5% (n = 3964), with 11.18 % (225/2013) of the cows and 9.84 % (191/1942) of the calves testing positive. Each one of the farms was positive to *Salmonella* in at least one sample point. The overall prevalence of environmental samples was 10.78% and showed differences ($P < 0.01$), being the sources of water (P = 25% and WT = 18.64%), S (15.15%), CL (14.28%) and MH (11.46%) which showed the higher prevalence.

Significance: Data showed that 100% of evaluated farms had cattle shedding *Salmonella* and that the surrounding farm environment was heavily contaminated, which contribute to the cycling of the pathogen at the farm and further contamination of milk.

P3-69 *Salmonella* and *Escherichia coli* O157:H7 Prevalence in Cattle and on Carcasses in a Vertically Integrated Feedlot and Harvest Plant in Mexico

CLAUDIA NARVAEZ BRAVO, Markus F. Miller, Alejandro Echeverry, Tanya Jackson, Kevin Pond and Mindy Brashears
Texas Tech University, Lubbock, TX, USA

Introduction: There are little data reported on the prevalence of *E. coli* O157:H7 and *Salmonella* in feedlot cattle and the impact on subsequent contamination on the carcasses in Mexican harvest facilities.

Purpose: To determine the prevalence of *Salmonella* and *E. coli* O157:H7 in a Mexican feedlot and the contamination of the cattle during the harvest process

Methods: A total of 58 fecal pats (FP) were collected in the feed yard from animals as they were shipped to the harvest facility. One hundred animals were randomly targeted and composite samples were taken from three anatomical carcass sites (inside round, hind shank and fore shank). In the facility, samples were taken on the hides, at pre-evisceration (PE), prior to entering the hot box (PHB) and after 24 hours of dry chilling (DC). Additionally, fecal and water samples were taken from lairage, and fecal grabs (FG) from each tagged animal. *E. coli* O157:H7 and *Salmonella* detection was carried out using BAX, IMS and conventional methods.

Results: The overall prevalence of *Salmonella* for carcasses from all sampling locations was 39%. *Salmonella* prevalence for hides was 100%. *Salmonella* prevalence at PE, PHB, and DC was 60, 43, and 14%, respectively. For fecal samples, 63% of fecal grabs in the plant were positive for *Salmonella*, feedlot fecal pats: 65.52%, holding pen fecal samples: 89.61% and for lairage water: 97.22%. A total of 25% of hide samples were positive for *E. coli* O157, while for carcasses the overall prevalence was 1.33%. *E. coli* O157:H7 prevalence in fecal samples was as follows: FG in plant: 2%, feedlot FP: 1.72% holding pen: 1.30%, and lairage water: 8.33%.

Significance: While *Salmonella* was highly prevalent in the all areas, *E. coli* O157 was rarely transferred to carcasses. Interventions will be implemented to decrease *Salmonella* prevalence

P3-70 Advances in the Implementation of Quality Systems in Mexican Beef Packers

EMA MALDONADO-SIMAN, Angelica Perez-Mendez, Pedro A. Martínez-Hernández, Jose A. Cadena-Mensese, Agustín Ruiz-Flores and Gilberto Aranda-Osorio
Universidad Autonoma Chapingo, Zootecnia/Posgrado, Texcoco, Mexico

Introduction: International food trade has brought an increased public and governmental interest in the application of protocols that assure safety and quality of food products in international trade.

Purpose: This research aimed to report the level of adoption of HACCP and the ISO 9000 System in registered Mexican beef packers (TIF-packers) and to identify their sale destinations. Along with this was the description of costs, benefits, and obstacles in the implementation of those protocols.

Methods: Fieldwork consisted of structured questionnaires applied to TIF-packers that showed export sales. Statistical analyses were conducted using univariate and tabulate procedures of SAS.

Results: Of the 26 TIF-packers, 65.4% answered the questionnaire. HACCP was in full operation in 88.2% of the plants, while only 5.9% had ISO 9000, making this last protocol of minor importance within the Mexican beef industry. Korea, Japan, and USA take over 70.0% of the export sales of these plants; other destinations are Honduras, Costa Rica, Nicaragua, Guatemala, and Uruguay. Main buyers in the domestic market are supermarkets, wholesalers, and restaurants. All TIF-packers adopted HACCP to obtain certification by specialized agencies and then access to overseas markets. In the process of implementing HACCP, major investments were certification fees and staff training, while for running HACCP in the plant, microbiological tests represented the higher cost. All TIF-packers agreed that increased shelf life, lower microbial counts of meat products, and a better chance to reach new markets were the major benefits of having HACCP.

