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Ivan Parkin Lecture Abstract

"A Progress Paradox: If We Have the Safest Food Supply, Why am I Working so Hard?"

Presented by

Dr. Arthur P. Liang
Acting Associate Director for Food Safety
National Center for Zoonotic, Vectorborne, and Enteric Diseases
Centers for Disease Control and Prevention
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The public health bottom line on the safety of the food supply is whether people are getting sick from eating. From this perspective, the US has among the safest food supplies in the world. Mortality and serious morbidity due to infectious diseases, in general, and foodborne diseases, in particular, have declined dramatically in the last 150 years. During the early 20th century, contaminated food, milk and water caused many foodborne infections. Investments in urban public health, improvements in food technology, rising standard of living, improved nutritional status, and availability of vaccines and antibiotics all contributed to this decline. A review of public health textbooks from this time period identified the following as foodborne diseases: amoebiasis, botulism, brucellosis, cholera, hepatitis, salmonellosis, scarlet fever (*Streptococcus*), septic sore throat (*Streptococci zooepidemicus*), staphylococcal food poisoning, tapeworms, trichinosis, tuberculosis (bovine), and typhoid fever. Today, one's chance of becoming infected with many of these is one in a million or less.

So if we have conquered most of the major foodborne disease problems, why are we working so hard? Common explanations include that it is because we are working hard that the food supply is as safe as it is or that new problems keep emerging because of a constant evolutionary arms race between us and the germs, or that improvement in the detection and investigation has led to identifying more foodborne illnesses and outbreaks that likely went unnoticed in previous years. So, we should not be surprised to be busy working on the control of agents such as noroviruses which are infectious at very low doses, induce no lasting immunity, are relatively chlorine-resistant, and for which we can now test in the laboratory. On occasion, we can actually identify a disease like hepatitis A that illustrates this paradoxical increase in work in the face of improvements in health. Currently, in the US, HAV rates are at an all-time low. However, about 8% of these cases are reported in foodhandlers. Although a minority of these foodhandlers is likely to transmit to co-workers or patrons, public health and industry may find themselves investing tremendous effort in notifying and offering immune globulin to those potentially exposed.

However, much of our workload in both industry and government is driven by society's demands for action based on perceived risk. Society tends to overreact to risks that are novel, "controlled-by-others," acute, and catastrophic as opposed to well known, "self-inflicted," chronic and recurrent. This is why an outbreak can disproportionately drive the food safety agenda more than huge numbers of sporadic cases, while rare but catastrophic illnesses like *Listeria* and *Enterobacter sakazakii* capture more headlines than *Salmonella*, and why consumers hypothesize industry as the source of their food complaints more than their own food-handling practices.

JOHN H. SILLIKER LECTURE ABSTRACT

Rising from the Ocean Bottom: The Evolution of Microbiology in the Food Industry

Presented by

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To a large extent, food microbiology has its roots in the clinical microbiology of the 19th and early 20th centuries. The first identified foodborne pathogens—*Clostridium botulinum, Salmonella* spp., *Staphylococcus aureus* and *C. perfringens*—were also important clinical pathogens. As the modern food industry emerged after 1945, the first influential food microbiologists, as exemplified by Dr. Silliker, had some clinical microbiology experience. At that time, microbiologists had little or no standing in the food industry. Dr. Silliker once said, "In the eyes of early food industry executives, the only object on the planet worthy of less esteem than a food microbiologist was whale feces at the bottom of the ocean!" Since those dire times, several significant phenomena have greatly improved the status of microbiologists in the food industry, and these continue to provide benefits for all parties. The phenomena include: management systems, scientific professionalism, pathogen evolution and globalization, and technological advances.

Management systems

Good manufacturing practices (GMPs), as organized by FDA, and the HACCP system of food safety both originated in the 1960s. Based upon the design of food safety into a product and the process by which it is produced, HACCP proved to be far more effective in assuring food safety than the early quality control schemes that depended on product testing. Today, HACCP, supported by GMPs and other prerequisite programs, is used globally by the food industry and regulatory agencies. Numerous commercial success stories with the new systems have led to the rise of the "friendly microbiologist."

Scientific professionalism

In the beginning, it was understandable that scientists worked in relative isolation. The professionalism of food industry microbiologists has been greatly enhanced by meeting with other industry microbiologists in trade associations, and by meeting with food microbiologists in professional associations and academic and regulatory settings.

Pathogen evolution and globalization

At the beginning of food microbiology, the number of foodborne pathogens could be counted on one washed hand. Today, we can easily list several dozen foodborne pathogens. Because of increasing global travel and global trade of food ingredients and products, we are estimated to be on the verge of confrontation with several hundred additional foodborne pathogens.

Technological advances

The information technology and biotechnology revolutions together have driven many advances in the biological sciences. Food microbiologists now have laboratory methods that are far more rapid and accurate than the early methods adopted from the clinical microbiologists. The isolation and identification of salmonellae and *E. coli,* that once required 5 to 9 days, now can be completed in one day or less by advanced technologies such as polymerase chain reactions for pathogen identification, and by simple technologies such as dry rehydratable films for quantification of microbes. Until recently, only larger outbreaks of foodborne illness could be detected by conventional epidemiological procedures. Today, even very small outbreaks can be detected because of the electronic sharing of pulsed-field gel electrophoresis patterns between laboratories around the world.

All of these phenomena have elevated the importance of microbiologists in the food industry. The terms "food microbiology" and "food safety" have become nearly synonymous and the reputations of food microbiologists have risen far above the early status described by Dr. Silliker. One can hope that the early, surly food industry executives would be pleased to witness today's friendly microbiologists.

RT1 Issues Regarding Raw Milk Sales and Consumption

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Currently, pressures are being applied on US and Canadian regulatory agencies as well as legislative bodies to allow the sale of raw milk. While there is variation between agencies, most regulations prohibit the sale of raw milk for human consumption. Others allow sale, but with certain restrictions. Restrictions on the sale of raw milk are supported, in general, by public health officials and microbiologists, based upon the potential risks of pathogenic microorganisms and are substantiated by association with raw milk in foodborne illness outbreaks. Many consumer groups also support the position taken by public health officials. However, raw milk proponents extol the virtues of raw milk by presenting a variety of nutritional, health and safety claims for raw milk as opposed to pasteurized milk. The objective of this roundtable is to have an open discussion to examine all sides of the issues, to open these issues for discussion from an objective scientific perspective, to examine the legitimacy of the perceived benefit claims for raw milk, and to formulate a meaningful regulatory approach.

RT2 Refrigerated Ready-to-Eat Foods: Microbiological Concerns and Control Measures

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Refrigerated ready-to-eat (RTE) foods contaminated with *Listeria monocytogenes* have been linked to several severe listeriosis outbreaks. The contamination was mainly traced to recontamination of product from the environment. Due to the ability of *L. monocytogenes* to grow at refrigerated temperatures and the non-necessity for cooking before consumption, RTE foods contaminated with *L. monocytogenes* that support growth can be a great health concern. Other pathogens, including *Clostridium botulinum*, are also a concern, particularly in vacuum-packed RTE foods. To address these concerns, the manufacturers must establish control programs for meat and poultry that include a Hazard Analysis Critical Control Point system and sanitation standard operating procedures, and in many cases include additional heat processes, addition of antimicrobials compunds and/or product/environmental sampling programs. These control programs have been effective, as the number of cases of listeriosis have decreased in the past years, but further reductions are desired. The objective of this roundtable discussion is for the regulatory agencies, producers of refrigerated RTE foods, consumers, and researchers to review and discuss the approaches to further improve the safety of refrigerated RTE foods. The roundtable will also discuss the possibility of other food safety issues that might be associated with RTE foods due to gross abuse conditions.