Significance: The decision of implementing quality systems faces high costs and the often intangible nature of the benefits; therefore, a better understanding of the relation of HACCP protocol with food safety and quality and trade competitiveness is needed. Thus, this study shows the willingness of registered Mexican beef packers to implement food safety protocols.

P3-71 Validation of Pepperoni Process for Control of Shiga-toxin Producing *Escherichia coli*

KATHLEEN A. GLASS, Charles W. Kaspar, Jeffrey J. Sindelar, Andrew Milkowski, Brian M. Lotz, Jihun Kang, Thomas Mand, Nancy G. Faith, Elena Enache, Ai Kataoka and Craig Henry
University of Wisconsin, Food Research Institute, Madison, WI, USA

Introduction: Certain non-O157 strains of shiga-toxin producing *E. coli* (STEC) have caused occasional foodborne outbreaks, sporadic cases, and hemolytic uremic syndrome. However, their relative sensitivity to current practices used to control *E. coli* O157:H7 in foods is largely unknown.

Purpose: The objective of this study was to compare the survival of non-O157 STEC with *E. coli* O157:H7 during pepperoni production.

Methods: Pepperoni batter was inoculated with 7-log CFU/g of a 7-strain STEC mixture including O26, O45, O103, O111, O121, O145, and O157. Sausages were fermented to pH $\leq$ 4.8, heated to 58.3°C and held at that temperature for one hour, followed by drying up to 20 days until a maximum moisture:protein 1.6:1 was reached. STEC populations were enumerated at designated intervals by plating on sorbitol MacConkey (SMAC) and Rainbow (RA) agars; enrichments were completed in modified EC (mEC) broth and non-selective trypticase soy broth (TSB). The experiment was replicated twice.

Results: When plating on SMAC, total *E. coli* populations decreased 2.6-3.5 at the end of the 1 h heating step at 58.3°C, and a 4.9-5 log reduction was observed after 7 days drying. RA was substantially more sensitive in recovering survivors; log reductions on RA were 1.9-2.6, 3.8-4.2, and 4.6-5.3 at the end of cook, and at days 7 and 14, respectively. Freezing the D-7 dried sausage for 2-3 weeks generated an additional 1-1.5 log kill. When populations were less than levels detectable by direct plating on days 14 and 21 (representing 5 log kill), no more than 1/3 samples for each treatment was positive by enrichment with mEC broth, however STEC were consistently recovered when using TSB as the enrichment. Confirmation by PCR revealed that serotypes O103 and O157 had the greatest survival rates, but all serotypes except O111 and O121 were occasionally recovered. Results from survival studies at reduced pH and water activity provide insight into persistence of the O103 and O157 serotypes in pepperoni.

Significance: This study suggests that non-O157 STEC strains have comparable or less survival characteristics as O157 in the manufacture of pepperoni. Methods which are deemed to be suitable to control O157 will similarly inactivate the other strains of STEC tested in this study.

Poster

P3-72 Wine-based Marinades Prevent the Growth of *Salmonella* Typhimurium and Background Flora in Beef Fillets

Aspasia Nisiotou, Antonia Gounadaki, Vasilios Iliopoulos, PANAGIOTIS SKANDAMIS, John Sofos and George Nychas
AUA, Food Science and Technology, Athens, Greece

Introduction: *Salmonella* may be introduced in fresh beef during slaughter or handling processes. Methods that can control its growth on meat surfaces are important for industry and authorities. Among them, marination may serve as a "natural" processing method in beef safety/spoilage.

Purpose: To evaluate wine-based marinades in controlling the survival of acid-adapted and non-acid-adapted *Salmonella* Typhimurium and extending shelf life of meat stored aerobically or under modified atmosphere.

Methods: Beef slices were inoculated with a 3-strain cocktail of acid or non-acid adapted *Salmonella* Typhimurium strains DT 193, 4/74 and DSM 554 and marinated by immersion in i) wine and ii) wine supplemented with 0.3% thyme oil, for 12 h at 4°C. Marinated slices were then stored at 5°C under air or modified atmosphere. *S.* Typhimurium and microbial association, the pH and the sensory characteristics were followed for a 19-day period of storage. Survival of *S.* Typhimurium individual strains were followed by pulsed field gel electrophoresis.