RT3 Water Safety and Quality Roundtable: Global Water – HACCP Issues

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Applying HACCP to water safety and quality is complex and has often been overlooked. With the increased exchange of food products, the need for globalization of water regulations will be being discussed at this roundtable. Representatives from the United Kingdom, Canada, the United States, Mexico, and a US company that purchases seafood from Asia will discuss regulations or the lack thereof with regards to water safety and quality, and expose gaps in water HACCP concerns. In the UK, water is regarded as a food and therefore is regulated differently. A Canadian representative will talk about water hazard analysis and how the resulting analysis is put into a HACCP plan. A representative of Mexico will report the results of a joint project between their university and government to assure water quality. The US Environmental Protection Agency representative will discuss the HACCP issues surrounding the use of public water supplies as an ingredient in the foodservice industry as well in food manufacturing. The lack of regulations in water safety and quality will be discussed by a representative from a US company. After the five presentations, the panel will be questioned. A representative from Kraft Foods, who is working on globalizing their water safety and quality HACCP program, will ask questions about the impact of the various countries' rules on such a project. Senior management from a company who consults global foodservice corporations about ingredient water quality will question the panel as to what governmental oversight exists in

various countries of public water supplies used by foodservice operations. A research scientist will question the panel as to what areas of research are lacking and what needs to be done to increase awareness of globalization of water safety and quality HACCP programs. Questions from the audience will be taken, time permitting.

S01 Making Foods Safer: How Outbreaks Can Influence Change

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The World Health Organization (WHO) suggests that in industrialized countries, the annual incidence of foodborne illness affects up to 30 percent of the population each year. The US Centers for Disease Control and Prevention have reported an average of more than 1,200 outbreaks per year in the US since 2000. Outbreaks can be used to evaluate management gaps and focus policy directions of both industry and government. The goal of this symposium is to provide an overview of the prevention, investigation, and reaction to foodborne disease outbreaks, focusing on specific positive and negative outcomes. Speakers will discuss why processors, ingredient suppliers, restaurant operators, and any operations involved in the growth, processing, and distribution of food products should understand the legal, social and business consequences of a foodborne disease outbreak. Through case studies of past outbreaks, speakers will discuss how data obtained from epidemiologic investigations can be used to focus outbreak prevention strategies. Barriers to investigations will be explored, as well as crisis management responses of firms impacted by outbreaks. An example of a full corporate strategy to create and foster an internal food safety culture will be described. Post-outbreak reactions of consumers, and their influence on attitudes, will be discussed. Media coverage and the development of storylines following incidents will also be explored. The symposium will end with a roundtable panel discussion on future directions and the potential opportunities for learning from outbreaks, with the focus on reducing incidence and improving public health.

S02 Bacterial Resistance to Antimicrobials: Current Trends and Future Perspectives

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Antimicrobials are commonly used in a variety of foods to guard against foodborne pathogens. Present day consumers demand fresh tasting foods with high nutritional quality as well as microbiologically safe foods. To meet this demand the food industry has adopted the hurdle concept wherein a combination of technologies is used to process foods. This allows use of low doses of antimicrobials or low intensities of each hurdle that will help maintain the sensory attributes and attack the microbes from several targets. Antimicrobials are an important part of the hurdle concept and these are often used with other technologies such as high pressure, heat, and irradiation to control microbial growth. Although antimicrobials have been used for the production of foods with extended shelf life, prolonged and extensive use has created bacterial strains that are resistant to these preservatives. As a result, today, antimicrobial resistance is a major problem faced by the food industry and the industry has incurred huge costs to overcome this problem. Research provides sufficient evidence that antibiotic resistant strains have emerged in meat animals. One example of such resistance is the multiple antibiotic resistant *Salmonella* Typhimurium DT104 that emerged among the livestock population in UK in the 1990s. Therefore, it is important to understand the emergence of such resistance among bacteria, the causes of resistance and how this can be prevented. This symposium will provide information to help understand the emergence of antimicrobial resistance among bacteria, causes for such resistance, foods that are primarily affected by this resistance emergence and what preventive measures can be taken to avoid or prevent such emergence.

S03 The Canadian Approach to Food Safety

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The Canadian approach to food safety along the supply chain is emerging as a unique combination of HACCP in registered establishments (e.g., meat processing, dairy, etc.), national, industry-led HACCP-based programs for non-registered establishments in many of the other segments of the chain, and official recognition of these programs based on agreements amongst the federal, provincial and territorial governments. The symposium will emphasize the activity in the non-registered sphere which is led by national industry associations in partnership with government. This activity currently involves the development of 26 national commodity-specific HACCP-based on-farm food safety programs and more than 15 similar programs for other segments of the supply chain from input suppliers to final marketers at retail. While HACCP-based programs are being developed in other countries, no where else is there such a broad involvement of the supply chain or such a high degree of collaboration. Finally, what makes the Canadian approach unique is the agreement by the federal, provincial and territorial governments to develop and implement an official recognition process for these national schemes that assesses both the technical soundness of the industry "standards/requirements" and their administrative effectiveness (overall management system, auditor training, certification scheme administration, etc.). The symposium will provide conference participants with an introduction to an approach that could easily be adapted to other national circumstances.

S04 Verification of Sanitary Design of Food Equipment

F. TRACY SCHONROCK, 3-A Steering Committee Chair, 11302 Alms House Ct., Fairfax Station, VA 22039, USA; JOHN HOLAH, Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire, GL55 6LD, UK; STEVEN SIMS, US-FDA-Milk Safety Branch, 5100 Paint Branch Pkwy., College Park, MD 20740-3835, USA; PHILIP WOLFF, USDA-AMS-Dairy Grading Branch, 1400 Independence Ave. SW, Washington, D.C. 20250, USA; PATRICIA L. JOHNSON, Ontario Ministry of Agriculture, Food and Rural Affairs, 1 Stone Road W., Guelph, ON N1G 4Y2, Canada

In the United States and throughout the world, many food equipment standards and related organizations are directly or indirectly involved with the sanitary design, construction, fabrication, and cleanability of food equipment. These include: 3-A Sanitary Standards, Inc. (3-A SSI), the National Sanitation Foundation (NSF), European Hygienic Equipment Design Group (EHEDG), Underwriters Laboratories (UL), and others. Standards for specific equipment have also been written and recommended by commodity trade associations. In addition, federal regulatory agencies (e.g., Food and Drug Administration (FDA), US Department of Agriculture (USDA), Canadian Food Inspection Agency (CFIA), European Union, and others) have established requirements for hygienic and sanitary design of food equipment. Under the Hazard Analysis Critical Control Point (HACCP) system, it may be required or recommended that sanitation standard operating procedures (SSOPs) be in place that address the sanitary construction, design, maintenance, and condition of food contact surfaces and equipment. Auditing or verification that the equipment is, in fact, constructed and designed to meet specific and appropriate standards is an important element of equipment sanitary design. There is, however, some variation in regard to the specific requirements of equipment auditing programs. This symposium is intended to provide an overview of auditing programs for equipment construction and design, standard operating procedures, and related topics, and provide a forum for discussion of consistency and differences among these programs across all food processing and food handling entities. Information gained by IAFP attendees may stimulate and encourage more food equipment standards development and more uniformity in regard to these standards.

S05 Practical Risk Assessment in the Food Industry

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Microbiological risk assessment is a well-recognized approach to food safety management, particularly amongst the regulatory community. As its use has widened, industrial risk managers have become interested in how risk assessment can help them understand and manage the food safety challenges they face. While there are proponents of the application of risk assessment in an industrial context, examples of this application are few and far between.This symposium will provide examples of the practical use of risk assessment concepts and techniques by industry to optimize processes and understand and manage microbiological risks. The focus will be on the practical application of risk assessment that is accessible to all food companies, regardless of size.

S06 Foodborne Viruses and Foodborne Viral Infections: Disease Burden, Epidemiology, Detection, and Transmission

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Enteric viral pathogens have been estimated to cause about 9.3 million foodborne disease cases, including 129 deaths annually in the United States alone. While foodborne disease surveillance and prevention efforts traditionally have focused on bacterial and parasitic pathogens, the importance of viral pathogens is increasingly recognized. This symposium will provide an overview of the nature, epidemiology and transmission of foodborne viral infections, including updates on surveillance systems used for viral foodborne diseases. Since enteric viruses can be transmitted by a variety of pathways, including foods, attribution of viral infections to specific transmission routes and in the case of foodborne disease, specific foods, will also be discussed. Examples of current research efforts to characterize survival, persistence, transmission, and risk of enteric viruses and their diseases also will be presented. Attendees of this session will gain a better understanding of foodborne viral infections and their human health impact as well as the challenges associated with the detection and characterization of viral foodborne pathogens.

S07 Surrogate Microorganisms: Selection, Use, and Validation

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Crucial to the implementation of an effective HACCP plan is validation of the Critical Limits (CLs) associated with the Critical Control Points (CCPs). This can be a daunting task given the dynamic nature of some processes that render laboratory-based challenge studies either impractical or too costly to conduct. Other approaches, to validation, such as extrapolation from disparate food matrices,

tradition, or a presumed lack of foodborne illness, may lack scientific rigor. In some instances it is undesirable to work directly with a pathogen in the laboratory due to risk or difficulty in its recovery. Thus there is a need for the use of non-pathogenic microbes with correlated growth or survival kinetics to the pathogen of concern. Use of appropriate surrogate microorganisms enables companies to perform pilot and perhaps factory scale CL validations without the risk associated with use of a pathogen or the need for an on-site pathogen laboratory. FDA and USDA have encouraged the use of surrogates for validation of CLs, however many technical questions must be considered. In other cases, the use of surrogates for undesirable spoilage organisms may also be desirable. This symposium will address these issues and provide examples of appropriate selection, validation and use of surrogate microorganisms.

S08 Spores, Spores, and More Spores...What is Spoiling My Ready-to-Drink Beverage? Is It *Alicyclobacillus* or Heat Resistant Mold?

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"Now you see it... now you don't." From fleeting images in holograms to disappearing coins in shell games, this well-recognized phrase has been associated with many events of a transitory nature, including intermittent spoilage of shelf-stable, non-carbonated, high-acid beverages by *Alicyclobacillus* spp. (ACB) and Heat Resistant Mold (HRM). These thermotolerant sporeformers are of concern to processors for a number of reasons, including: (a) the ability of the spores to survive conventional hot-fill and aseptic pasteurization treatments; (b) poor detection capability, due to low contamination levels in ingredients and manufacturing environments; (c) increased growth potential in higher-oxygen environments, such as large headspace and PET packaging; (d) spoilage onset after products are already in the distribution chain; and (e) lack of overt signs of spoilage (for ACBs in general, and for HRMs in cloudy beverages). This symposium will address the sources and significance of ACBs and HRMs in high-acid beverage plants and products, provide an overview of organism growth characteristics and spoilage manifestations, identify processing steps and operational practices that select for ACBs and HRMs, review current detection methodologies, and offer potential strategies for controlling these organisms throughout the supply chain.

S09 BioSecurity at Retail

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The abundance and availability of safe food in the US has become expectation for consumers. Maintaining the safety of the food supply has been focused primarily on the control and elimination of unintentional contamination. Great strides have been made by food safety professionals and the food industry in successfully managing these natural food safety issues and crises. However, recent global events have raised concern that intentional contamination of food is not only possible but highly probable. Although food security and food safety share some elements in terms of crisis responses and monitoring, crisis prevention techniques are very different for food security versus food safety. This symposium will focus on food security at the retail level (foodservice and food retail). The primary emphasis of the presentations will be to discuss some of the unique food security issues retail operators face in an effort to enhance understanding between retailers, producers and suppliers. The food security threat and government response to it will be discussed as will some of the vulnerabilities that are inherent in retail food operations. In addition to outlining the problem, the presentations will discuss ways to mitigate the threat, respond to it, and how to plan and practice for a crisis. While the subject of this symposium is retail food defense related, a key portion of its audience is producers and suppliers to the food retail industry. The focus of the symposium is to provide the audience with a view of the challenges and issues of food security from the retail perspective. The goal of the symposium is to improve the understanding between producer/processors and retail while fostering a dialogue on food defense strategies and solutions that can be applied along this important link in the food chain. This subject is particularly relevant to US government initiatives in food defense and its recent announcement to assess the security of all major sectors of the food supply chain.

S10 Disaster Preparedness and Response

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Recent events such as the Tsunami of 2004, Hurricanes Katrina and Rita in 2005 and the earthquake in Pakistan in 2005 have made clear the vulnerability of all countries to natural disasters. Recent studies published in *Science and Nature* have reported that hurricane intensity has been increasing during the past 30 years.The inevitability of such natural disasters makes preparing for them and their impact on food safety critically important. A disaster such as a hurricane or earthquake can be devastating to a community and the need to focus on basic survival can make issues like food safety seem unimportant. However, during a natural disaster, disruptions to basic food safety measures such as refrigeration, cleaning and sanitation routines, access to potable water, and safe sanitary sewer systems can actually elevate the risk of food borne illness. This risk can continue long after the disaster is over. Rebuilding a community and getting basic community infrastructures like food retail and food service business back in operation can be

extremely problematic. This symposium will focus on food safety issues that can arise before, during, and after disasters. Discussions will include how to prepare a food service, food retailer, or food processing operation for a disaster; how to clean and sanitize after a disaster; and what to do if the recovery of the food business will take more than just soap and water. Case studies will also be presented from food businesses that have been through disasters. The case studies will discuss what went right and wrong before, during, and after the disaster.

S11 Symposium on *Enterobacter sakazakii*

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Enterobacter sakazakii is an emerging pathogen notable for its severe morbidity and mortality in human infections. It has caused meningitis in infants and bacteremia in adults and these infections have been notable for very high fatality rates despite treatment. In a few outbreaks in infants, powdered infant formula has been identified as a vehicle for transmission of this pathogen both in the United States and internationally. Overall however, relatively little is known about the clinical epidemiology of human infection with *E. sakazakii,* its environmental niche and its presence and survival in infant formula and foods. This symposium will provide the most current information on the epidemiology and clinical significance of *E. sakazakii* as a human pathogen. The design of the current US Food and Drug Administration study to investigate clinical cases of *E. sakazakii* infection will be discussed along with its strengths, limitations and preliminary data. The survival and persistence of this pathogen in powdered infant formulas and cereals will be described. Studies of the pathogenicity of *E. sakazakii* in murine and other non-primate animal models will be presented. A perspective on industry methods to ensure the safety and integrity of powdered infant formula will be presented. These talks will aim to advance our understanding of the pathogenicity of *E. sakazakii* and the ways to decrease transmission of this emerging pathogen.

S12 *Campylobacter* – From Gate to Plate

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Campylobacter is an important bacterial foodborne pathogen. While the severity of most cases of human campylobacteriosis cases is usually slight, the prevalence of human infection, the potential for the emergence of antimicrobial resistance, and the gravity of long-term sequelae such as Guillain-Barré Syndrome require that producers of raw foods make a concerted effort to reduce the exposure of consumers to this significant foodborne hazard. This symposium will give basic information about the prevalence and tracking of *Campylobacter* at the farm and in food processing facilities. It will include a discussion of current regulatory thinking about controlling this hazard, and provide information on some of the emerging technologies and strategies for managing the risk of *Campylobacter* in raw foods of animal origin.

S13 Hygiene and Sanitation Solutions to Manage Evolving Risks

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The abundance and availability of safe food in the US has become an accepted expectation for consumers. The challenge to maintaining the safety of the food supply continues to increase with larger facilities, faster production and more demands for longer shelf life. Proper cleaning and sanitation is imperative to the production of safe food. Cleaning fast and efficiently is imperative to a company's profitability and an ever growing challenge.This symposium will show how food safety can be enhanced through effective and efficient cleaning programs aimed at eliminating the environmental risks associated with food processing facilities. It will provide useful knowledge to better understand not only the challenges the industry faces, but how to measure your program to enhance its effectiveness while meeting compliance. It will answer whether contract cleaning is a viable option for your facility and provide actual time management case studies that have been shown to be effective. Producers, suppliers and sanitation mangers will benefit by understanding the challenges and what can be done to improve their cleaning programs.

S14 International Food Law – A Global Overview

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Food production, distribution and retail sale are now global activities. Every country relies upon both the import and export of foodstuffs for trade. Operating in this market is potentially problematic due to very distinct differences in food laws and food law enforcement in each country. Technical, quality, purchasing and general managers in the food industry all need to be aware of the

intricacies of food legislation. While it is difficult to be conversant about such a diverse topic, it is the responsibility of these managers to ensure that they are fully compliant with the appropriate legislation. This can be particularly difficult; for example additives permitted in certain countries are banned in others. A single theme will run through each presentation in the symposium with the aim of critically evaluating the differences and similarities between food safety legislation across the globe. The panel will be made up of six speakers, each covering a different geographical area of the world. The speakers will give an overview of key food safety legislation, make reference to other relevant legislation (e.g., compositional), and discuss enforcement strategies and practices in their region. While it is difficult to cover every country, it is hoped that the major food producing regions will be represented. This symposium draws on a wide range of expertise in the area of food law and enforcement issues. The topics covered will ensure that the attendees will be more thoroughly informed of global legislation, enforcement practices and will gain an awareness of this complex subject.