Results: Marination significantly reduced the levels of *S.* Typhimurium and the background flora of beef compared to the control (non-marinated). The survival of *S.* Typhimurium and microbial association was affected by the addition of thyme oil in the marinade. There were not any important differences between acid and non-acid adapted cells during the time-course. Monitoring of *S.* Typhimurium DSM 554, DT 193 and 4/74 during storage of fillets revealed similar strain distribution in different storage conditions throughout time-course. The epidemic multi-drug resistant DT 193 could compete other strains, like 4/74 originating from calf bowel.

Significance: Present results show that wine-based marinades are efficient, from a safety and shelf-life standpoint, in reducing pathogen's levels as well as the background beef flora.

P3-73 Efficacy of Brine Spray Chilling on the Reduction of *Escherichia coli* O157:H7 and *Salmonella* on Hot Beef Carcass Surfaces

WILLIAM E. CHANEY, Mark F. Miller, Alejandro Echeverry, Henry Ruiz and Mindy M. Brashears
Texas Tech University, Lubbock, TX, USA

Introduction: Spray chilling may decrease the time margin for post-slaughter carcass chilling. Utilizing brine may impart antimicrobial characteristics on the carcass.

Purpose: To validate pathogen reduction of sodium chloride brine chilling methods on beef surfaces.

Methods: Thirty 4" x 10" surfaces from the brisket and foreshank fascia were collected at slaughter, packaged in sterile bags, and transferred to the laboratory. At 10°C, fifteen of each surface type were randomly selected for dip inoculation into *Escherichia coli* O157:H7 or *Salmonella* cocktails, both at surface target concentrations of 10^5 CFU/cm^2 followed by 30 minute attachment. Three samples per surface were placed onto trays with one of 5 treatments assigned as control, positive, brine spray, brine dip, or sterile water spray. Brine consisted of saturated sodium chloride in sterile 5°C deionized H_2O. Fifty cm^2 surface samples were collected at 0, 12, and 24 hours. Pathogen counts were determined by plating onto MacConkey and XLD agars with TSA overlays and incubation at 37°C for 24 hours.

Results: Foreshank fascia counts of *E. coli* O157:H7 declined from 4.76 to 4.02 log CFU/cm^2 for the brine dip, 5.29 to 4.50 log CFU/cm^2 for the water spray, 4.94 to 4.43 log CFU/cm^2 for the brine spray, and 5.26 to 4.93 log CFU/cm^2 for the positive. *E. coli* brisket counts declined from 4.73 to 2.87 log CFU/cm^2 for the positive, 4.50 to 2.89 log CFU/cm^2 for the brine dip, 4.37 to 3.03 log CFU/cm^2 for water spray, and 4.06 to 3.44 log CFU/cm^2 for brine spray. *Salmonella* foreshank fascia counts declined from 4.68 to 2.05 log CFU/cm^2 for brine dip, 4.81 to 4.16 log CFU/cm^2 for water spray, 4.71 to 4.12 log CFU/cm^2 for brine spray, and 4.78 to 4.38 log CFU/cm^2 for positive. *Salmonella* brisket counts declined from 4.71 to 3.23 log CFU/cm^2 for positive, 4.29 to 3.52 log CFU/cm^2 for brine dip, 3.67 to 3.45 log CFU/cm^2 for brine spray and no reductions for water spray.

Significance: These data do not support sodium chloride brine spray chilling as an efficient antimicrobial to reduce *Escherichia coli* O157:H7 and *Salmonella* from beef surfaces.

P3-74 Efficacy of High Intensity UV/Photohydroionization Cell for the Surface Decontamination of Beef Subprimals Inoculated with *Escherichia coli* O157:H7 and *Salmonella* spp.

JASDEEP K. SAINI and James Marsden
Kansas State University, Manhattan, KS, USA

Introduction: The interior of the whole muscle cut, otherwise pathogen free, may become contaminated due to translocation of surface contamination during mechanical tenderization. The translocation of surface contamination may pose a serious problem. Photohydroionization is an advanced oxidation process that may be effectively utilized as a non-thermal pasteurization technology for raw ground beef and processed beef products.

Purpose: This study validates the use of High Intensity Ultraviolet/photohydroionization cell to decontaminate beef subprimals, intended for non-intact processes, surface inoculated with *Escherichia coli* O157:H7 and *Salmonella* spp.