S15 Foodborne Disease Update

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The Foodborne Disease Update Symposium provides attendees with updates on significant foodborne disease outbreak investigations and other related foodborne disease issues. This year's symposium will cover two themes: outbreaks of *Cyclosporiasis* in the US and Canada in 2005 linked to basil and *Vibrio* diseases related to the consumption of raw shellfish.

S16 Contamination of Ready-to-Eat Foods: Transfer and Risk: *Listeria monocytogenes* and Other Microorganisms

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This symposium brings together some of the most recent research conducted within Europe and the US, some funded through the National Alliance for Food Safety and Security, and will provide new and updated information with regard to microbiological contamination and its transfer to ready-to-eat (RTE) foods for the development of appropriate risk assessment models. It will offer academic, industry and government food scientists with the opportunity to share experimental data and exchange experiences and expert opinion relating to microbial contamination and transfer to RTE foods and the use of transfer data in risk assessments. The symposium will focus on *Listeria* contamination levels in RTE meat and poultry, the dissemination of microbial transfer rate data from food contact vectors (air, surfaces and liquids) to the food, the factors affecting these transfers and the use of these data in the development of models for use in risk assessment. The symposium will provide: Information on the quantification and likelihood of contamination transfer to select RTE foods from select food contact vectors; guidance on how to assess these factors within a RTE food production and handling environment; information that will allow RTE food producers and handlers to make informed decisions on the risk of contamination from different vectors and prioritization of their hygiene control measures; information on the availability of models to help quantify and determine the likelihood of microbial contamination transfer in general and allow RTE food producers and handlers to undertake microbial risk assessments of their own; and transfer rate data that could be used in the development of more complex microbiological risk assessment models. The work presented addresses areas pertinent to the IAFP with regard to microbiological food safety and quality and general and applied food protection with respect to quality assurance and risk analysis.

S17 Role and Application of International Standards in Supporting Food Safety Management and Testing

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ISO 22000 food safety management along with ISO 17025 laboratory quality control accreditation conformity assessment criteria work in harmony to help maintain food product quality and safety. Together these help to provide to the consumer confidence that the food supply is as safe and secure as possible. ISO 22000:2005 is designed to allow all types of organizations within the food chain to implement food safety management systems. This includes organizations ranging from feed producers, food manufacturers, transport operators, and subcontractors to retail and food service outlets, together with related organizations such as producers of ingredients and ancillary materials. ISO 17025:2005 represents a significant advance in the application of HACCP and other food safety management principles. ISO 17025:2005 accreditation in a food-testing laboratory provides third party demonstration of competence and the ability to produce technically valid results. Sharing similar quality system management criteria with that of ISO 9001 and ISO 22000, ISO 17025:2005 extends these standards with additional requirements for the technical management of the product testing process. Requirements include criteria for equipment, environmental conditions, method validation, sampling, handling of test items,

measurement traceability, reference standards/reference materials, control of data, and assuring the quality of test results. After a brief introduction, there will be three 25-minute presentations covering the new international standards: ISO 22000:2005 Food safety management system requirements and ISO 22003 requirements for audit and certification, followed by three 25-minute presentations covering the structure and impact of ISO 17025:2005 laboratory accreditation in both a governmental and commercial food testing laboratory.

S18 A New Crack at Egg Safety: From the Hen House to Your House

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In many ways, the egg industry is the bald headed step child of US agriculture. Though eggs and egg products are an important part of most American diets, with per capita consumption for 2003 of 254.1, many people are unfamiliar with the egg industry or its current regulation status. As a research subject, eggs seem to go in and out of scientific awareness. Since passage of the Egg Products Inspection Act in 1971, most of the regulations for the egg industry have been focused on providing high quality eggs to consumers and in the process, egg safety was improved. Currently, safety-based egg regulations are being drafted for the US egg industry. In September of 2004, FDA released a proposed rule for on-farm egg production. In 2006, FSIS is expected to publish a proposed rule for liquid egg processing. As eggs move to the regulatory fore-front, consumers, industry, and researchers will be more concerned with the safety of eggs and egg processing as well as issues of sanitation in egg processing plants. In this symposium, FDA will provide highlights of the proposed rule and FSIS will discuss their risk analysis of egg-borne salmonellosis and aspects of their upcoming proposed rule. This symposium will provide an overview of the egg industry, a description of modern egg processing and how it affects microbial populations, information on egg plant sanitation, and efforts to minimize contamination by important pathogens such as *Salmonella* Enteritidis.

S19 Cleaning and Sanitation for Retail Food Safety – Identifying the Issues

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Much of the focus of food safety efforts from government and academia in the past has been on food production and processing. Comparatively little attention has been given to food safety at the retail level. Cleaning and Sanitation (C&S) are critical elements of any plan to prevent foodborne illness and are as important at food retail as they are to the food processor. A good C&S program helps prevent cross contamination, restricts environmental conditions in which microorganisms can grow, and creates a safer more appealing environment for customers and employees. Although the importance of C&S is generally well recognized by the food industry, there are many practical barriers to implementation of a strong C&S program, particularly at the retail level. These can include high employee turnover, a comparatively uncontrolled environment, inadequate understanding of the importance of C&S on the part of store management and the operators responsible for C&S activities, varying regulations, poor facility and equipment design, and difficulties in developing good training programs. This symposium will highlight the current situation and best practices for C&S in the food retail environment. It will focus on issues, barriers, and concerns faced by the food retail industry. Topics covered will include an introduction to the grocery and food service retail environments and present day C&S best practices and solutions for those environments, a retail regulatory update, equipment and facility design issues associated with the food retail environment, and some of the challenges and opportunities associated with training of food retail workers.

S20 Public Health and Environmental Impact Assessments in the Aftermath of Hurricanes Katrina and Rita

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Hurricanes Katrina and Rita caused extensive damage in the central Gulf States region in August and September of 2005. High winds, heavy rainfall and storm surge associated with the hurricanes, and the de-watering of New Orleans into Lake Pontchartrain in their aftermath, were causes for national concern regarding the welfare of the affected population and the coastal ecosystems. Secondary only to human safety and welfare, concerns about the environmental impact of possible widespread industrial and wastewater contamination in the region resulted in the mobilization of state and federal public health and environmental health agencies. The agencies coordinated public health and environmental impact assessments of biological conditions, fisheries, water quality, sediment quality, seafood safety and human health risks in coastal ecosystems of Louisiana, Mississippi, and Alabama. This effort characterized the magnitude and extent of coastal contamination and ecological effects resulting from the unprecedented storms. This symposium provides a brief description of the various components of this comprehensive, coordinated, interagency effort.

S21 Assuring Microbiological Safety of Organic Products

EWEN C. D. TODD, Michigan State University, 165 Food Safety & Toxicology Bldg., East Lansing, MI 48823, USA; HARSHAVARDHAN THIPPAREDDI, University of Nebraska-Lincoln, Dept. of Food Science and Tech., 236 Food Industry Complex, Lincoln, NE, 68583, USA; CRAIG HARRIS, Michigan State University, Dept. of Sociology, East Lansing, MI 48823, USA; STAN BAILEY, USDA-ARS-SAA, 950 College Station Road, Room 551, Athens, GA 30605-2720, USA; TREVOR SUSLOW, University of California-Davis, Dept. of Plant Sciences, Mail Stop 3, One Shields Ave., Davis, CA 95616-8780, USA; FRANCISCO DIEZ-GONZALEZ, University of Minnesota, Dept. of Food Science and Nutrition, St. Paul, MN, 55121-1560, USA

The total organic food sales in the US is estimated to be $10 billion, with over 5,000 new food and beverage products introduced to the US retail market in 2003. While most of the organic products presently marketed are raw agricultural commodities that require minimal processing, processed agricultural products are increasingly being introduced. Assuring the safety of these products is a challenge as the ingredients for use in organic products are regulated. Microbiological risks and the tools to assure safety of organic products are limited, although food safety performance standards for most of these products are the same as conventional products. The proposed symposium addresses some of these food safety issues and challenges posed by a variety of organic foods, and identifies possible antimicrobial intervention technologies for use during processing to assure the safety of organic products.

S22 *Salmonella*: The Saga Continues

MICHAEL LYNCH, CDC, 1600 Clifton Road, NE, Mail Stop A38, Atlanta, GA 30329, USA; HEEJEONG LATIMER, USDA-FSIS, 10004 Woodrow St., Washington, D.C. 20036, USA; STAN BAILEY, USDA-ARS-SAA, 950 College Station Road, Room 551, Athens, GA 30605-2720; USA; KEITH WARRINER, University of Guelph, Dept. of Food Science, Guelph, ON N1G 2W1, CANADA; PAULA FEDORKA-CRAY, USDA-ARS, 950 College Station Road, Athens, GA 30605, USA; MARTA HUGAS, European Food Safety Authority, Largo N. Pailli 5/A, Parma, I-43100, Italy

Salmonella has re-emerged as the leading cause of foodborne bacterial enteric disease in humans and is the only major enteric bacterial pathogen that has not seen reductions in human illnesses in recent years. Despite the implementation of new regulations by the US Department of Agriculture's Food Safety and Inspection Service, and large expenditures by the poultry industry, the level of *Salmonella* in processed poultry has not been significantly reduced. In addition, better attribution models have shown that tomatoes and other fruits are responsible for a large number of outbreaks of human salmonellosis. The use of antibiotics has been reduced, but there are continuing concerns about the development of antimicrobial resistance in bacteria associated with animal production systems. In Europe, *Salmonella* Enteritidis continues to be the predominant serotype of concern, but many new issues are emerging. A distinguished panel of experts will discuss these and other issues surrounding an old pathogen, *Salmonella*: The Saga Continues.

S23 How Risk Managers Decide on Microbiological Risks from Different National Perspectives

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The framework of risk analysis is being adopted by many nations in the world. A significant number of microbial risk assessments (MRAs) have been developed over recent years under the auspices of (inter-) governmental bodies that fit within this framework. These assessments typically would be commissioned by risk managers in order to obtain pertinent information on which to base their decisions, for instance, whether or not interventions are needed to reduce a risk in light of other risk management priorities, and which interventions would then be most favorable, comparing the possible outcome of different intervention scenarios. When a decision on interventions has been made, risk managers would need to consider providing specific guidance to the food industry and possibly, consumers, including adopting new standards in legislation where these are deemed necessary. Until now, only a few countries have gained experiences in the uptake of MRA studies and their use in making policy decisions. This symposium is intended to share and discuss risk management experiences more broadly and to highlight both successes and problems encountered in the process of using MRAs in risk management.

S24 Food Allergen Control at Retail and Food Service

KATHLEEN O'DONNELL, Wegmans Food Markets, Inc., 1500 Brooks Ave., P.O. Box 30844, Rochester, NY 14603, USA; GALE PRINCE, Kroger Company, 1014 Vine St., Cincinnati, OH 45202-1100, USA; DONNA GARREN, National Restaurant Association, 1200 – 17th St. NW, Washington, D.C. 20036, USA; CHRISTINE BRUHN, University of California-Davis, 1 Shields Ave., Davis, CA 95616-8598, USA

The Food Allergen Labeling and Consumer Protection Act (FALCPA) effective January 1, 2006 requires food allergens to be declared in plain English on food packages sold to consumers. Less clear for consumers concerned about food allergens is food allergen declaration and management in the retail and food service environments. It is estimated that 11 million Americans have food allergies, resulting in 30,000 emergency room visits and up to 200 deaths each year. The only action certain to prevent an allergic reaction is for clear communication to the food allergen-sensitive individual regarding the allergen composition of the food. This symposium will present the unique challenges along with best practices for allergen management and consumer communication in the retail and food service environments.

S25 Hot Topics in Food Safety

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The field of food safety continues to change and evolve. Accordingly, it is critical that today's food safety professional stay informed of the latest trends and science. This symposium will cover a host of hot topics ranging from an update on the current status of avian influenza and what it means to the food industry, to trends concerning outbreaks of non O157:H7 *E. coli* in Europe and the potential implications for other parts of the world. In addition, you'll hear leading experts discuss recent food safety developments in the European Union and an enlightening discussion on the reality of politics in food safety with emphasis on the need to move beyond politics to science-based decisions and policies.

S26 Quality Control in Research Labs

CHRISTINA OSCROFT, Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire, Gl55 6LD, UK; ARLENE FOX, AOAC International, 481 North Frederick Ave., Suite 500, Gaithersburg, MD 20877, USA; MARK CARTER, Silliker, Inc., 2057 Builders Place, Columbus, OH 43204, USA; LORALYN LEDENBACH, Kraft Foods, 801 Waukegan Road, Glenview, IL 60025, USA

Food manufacturers use microbiological data to ensure the quality and safety of their products. These data may be generated by the manufacturers' internal laboratory or by contract laboratories. The manufacturers must use due diligence to ensure that these laboratories provide accurate, reliable data that is capable of withstanding legal scrutiny. Erroneous data can lead to negative and costly consequences. Assurance of accurate data is generally achieved through a laboratory quality assurance program of some kind. With increasing globalization within the food industry, an understanding of what constitutes a good and acceptable laboratory quality assurance program from a global perspective is essential. In this symposium, speakers from industry, research (CCFRA) and AOAC will discuss the basic requirements for a good laboratory program, ideas on harmonization of global standards, and tools for measuring the effectiveness of these programs and international standards.

T1-01 Enrichment Protocols Containing Specific Bacteriophage Reduce False Positive and Negative Results in Food Pathogen Detection Methods

JAMES W. STAVE, Meredith Sutzko, and George B. Teaney, Strategic Diagnostics Inc., 128 Sandy Drive, Newark, DE 19713, USA

Antibodies to *Salmonella lipopolysaccharide* (LPS) were used to develop immunochromatographic assays for the detection of *Salmonella* spp. in food samples. Cross-reactive bacteria expressing *Salmonella*-specific LPS determinants were isolated using standard food sample enrichment protocols. Lytic bacteriophage specific for the cross-reactive bacteria were tested to assess their capacity to prevent the growth of undesirable bacteria during enrichment. The kinetics of *Salmonella* Enteritidis growth (0, 0.02, 0.1, 2.0, and 10 CFU/mL initial concentrations) was determined in the presence of *Citrobacter freundii* (10^5 CFU/mL) and bacteriophage lytic for *C. freundii* (10^8 CFU/mL). Without phage, initial *Salmonella* concentrations of 0.02 and 0.1 CFU/mL in the presence of 10^5 CFU/mL *C. freundii* did not reach levels detectable by the immunochromatographic test during the course of the experiment (24 h). With phage, *Salmonella* was detected in the same treatments in 12 h. At a starting concentration of 2 CFU/mL, *Salmonella* was detected 6 h earlier in a culture containing phage compared to the same treatment without phage. *Salmonella* concentrations determined by differential plate counts at the completion of the study were 2–3 orders of magnitude higher in the presence of phage. To assess the utility of this approach for routine analysis of food, five different bacteriophage specific for a large host range of cross-reactive bacteria were incorporated into the media of a sample enrichment protocol. Thirty-five samples of beef were enriched with and without phage and tested using an immunochromatographic test strip specific for *Salmonella*.Thirteen of the 35 samples from the non-phage enrichment were positive by the immunochromatographic test but the results could not be confirmed by cultural methods. No positive results were reported from samples enriched using the phage-supplemented media. These results demonstrate that the use of bacteriophage may control competing and crossreacting bacteria in sample enrichment protocols and provide a basis for improved methods with reduced false positive and negative results.