Methods: The subprimal meat cuts were inoculated with a five-strain cocktail of the two bacteria individually, and treated for 0, 10, 20 and 30 s. Excision sampling was carried out. Two core samples were obtained from the top and bottom of each beef subprimal. The tissue was excised (ca. 3 mm depth) from the outside (15.9 cm^2) surface of each beef subprimal cut, and placed in 90 ml 0.1% peptone diluent in a stomacher bag, and stomached for 1 min. Samples were then plated on the selective media, MacConkey Sorbitol Agar (MSA) and Xylose Lysine Desoxycholate agar (XLD) for *E. coli* O157:H7 and *Salmonella* spp., respectively, for enumeration.

Results: Results indicate that *E. coli* O157:H7 counts reduced by 1.035 log CFU/cm^2 after 10 s treatment. Reductions of 2.14 and 2.56 log CFU/cm^2 of *E. coli* O157:H7 were seen after 20 and 30 s treatment, respectively. The 10 s treatment showed 1.19 log CFU/cm^2 reductions in bacterial populations of *Salmonella* spp., while 20 and 30 s treatments showed 2 and 2.45 log CFU/cm^2 reductions, respectively.

Significance: This study demonstrates the efficacy of the High Intensity UV/photohydroionization cell for surface decontamination of subprimals and its potential use as an antimicrobial treatment.

P3-75 Thermal Inactivation of Shiga-toxin Producing Cells of *Escherichia coli* in Chemically-injected Beef Steaks Cooked on a Commercial Open-flame Gas Grill

JOHN B. LUCHANSKY, Anna C. S. Porto-Fett, Bradley Shoyer, Jeff Call, Christopher Sommers, Wayne D. Schlosser, William K. Shaw, Nathan E. Bauer and Heejeong Latimer
USDA-ARS, Microbial Food Safety Research Unit, Wyndmoor, PA, USA

Introduction: Previous studies have shown that chemical or mechanical tenderization transfers *Escherichia coli* O157:H7 (ECOH) and non-O157:H7 verocytotoxigenic *E. coli* (STEC) throughout the interior of beef subprimals.

Purpose: Evaluate the viability of ECOH or STEC in brine-injected beef subprimals during storage and subsequent cooking on a commercial open-flame gas grill.

Methods: Beef subprimals were inoculated on the lean side with ca. 5.7 log CFU/g of a five-strain cocktail of rifampicin resistant ECOH strains or kanamicin resistant STEC strains and then passed once through an automatic brine-injector tenderizer with the lean side facing upwards. Brine solutions were formulated with: i) 3.3% (w/v) of sodium tripolyphosphate and 3.3% (w/v) of sodium chloride or ii) 3.3% of sodium tripolyphosphate, 3.3% (w/v) of sodium chloride, and 25% (v/v) of a 60% potassium lactate-sodium diacetate syrup. The brine was injected into subprimals to a target level of 9.1 ± 0.8% over fresh weight. Following injection, subprimals were cut into 2.54 cm (1 inch) thick steaks and then stored for up to 15 days at 4°C. Brine-injected steaks were cooked on a commercial open-flame gas grill to internal endpoint temperatures of either 37.8°C (100°F), 48.8°C (120°F), 60°C (140°F), or 71.1°C (160°F).

Results: Regardless of brine formulation, storage of brine-injected steaks for up to 15 days at 4°C resulted in a 0.3- to 1.4-log CFU/g reduction of ECOH and STEC. Regardless of storage time, brine formulation, or cooking temperature, cooking achieved log CFU/g reductions of 1.1 to 4.8 of ECOH and 1.5 to 4.3 of STEC. At 71.1°C (160°F) the percent of organisms recovered ranged from 0.0013% to 4.0% for ECOH and from 0.0034% to 0.12% for STEC.

Significance: Cooking highly contaminated, brine injected steaks on a commercial gas grill to 71.1°C (160°F) is insufficient to kill all cells of ECOH and STEC.

P3-76 Translocation of Shiga Toxin-producing Cells of *Escherichia coli* in Chemically-injected Beef Subprimals

JOHN B. LUCHANSKY, Anna C. S. Porto-Fett, Bradley Shoyer, Jeffrey E. Call, Christopher Sommers, Wayne D. Schlosser, William K. Shaw, Nathan E. Bauer and Heejeong Latimer
USDA-ARS, Microbial Food Safety Research Unit, Wyndmoor, PA, USA

Introduction: Relatively little information is available regarding the translocation of *Escherichia coli* O157:H7 (ECOH) and non-O157:H7 verocytotoxigenic *E. coli* (STEC) into beef subprimals following chemical tenderization.

Purpose: Quantify translocation of ECOH or STEC from the surface into the interior of beef subprimals following tenderization by chemical injection.

Methods: Beef subprimals were inoculated on the lean side with ca. 6.6 log CFU/g of a five-strain cocktail of rifampicin resistant ECOH or kanamycin resistant STEC and then passed once through an automatic brine-injector tenderizer with the lean side facing upwards. Brine solutions were as follows: i) 3.3% (w/v) of sodium tripolyphosphate and 3.3% (w/v) of sodium chloride (Lac-) or ii) 3.3% of sodium tripolyphosphate, 3.3% (w/v) of sodium chloride, and 25% (v/v) of a 60% potassium lactate-sodium diacetate syrup (Lac+). Brine was injected into subprimals to a target level of 9.1 ± 0.8% over fresh weight. Six core samples were removed from each subprimal and cut into six consecutive segments starting from the inoculated side: segments 1 to 4 comprised the top four cm and segments 5 and 6 comprised the deepest four cm.

Results: For samples that were injected with Lac- and Lac+ brine, levels of ECOH recovered from segment 1 were ca. 6.5 and 6.2 log CFU/g, respectively, whereas levels of STEC recovered from segment 1, were ca. 6.3 and 6.0 log CFU/g, respectively. Regardless of brine formulation, the percentage of ECOH and STEC recovered from segment 2 for brine-injected subprimals were 5- to 65-fold lower than levels recovered from segment 1. It was possible to recover ECOH or STEC from all six segments of all cores tested.

Significance: These results validate that chemical tenderization transfers ECOH and STEC throughout the interior of beef subprimals. However, appreciably more cells were transferred to the topmost 1 cm compared to the deeper segments.

P3-77 Frequency and Antimicrobial Resistance of *Salmonella* spp. in Beef Carcasses at Slaughterhouses in Jalisco, México

JULIA A. PÉREZ-MONTAÑO, Delia González-Aguilar, Carlos Pacheco-Gallardo, Carlos Campos-Bravo, Jeannette Barba-León, Santos García, Norma L. Heredia and Elisa Cabrera-Díaz
Universidad de Guadalajara, Departamento de Salud Pública, CUCBA, Zapopan, Mexico

Introduction: *Salmonella* is an important foodborne pathogen that has been isolated previously from 21% of cattle feces samples collected in holding pens and from 15 to 54% of beef samples obtained at retail stores in México. *Salmonella* isolates from those studies showed resistance to tetracycline (92.5%), streptomycin (87.7%), and sulfixosazole (56.1%). However, investigations about the frequency and antimicrobial resistance patterns of *Salmonella* on beef carcasses at slaughterhouses in México have not been reported.

Purpose: The purpose of this study was to determine the frequency and antimicrobial resistance of *Salmonella* isolates obtained from beef carcasses at slaughterhouses in México.

Methods: Utilizing USDA-FSIS protocols, *Salmonella* spp. were isolated from beef carcasses (n = 505) at four municipal slaughterhouses (A, B, C and D) during a 10-month period. Antimicrobial resistance was determined using the disk diffusion method described by the Clinical and Laboratory Standards Institute (CLSI, 2008).

Results: *Salmonella* was isolated from 85 (16.8%) beef carcass samples with a distribution by slaughterhouse as follows: A (4.0%), B (1.6%), C (7.5%) and D (3.7%). Antimicrobial resistance analysis indicated that 41.2% of the isolates were resistant to tetracycline (TET), 36.5% to streptomycin (STR), 21.2% to chloramphenicol (CHL), 21.2% to trimethoprim-sulfamethoxazole (SXT), and 18.8% to gentamicin (GEN). Thirty percent of the isolates demonstrated multi-resistance to ≥3 antimicrobials. The most common multi-resistance pattern was GENTETSXTCHLSTR.

Significance: These findings highlight the importance of implementing measures to assure the proper use of antimicrobials in production of animals intended for human consumption and the need to reduce the presence of *Salmonella* on beef carcasses in México.

Poster

P3-78 Sensitive Detection of Bovine Plasma-derived Proteins in Ground Beef

JACK A. OFORI and Yun-Hwa P. Hsieh
Florida State University, Nutrition, Food and Exercise Sciences, Tallahassee, FL, USA

Introduction: Plasma-derived proteins are usually preferred over hemoglobin-containing blood proteins and more widely used in food products because they are odorless and have a neutral taste. Certain individuals, however, avoid blood-tainted foods for religious, cultural, ethical or health reasons, necessitating the need for methods to detect the presence of these proteins in food. Such methods are also important in preventing fraudulent use of these non-meat proteins in meat products to increase the apparent meat content.