T1-02 Enrichment Time, Media Ratios, and Immunomagnetic Separation as Factors in the Rapid Detection of Very Low Levels of *Escherichia coli* O157:H7 in 375 g Trim Samples

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Current validation studies by manufacturers who market rapid enrichment and detection methods for *Escherichia coli* O157:H7 in beef trim utilize a smaller amount of material than most ground beef producers test. 375 g samples are usually enriched and tested instead of the smaller amounts validated. Because of the limited amount of product assayed in validation studies, publicly available data is limited on the behavior of low contaminating levels of *E. coli* O157:H7 in these larger samples. This study documents the behavior of approximately 1–2 CFU *E. coli* O157:H7 when enriched in the presence of 375 g trim samples, using three different media ratios, and different enrichment times, using the DuPont Qualicon BAX® System *E. coli* O157:H7 and *E. coli* O157:H7 MP test kits. Beef trim samples were inoculated at a level such that some samples would receive no cells and those which did receive the pathogen would be expected to have only 1 or 2 cells. Trim samples were then enriched in trim to media plus trim ratios of 1:10, 1:5, and 1:2.7. BAX® assays (*E. coli* O157:H7 and *E. coli* O157:H7 MP and MP express) were conducted at 8, 10, 12, and 24 h using standard BAX® system protocols. Additionally, at the 8, 10, and 12-h time points an immunomagnetic separation was conducted and the three assays were performed on the resulting material. Our results indicate that enrichment of 375 g trim samples for 10 h would reliably detect very low levels of cells. Additionally, enrichment in 4 and 9 parts media to 1 part trim gave equivalent results while enrichment in 1.7 parts media to 1 part trim gave unacceptable results. The study will give valuable guidance to industry in terms of tradeoffs involving enrichment time, media costs, and sensitivity of their testing programs.

T1-03 Comparison of Two Enrichment Broths for the Recovery of *Campylobacter* spp. from Carcass Rinses from Several Commercial Processing Plants

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Campylobacter is the leading cause of acute bacterial gastroenteritis worldwide. Better cultural recovery methods for *Campylobacter* spp. are needed in order to accurately develop an understanding of the epidemiology and ecology of this organism in poultry. The objective of this study was to compare a traditionally used culture method (Bolton's enrichment broth containing lysed horse blood) to a newly developed proprietary Tecra® *Campylobacter* enrichment broth for the recovery of *Campylobacter* spp. from chicken carcass rinses. A total of 390 carcass rinse samples from four different locations on the processing line were taken from 17 processing plants located throughout the United States. An aliquot of the rinse from each sample was put into Bolton's and Tecra® enrichment broths and standard laboratory procedures using Campy Cefex plates were followed for the recovery of *Campylobacter* spp. For all carcass rinse samples, *Campylobacter* spp. were recovered from 84% (328/390) of the rinse samples in Tecra® enrichment broth and from 67% (261/390) of the rinse samples in Bolton enrichment broth. Evaluation of background microflora on Cefex plates showed that Tecra® enrichment broth significantly ($P > 0.05$) reduced the background microflora when compared to Bolton's enrichment broth. Tecra enrichment broth yielded 17% more positive samples than did Bolton's enrichment broth, primarily because the level of background microflora on Cefex plates were reduced making it easier to identify suspect *Campylobacter* colony forming units. In this study, Tecra enrichment broth proved to be superior overall to the presently used conventional cultural method for the detection of *Campylobacter* spp. in chicken carcass rinses.

T1-04 A New Immunochromatographic Strip-based Method for the Determination of *Salmonella* in Meat and Poultry

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Immunochemical methods for the detection of *Salmonella* in food samples typically rely on the analysis of flagella or lipopolysaccarides (LPS) present on the cell surface and/or released into the surrounding media. Although highly sensitive, these methods are historically complicated by the fact that several closely-related *Enterobacteriaceae* (e.g., *E. coli, Citrobacter* sp.) also share some of these same antigenic factors and may be detected by the *Salmonella* immunoassay, resulting in a false positive result. For the end user, false positive results can result in incurred costs in confirmation, labor, lost time, and food product quarantine thereby disrupting the release of food product. New advances in media technology incorporate the use of specific bacteriophages that prohibit the growth of potentially cross-reactive bacteria while encouraging the growth of *Salmonella.* Further improvements in selectivity was achieved through the use of antibiotics. In a ground beef study, 20 out of 100 samples were found to be falsely positive using non-selective pre-enrichment media whereas zero (0) were found when specific bacteriophage were included. By combining antibiotics, specific bacteriophage, and *Salmonella* spp. antibodies in a immunochromatographic test strip, a highly selective method was developed resulting in improvements from both a performance and ease-of-use perspective. The new method was compared to the USDA-FSIS cultural method, and two commercially-available methods (PCR and automated ELISA) for the analysis of raw ground turkey spiked to 0.3 and 1 CFU per 25 g. The new method gave 37 positive results out of 37 confirmed positives whereas the PCR and ELISA gave 33 and 36 positive results out of 37 confirmed positives by the USDA method, respectively. Though the performance data between the methods were comparable, the new immunochromatographic strip-based method was advantageous from a sample handling perspective, in requiring only a single transfer step versus 6, 3, and 7 for the USDA-FSIS, PCR, and ELISA methods, respectively.

T1-05 Detection of *Salmonella* in Chicken Carcass Rinses Using a Chromogenic Agar Plating Medium

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Salmonella remains one of the most important foodborne pathogens and its association with poultry is still considered significant. While an extensive range of methods exist for the detection of the bacterium, many laboratories still employ conventional cultural methods. There is a diversity of plating media available, though most recent developments for the detection of *Salmonella* (and other foodborne pathogens) have focussed on chromogenic media, incorporating a substrate which, upon cleavage by a specific enzyme activity, yields a coloured compound. The aim of this study was to evaluate the performance of one such medium, CHROMagar *Salmonella* (CS), against the USDA conventional cultural method for the detection of *Salmonella* in rinses of whole chicken carcasses. Rinsate from each carcass was pre-enriched in buffered peptone water, and then enriched selectively in Hajna's tetrathionate broth (TT) and Rappaport-Vassiliadis (RV) medium. Each selective enrichment was plated to brilliant green sulfa (BGS) and modified lysine iron agar (mLIA), as standard media, as well as CS. Colonies typical of *Salmonella* were confirmed biochemically using slants of TSI and LIA and those showing atypical reactions were tested using latex agglutination. Based on analysis of 480 samples, CS proved highly effective, with a sensitivity of 96.7% when compared to the combination of the two standard media. The sensitivity of CS was identical (100%) to that of BGS alone and superior (134%) to that of mLIA alone. While the level of background on CS was generally very low, the specificity of the medium was lower than that of BGS or mLIA. While more positives came from TT than RV enrichment, the latter proved more specific. As CHROMagar *Salmonella* is marketed as a single plating medium for *Salmonella*, this study shows that to be feasible for the detection of the bacterium from whole chicken carcass rinses.

T1-06 Evaluation of the Oxoid Biochemical Identification System (OBIS) *Salmonella* Colony Confirmation Test for Use in Veterinary Laboratories

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Recent improvements in culture methods involving semi-solid selective media and chromogenic agars have increased the sensitivity and specificity of detection of *Salmonella* from fecal and environmental samples and in many cases allow for more rapid identification of the organism. Conventional biochemical and serological confirmatory tests for colony confirmation are still often laborious, time consuming and expensive and there is a need for a more pragmatic but accurate approach. The OBIS *Salmonella* test is a rapid colorimetric test for the determination of pyroglutamyl aminopeptidase (PYRase) and nitrophenylalanine deaminase (NPA) activity. The lack of PYRase and NPA activity can be used to differentiate *Salmonella* from other organisms. A panel of 305 different *Salmonella* serovars from the Weybridge culture collection were tested with the OBIS test and all gave the expected result. 18 non-*Salmonella* isolates which had been erroneously submitted as *Salmonella* by diagnostic laboratories were also tested and 4 of these, all *Hafnia alvei*, gave false positive results. In parallel with this, 102 suspect *Salmonella* field isolates were tested direct from the primary isolation medium, Brilliant Green Agar. The isolates were also tested by slide agglutination, ONPG, Urea and API 20E. 46 of these isolates were identified as *Salmonella* and confirmed by full serotyping. 44 isolates, representing 15 different serovars, gave the expected results with the OBIS test. 2 of 4 isolates of *S.* Senftenberg gave inconclusive reactions in terms of very weak positive reactions with the PYR reagent. 53 non-*Salmonella* isolates were correctly identified. 3 non-*Salmonella* organisms gave false positive reactions but these were also oxidase positive so would have been excluded because of this. The combination of the OBIS test plus direct slide agglutination with poly H and, where indicated, somatic antisera proved to be a very rapid and accurate test and was adopted for routine diagnostic use throughout the organization.