Purpose: The objective of this study was to detect different commercially produced bovine plasma-derived food grade proteins using a sandwich enzyme-linked immunosorbent assay (ELISA) where monoclonal antibody (MAb) 6G12 was used as the capture antibody and biotinylated MAb 3D6 as the detection antibody.

Methods: Soluble proteins were extracted from raw or heated (100°C for 15 min) blood plasma-fraction (Plasma Powder FG, Fibrimex® and ImmunoLin®) and cellular-fraction (Hemoglobin Powder and Hydrolyzed Globin) proteins of both bovine and porcine origin. Soluble proteins were also extracted from raw or heated (100°C for 15 min) ground meat samples spiked with different bovine plasma-derived proteins.

Results: All raw and cooked bovine plasma containing samples showed strong reaction signals (OD: 1-3). The detection limit for spiked samples was 0.5%(v/v), 1%(v/v) and 0.01%(v/v) Plasma Powder FG, Fibrimex® and ImmunoLin® in raw ground beef, respectively; and 0.5%(v/v), 3%(v/v) and 0.5%(v/v) Plasma Powder FG, Fibrimex® and ImmunoLin® in cooked ground beef, respectively. The assay did not react with any of the porcine-based blood proteins but showed a moderate reaction (OD: 0.5-1) with hemoglobin powder, which may be as a result of contamination with plasma material as a result of poor separation.

Significance: This study demonstrated for the first time that diverse bovine-plasma derived proteins can be effectively and rapidly detected in both raw and heat-processed meat products with the sandwich ELISA.

P3-79 Inhibition of *Listeria monocytogenes* Growth on Kielbasa and Ham by Buffered Vinegar

CAROL VALENZUELA MARTINEZ, Mauricio R. Solano, Nageswara Rao Korasapati and Harshavardhan Thippareddi
University of Nebraska, Lincoln, NE, USA

Introduction: Listeria monocytogenes has been implicated in several foodborne illness outbreaks related to Ready-to-Eat meat and poultry products. The USDA-FSIS requires RTE meat and poultry processors to control the organism in the processing environment and in the product.

Purpose: The inhibitory effect of buffered vinegar on *L. monocytogenes* growth on kielbasa and ham was evaluated.

Methods: Kielbasa and ham products containing .5, 2.5 and 3.5% concentrations of buffered vinegar, along with a control (no antimicrobial) were obtained from a meat processor. Kielbasa and ham samples were cut or sliced, respectively, surface inoculated with a five-strain cocktail of *L. monocytogenes* to ca. 2.0 log CFU/cm^2, vacuum packed, and stored at 8 or 4°C. *L. monocytogenes* populations on the product were enumerated at regular intervals.

Results: L. monocytogenes population reached 7.28 log CFU/cm^2 on kielbasa (control) stored at 8°C, within 56 days. At 4°C, an increase of 3.10 log CFU/cm^2 was observed after 84 days of storage. Increasing the concentration of buffered vinegar to 2.5 and 3.5% minimized *L. monocytogenes* growth to < 1 log CFU/cm^2 for 84 and 112 days, respectively. *L. monocytogenes* population reached 7.49 log CFU/cm^2 in control ham within 56 days during storage at 4°C. The addition of buffered vinegar at 1.5, 2.5 and 3.5% concentrations resulted in increases of 1.96, 1.0 and 1.94 log CFU/cm^2 in 14 days at 4°C of storage.

Significance: Differences in *L. monocytogenes* growth were observed on kielbasa and ham products, containing similar concentrations of buffered vinegar. Distribution of the antimicrobial within the product and the concentration of the antimicrobial are important for control of *L. monocytogenes* growth on RTE meat products.

P3-80 Inhibition of *Listeria monocytogenes* Growth on Ready-to-Eat Turkey Products by Buffered Lemon Juice Concentrate and Vinegar Mixtures

CAROL VALENZUELA MARTINEZ, Nageswara Rao Korasapati and Harshavardhan Thippareddi
University of Nebraska, Lincoln, NE, USA

Introduction: Listeria monocytogenes has been implicated in several outbreaks related to Ready-to-Eat (RTE) meat and poultry products. The USDA-FSIS requires RTE meat and poultry processors to control this organism in the RTE meat processing environment and in the product.

Purpose: The inhibitory effect of MOstatin® V (buffered vinegar) and MOstatin® LV1X (buffered of lemon juice and vinegar mixtures) in Ready-to-Eat turkey products without the addition of phosphates and nitrites on the growth and inhibition of *L. monocytogenes* was evaluated.

Methods: The RTE turkey was formulated to include sea salt (1.5%), turbinado sugar (0.5%), with either MOstatin® V (0.75, 1.25 and 2.5%) or MOstatin® LV1X (1.5, 2.5 and 3.5%), along with a control (no antimicrobial). RTE turkey slices were surface inoculated with a five-strain cocktail of *L. monocytogenes* to ca. 2.0 log CFU/cm^2, vacuum packed, and stored at 10 or 4°C. *L. monocytogenes* population on the products was enumerated at regular intervals.

Results: L. monocytogenes population reached 8.19 and 7.67 log CFU/cm^2 in 7 and 28 days of storage at 10 and 4°C, respectively. At 4°C, MOstatin® V at 2.5% was the most effective concentration and inhibited *L. monocytogenes* growth to < 1 log CFU/cm^2 up to 126 days of storage. MOstatin® LV1X inhibited the growth of *L. monocytogenes* up to 84 days, with an increase of 0.97 log CFU/cm^2. Greater increases in *L. monocytogenes* was observed in products stored at 10°C.

Significance: Buffered lemon juice concentrate and Vinegar mixtures inhibited growth of *L. monocytogenes* on RTE turkey products in natural, turkey product.

P3-81 Inhibition of *Clostridium perfringens* Spore Germination and Outgrowth by Buffered Sodium Citrate and Buffered Vinegar in Corned Beef

CAROL VALENZUELA MARTINEZ, Nageswara Rao Korasapati and Harshavardhan Thippareddi
University of Nebraska, Lincoln, NE, USA

Introduction: Traditional thermal processing for the manufacture of processed meat is not sufficient to destroy spores of foodborne pathogens. The surviving spores are heat activated, and can potentially germinate and grow to high populations if product are temperature abused.

Poster

Purpose: The inhibition of germination and outgrowth of *C. perfringens* spores by Ional™ LC (buffered sodium citrates containing sodium diacetate) and MOstatin® V (buffered vinegar) was evaluated in corned beef products during abusive chilling.

Methods: Prepared, non-cooked corned beef was obtained from a meat processor. Three treatments, Ional™ LC (1 and 1.5%), MOstatin® V (1.5 and 2.0%) along with a control (with no antimicrobials) were used. Prepared corned beef was inoculated with a three strain cocktail of *C. perfringens* to obtain 2.5 log CFU/g. The inoculated product was heat activated and chilled from 54.4 to 4.0°C, in 6.5, 9.0, 12, 15, 18 or 21 h.

Results: Addition of Ional™ LC, regardless of concentration, decreased *C. perfringens* populations subsequent to chilling for all cooling profiles. Addition of MOstatin® V at 1.5% inhibited the germination and outgrowth of *C. perfringens* spores. A maximum increase of 0.73 log CFU/g was observed following 21 h chilling profile. Incorporation of MOstatin® V at 2.0% in the corned beef product resulted in no increase in *C. perfringens* population.

Significance: Incorporation of Ional™ LC and MOstatin® V into corned beef formulations were effective in controlling *C. perfringens* spore germination and outgrowth during abusive chilling.

P3-82 Thermal Inactivation of Acid, Cold, Heat, Starvation and Desiccation Stress-adapted *Escherichia coli* O157:H7 in Nonintact Beef Moisture-enhanced with Various Brine Ingredients

CANGLIANG SHEN, Ifigenia Geornaras, Keith E. Belk, Gary C. Smith and John N. Sofos
Colorado State University, Animal Sciences, Fort Collins, CO, USA

Introduction: Escherichia coli O157:H7 cells contaminating nonintact beef may have been exposed to food processing-related stresses. As such, they may be less or more sensitive or cross-protected when exposed to heat during product cooking.

Purpose: This study compared thermal inactivation of stress-adapted and unstressed *E. coli* O157:H7 cells in nonintact beef moisture-enhanced with various brine formulations and cooked to an internal temperature of 65°C.