T1-07 Validation of a New Alternative Automated Immunoassay Method for the Simultaneous Detection of *Listeria monocytogenes* and *Listeria* Species in Food and Environmental Samples

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Listeria screening methods often give only limited information such as in the presence of *Listeria* species or in the specific presence of *Listeria monocytogenes*. Simultaneous screening for both *Listeria* genus and presence or absence of *L. monocytogenes* is valuable information to better manage *Listeria* risk in the production process. To answer this need, a new immunoassay method, the VIDAS® *Listeria* DUO assay, was developed, based on the use of a cocktail of antibodies against *L. monocytogenes* and all *Listeria* species. A study was conducted by AFNOR to validate the performance of this new method in comparison with the ISO 11290-1 reference method, according to the ISO 16140 guidelines. The inclusivity study, performed on 50 *Listeria monocytogenes* and 30 *Listeria* non-*monocytogenes* strains, and the exclusivity study performed on 30 *Listeria* non-monocytogenes (for the LMO detection of the test) and on 31 non *Listeria* strains demonstrated 100% specificity. Inoculated studies were performed on whole milk, potted meat, smoked salmon, red cabbage and process water and 6 pure culture *Listeria* isolates. Fractional positives were observed with inoculum levels below 1 CFU/25 g. The detection limit was calculated by Spearman Kärber method to be situated between 0.3 and 2.5 CFU/25 g for both methods. A comparative study was conducted on 374 food products and 92 environmental samples. The immunoassay method detected 265 *Listeria* positive samples (94.6% sensitivity) compared to 254 for the reference method (90.7% sensitivity) and 171 *Listeria monocytogenes* positive samples (96.1% sensitivity) compared to 162 (91.0% sensitivity) for the reference method. Data presented in this study demonstrated equivalency of the VIDAS *Listeria* DUO with the ISO reference method and was granted AFNOR approval. The new method fulfils the European criteria for its use as an alternative method for the simultaneous detection of both *Listeria monocytogenes* and *Listeria* species in food and environmental samples.

T1-08 DSC Verification of the Reliability of the Time Temperature Integrators Made from the á-amylase of the *Bacillus amyloliquefaciens* for Assuring the Safety of Various Thermal Processes

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The use of Time Temperature Integrators (TTIs) based on the thermal properties of á-amylase from *Bacillus amyloliquefaciens* as food safety and process validation tools have been studied. TTIs and thermocouples were exposed to various non isothermal industrially relevant heat treatments in order to verify the accuracy of the TTIs. The various processes were delivered by a Peltier thermoelectric module; which was shown to be successful in reproducing the various non isothermal heat treatment profiles. The TTIs' responses correlated well with the thermocouples for critical non isothermal processes. However, the TTI error increased compared to the thermocouples as the holding time of the heat treatment increased, but it still represents a valuable process validation tool. Conversely, for very short and potentially not industrially relevant heat treatments, the responses of the TTIs presented poor accuracy. This work shows that the TTIs can be successfully used as a tool for process validation, particularly when the processing environment is inaccessible for the thermocouples. Beyond very short processes, *P*-value of 1 to 2 min, the accuracy of the TTIs is excellent; however, when the *P*-value is above 8 min, their accuracy diminishes. After 8 min, the assured safety is delivered since the food has received more and more cooking and so the absolute accuracy of TTI becomes less imperative.

T1-09 A Comparison of Pulsed-field Gel Electrophoresis Patterns Obtained from FSIS Routine and Intensified Verification Testing Programs for *Listeria monocytogenes*, 2002–2005

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As part of its strategy to control *Listeria monocytogenes* (*Lm*) in Ready-to-Eat meat and poultry products, the Food Safety and Inspection Service (FSIS) collects product samples through routine sampling programs and product, food-contact surface (FCS) and nonfood-contact (NFCS) samples through the Intensified Verification Testing (IVT) program. Establishments are identified for IVT sampling based on previous positives or evidence of ongoing sanitation problems. Following routine cultural confirmation, FSIS performs pulsed-field gel electrophoresis (PFGE) on all *Lm* isolates. Patterns from routine and IVT sampling were compared within and across establishments and over time. We identified 273 samples collected from 59 establishments with at least one IVT positive during a three-year period beginning November 2002. Patterns from routine and IVT sampling were compared using an algorithmic method followed by visual inspection. We identified fifty clusters with identical patterns using two-enzyme PFGE. Thirty-seven (74%) clusters were collected from single establishments and 23 (46%) clusters were collected on single days. The high proportion of clusters from single establishments in this dataset may indicate that *Lm* PFGE types are more likely to be establishment specific than distributed across establishments. Among the 37 clusters collected from single establishments, 10 (27%) were from both FCS and product samples, 12 (32%) were from both NFCS and product samples, and 6 (16%) were from all three sample types. These data indicate that NFCS may represent important sites for direct or indirect product contamination. Twelve clusters were identified from routine samples followed by IVT samples collected at the same establishment at a later date (range: 36–206 days). The repeated finding of identical patterns in establishments over time indicates that sanitation may be ineffective. PFGE can be used by FSIS to identify cross contamination and harborage and provide useful information in ongoing efforts to protect public health.

T1-10 Quantitative Transfer of *Listeria monocytogenes* from Conveyor Belt Materials to Deli Ham

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Listeria monocytogenes is a serious foodborne pathogen that continues to threaten the processed meat industry with conveyor belts identified as a major vector for *Listeria* transfer. To assess this problem, polypropylene, high-density polyethylene, and acetyl conveyor belt pieces measuring 10 × 9 cm were inoculated with a 6-strain *L. monocytogenes* cocktail to yield ~10^6 and 10^3 CFU/cm^2. After 45 min of drying, one ham slice (20 g, 72.5% or 60.6% moisture) was placed on the inoculated surface, followed by a 12-oz

deadweight for 1 min and then removed. This process was repeated until 15 (10^3 CFU/cm^2) or 30 (10^6 CFU/cm^2) slices consecutively contacted the original inoculated surface. Each slice was diluted 1:5 in phosphate buffer solution or University of Vermont medium (UVM) for enrichment and stomached for 1 min. Thereafter, aliquots were spread-plated (0.1 ml) on Modified Oxford agar (MOX) or pour-plated (5 ml) in 20 ml of MOX, using 150-mm diameter Petri plates, and incubated at 37°C for 48 h, with the latter protocol giving a detectable limit of 0.5 CFU/g (10 CFU/slice). Regardless of the type of ham or conveyor belt material, greater *Listeria* transfer was seen from slice 1 to 15 than from slice 16 to 30 at 10^6 CFU/cm^2 and from slice 1 to 10 than slice 11 to 15 at 10^3 CFU/cm^2. Overall, > 90% of the *Listeria* population transferred to ham during the first 10 contacts with no significant differences ($P > 0.05$) seen between the three conveyor belt materials or two product moistures. These findings suggest that discarding the first 10 slices of deli meat could reduce the risk of *Listeria* contamination, and that conveyor belt materials and product moistures are unlikely to significantly impact the rate of *Listeria* transfer during manufacture of deli meats.