Methods: Batches (1.5 kg) of coarse-ground beef (5% fat) were mixed with an 8-strain composite (30 ml) of acid, cold, heat, starvation, or desiccation stress-adapted or unstressed rifampicin-resistant *E. coli* O157:H7 (5 to 6 log CFU/g) and with brine solutions (120 ml; to simulate a 10% pump rate), and extruded into bags (10 cm diameter). Brines tested included distilled water (DW-control), sodium chloride (NaCl, 0.5%)+sodium tripolyphosphate (STP, 0.25%), oar NaCl+STP combined with cetylpyridinium chloride (CPC, 0.2%), lactic acid (LA, 0.3%) or sodium metasilicate (SM, 0.2%). The meat was semi-frozen (-20°C, 4.5 h) before cutting into 2.54 cm-thick steaks, and then vacuum-packaged, frozen (-20°C, 42 h), and tempered (4°C, 2.5 h) before cooking. Partially thawed (-1.8 ± 0.4°C) steaks were pan-broiled (Presto® electric skillet) to 65°C. Samples were analyzed for survivors after 4.5 h of freezing, and before and after cooking. Data (two replicates/three samples each) were analyzed using the Mixed Model procedure of SAS.

Results: After 4.5 h of freezing, pathogen counts of stressed and unstressed cells in DW-control (5.5 to 5.9 log CFU/g) and NaCl+STP-treated (5.5 to 5.8 log CFU/g) samples were similar, whereas CPC-, LA- or SM-treated samples had lower counts (by 0.4 to 1.0, 0.2 to 0.4, and 0.2 to 0.5 log CFU/g, respectively) compared to the DW-control. After 42 h of frozen storage, further reductions (0.2 to 0.6 log CFU/g) were observed in CPC-treated samples. Pan-broiling (30 ± 5 min to reach 65°C) reduced unstressed cells by 2.1 to 2.7 log CFU/g. Cold and desiccation stress-adapted cells had higher ($P < 0.05$) reductions of 3.0 to 4.5 and 2.6 to 3.9 log CFU/g, respectively, while acid stress-adapted cells had lower ($P < 0.05$) reductions of 1.3 to 1.9 log CFU/g, compared to unstressed cells. Reductions of heat and starvation stress-adapted cells were similar ($P \geq 0.05$) to those of unstressed cells, regardless of the brine treatment. Among all stressed cultures, CPC- (0.8 to 3.6 log CFU/g) and LA- (0.8 to 3.5 log CFU/g) treated samples had the lowest numbers of survivors after cooking.

Significance: These findings will be useful in risk assessments of nonintact beef products, and for development of cooking protocols for consumers and the foodservice industry.

P3-83 Survival of *Arcobacter butzleri* on Vacuum-packaged Beef Stored under Refrigerated Temperatures

SAMPATHKUMAR BALAMURUGAN and Lynda Baker
Agriculture and Agri-Food Canada, Lacombe, AB, Canada

Introduction: Arcobacter spp. have been isolated from a number of food products of animal origin. *Arcobacter butzleri* is the fourth most common *Campylobacter*-like organism after *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter upsaliensis* isolated from stool of human patients. It is also found more regularly in diarrhoeic stools than in non-diarrhoeic stools. *A. butzleri* does not grow at temperatures below 15°C and therefore, its survival rather than growth on meat is of concern. However, their survival on vacuum packaged beef is not know.

Purpose: The present study examined the survival of *A. butzleri* on vacuum packaged beef stored at refrigerated temperatures.

Methods: Sterile 10 cm^2 cores of beef muscle tissue were inoculated with ~10^6 CFU/cm^2 of *A. butzleri* ATCC 49616, vacuum packaged using commercial (40-50 cc m^{-2} 24 h^{-1}) and low oxygen transmission (0.4 cc m^{-2} 24 h^{-1}) barrier bags and stored with uninoculated controls for 6 weeks at -1.5 and 4°C. At predetermined time intervals samples were withdrawn and stomached in 90 ml sterile peptone water and serially diluted. Appropriate dilutions were plated on tryptic soy agar (TSA) and incubated microaerophilically for 48 hrs at 30°C to enumerate *A. butzleri*. Simultaneously, another sample was enriched microaerophilically for 48 hrs at 30°C in 90 ml Arcobacter selective medium (JM broth) and then swabbed on JM agar. Representative colonies from TSA and JM agar were confirmed as *A. butzleri* using PCR.

Results: A. butzleri numbers on beef muscle decreased by ~1.17-1.42 log cfu/cm^2 in the first week, but could be detected by direct plating on TSA up to 35 wks. *A. butzleri* could be recovered for the entire length of the study (6 wk) following enrichment of samples in Arcobacter selective media (JM broth). No significant ($P < 0.05$) difference in *A. butzleri* survival was observed on beef vacuum packaged using very low oxygen permeable barrier bags.

Significance: These results indicate that *A. butzleri* unlike *Campylobacter jejuni* can survive longer on meats stored under standard preservative packaging conditions.

Poster