T1-11 Identification of an Effective Strategy for Microbiological Reduction on Cattle Hides

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Responding to research results identifying the hide as the major source of *Escherichia coli* O157:H7 contamination of beef carcasses, the USDA-FSIS has suggested that *E. coli* O157:H7 is reasonably likely to occur on cattle entering the processing facility, requiring modification to their HACCP plans. This study compared the antimicrobial efficacy of several reportedly effective hide decontamination interventions. Whole beef hides were inoculated with *E. coli* O157:H7 and *Salmonella* and decontaminated with acetic acid (AA; 10%, 55°C), lactic acid (LA; 10%, 55°C), sodium hydroxide (SH; 3%, 23°C), sodium metasilicate (SM; 4%, 23°C), potassium cyanate (PC; 2.4%, 30°C), sodium sulfide (SS; 6.2%, 30°C), sodium hydroxide (1.5%), followed by high pressure washing with hypo-chlorinated (200 ppm) water (SHC; both applied at 23°C) or water (W; 23°C). The AA, LA, SH, SM and W treatments were applied for 30 s at 2.04 bar, allowed to dwell for 2 min and neutralized with a 30 s deluging water rinse. The PC and SS were dehairing treatments that consisted of a 3 L application, 90 s dwell, additional 3 L application followed by a 60 s dwell and neutralization. The SHC treatment incorporated a 30 s SH (1.5%) application at 2.04 bar followed by a hypo-chlorinated water wash for 30 s at 34 bar. Following neutralization, hide samples were obtained for microbiological analysis. The PC, SS and SHC treatments resulted in the greatest ($P < 0.05$) reductions of *E. coli* O157:H7 by 5.1, 4.8 and 5.0 log CFU/cm^2, respectively. Similarly, the SS and SHC also decreased *Salmonella* by 2.5 and 2.7 log CFU/cm^2 more than the water treatment ($P < 0.05$). The SH, AA and LA treatments also lowered *E. coli* O157:H7 and *Salmonella* by at least 2.0 log CFU/cm^2. These effective treatments deserve further consideration for commercial implementation as hide-decontamination interventions.

T1-12 Controlling *Listeria monocytogenes* on Ready-to-Eat Meat and Poultry Products Using Antimycotic Agents

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While the antimicrobials lactate and diacetate have been used effectively to control growth of *Listeria monocytogenes* (*Lm*) on cured Ready-to-Eat (RTE) meat and poultry products, alternate antimicrobials are still needed to provide choices for safe formulations, particularly for uncured products. This study was designed to identify specific levels of sorbate, benzoate, and propionate that prevent growth of *Lm* on sliced, cooked, uncured turkey breast and cured (with 156 ppm sodium nitrite) ham. Sixteen formulations of each product type were manufactured to include potassium sorbate, sodium benzoate, or sodium propionate, used alone and in combination (up to 0.3% w/w meat block), or with lactate-diacetate combinations. Products were inoculated with 5-log CFU *Lm* per 100 g package, stored at 4, 7, or 10°C for up to 12 weeks, and triplicate samples/treatment assayed biweekly by plating on Modified Oxford agar. Data revealed that 0.1% benzoate, ≥ 0.1% propionate, 0.3% sorbate prevented growth of *Lm* on cured ham stored at 4°C for 12 weeks, compared with >1-log increase in 4 weeks for the control ham without antimicrobials. When no nitrite was included, combinations of ≥ 0.1% propionate + 0.1% sorbate or ≥ 0.2% propionate were required to prevent listerial growth on uncured turkey stored at 4°C for 12 weeks. Inhibition was less pronounced when formulations were stored at abuse temperatures. When stored at 7°C, treatments delayed listerial growth for 4 weeks, but supported significant growth at 8 weeks. All treatments supported >1-log listerial growth when stored at 10°C for 4 weeks. These results verify that antimycotic agents inhibit growth of *L. monocytogenes* on RTE meats, but are more effective when used in combination with nitrite and at 4°C. Data collected will be used to petition USDA for approval to use propionate, benzoate, and sorbate as anti*Listeria*l agents in Ready-to-Eat meat and poultry products.

T2-01 Evaluation of a Process Specific Information Resource to Assist SME Food Manufacturers with Hazard Analysis

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In the UK the application of hazard- and risk-based quality management systems is a legal requirement. Small companies can have a poor understanding of such systems and provision of information resources has been identified as a strategy to assist small businesses. A process-specific information resource was developed, taking into account the requirements of the chilled and frozen soups and sauces sector. The differences between SMEs (10–250 employees) and microbusinesses (9 or fewer employees) were also investigated. An evaluation of the resource was performed. 123 companies were recruited (approximately 90% of eligible companies), and randomly divided into control and intervention groups. There were equal numbers of SMEs and microbusinesses in each group. Companies in the intervention group only were given the resource for a period of three months. A tool was developed to assess the knowledge, behavior and attitude of the person responsible for food safety with regard to specific food safety practices. A research team evaluated knowledge and behavior while attitude was assessed via a self-report questionnaire. All questions related directly to the information in the resource. All companies were visited and assessed after three months. Attrition was high, with 81 of 123 companies

withdrawing from the study before assessment. There were no statistically significant differences between the intervention and control groups in the knowledge, behavior or attitude components of the assessment tool, for either SMEs or microbusinesses. Additional data analysis, however, showed that, in the intervention group, SMEs scored significantly higher for both knowledge and behavior than microbusinesses. There were no significant differences in the attitude scores. There were no statistically significant differences between SMEs and microbusinesses in the control group in any of the three components of the assessment tool. The intervention strategy employed was not successful in improving the knowledge, attitudes or behavior of the participants.

T2-02 Understanding the Implementation of Enhanced Food Safety Controls among Non-federally Registered Food Processors in Ontario

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The challenge of ensuring food safety has become more important with increased awareness of the level and nature of foodborne illness and associated economic and social costs. This has motivated governments and the food industry to implement food safety controls such as GMPs and HACCP to strengthen consumer confidence and manage risk. Firms assess the costs and benefits of implementing food safety controls in the context of the regulatory environment and marketplace demands. At the same time, although there may be economic incentives to enhance food safety controls, barriers may prevent firms from actually implementing such systems. This study investigates the process through which controls are implemented in the Canadian food processing sector and quantifies the motives for and barriers to adoption of food safety controls. Non-federally registered food processors are largely small and medium-sized, and this sector, world-wide, presents significant risk. Data collection involved a postal survey of food processing plants in Ontario among different sizes of firm and sectors. A total of 4,001 plants were mailed a questionnaire in October 2005, and 714 responses were returned, of which 363 were eligible. The results indicate that the main motivators to implement HACCP were the desire to comply with 'good practice', the expected impact on product safety and the anticipation of future customer requirements. Expected reductions in legal and insurance costs were considered unimportant. Predominant barriers to the implementation of HACCP were associated with high external consultant fees, internal budgetary constraints and problems obtaining external funding. The results suggest a number of strategies to be designed and identified through which the motives to adopt enhanced food safety controls may be strengthened and/or barriers reduced. Also, ways in which non-federally registered food processors may gain commercial and/or market advantages from adopting enhanced food safety controls need to be explored.

T2-03 Staging the Implementation of HACCP among Small and Medium-sized Food Processing Establishments in Ontario

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HACCP is an internationally recognized food safety system, used to prevent food safety problems from occurring. Successful implementation of HACCP depends on an underlying foundation of prerequisite programs, or Good Manufacturing Practices (GMPs), that control basic food safety risks such as environmental contamination and personnel-related hazards. Around the world, small and medium-sized food processors (SMFPs) present a significant risk to food safety, and it is in this sector that HACCP implementation presents a major challenge. In Ontario, SMFPs represent a large proportion of the food processing sector. The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) worked extensively with industry to develop a HACCP approach that is feasible and practical for SMFPs, while maintaining high food safety standards. In 2004 the HACCP Advantage was launched, with auditing and certification services, provided by the Canadian General Standards Board (CGSB), becoming available in 2005. While the program was well-received by industry, with nine firms receiving certification in the first year, many smaller operations expressed the view that full HACCP is beyond their capacity or need and that GMPs alone may be sufficient to adequately control food safety risks. At the same time, there is increasing pressure for food processors to adopt food security measures and to implement traceability into food safety systems. In response, OMAFRA has expanded the HACCP Advantage program to stage the implementation of HACCP. Stage 1 consists of GMP Advantage (GMP programs alone), Stage 2 is HACCP Advantage (GMP programs plus HACCP plans) and Stage 3 is HACCP Advantage Plus+ (GMP programs, HACCP plans plus traceability and security). Certification is available at each stage of implementation. The introduction of GMP Advantage certification recognizes a significant milestone and provides an entry point for clients working their way towards full HACCP certification, particularly in sectors where industry readiness varies.

T2-04 Development of Egg HACCP Programs in Egg Processing and Further Processing Facilities

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Although the incidence is extremely low, *Salmonella* Enteritidis has been identified as a significant health risk with the consumption of contaminated and undercooked egg products. To address this risk, the Egg Safety Action Plan identified establishing a HACCP-based system for the egg industry as a high priority. Currently the egg industry is operating under the Egg Inspection Act of 1970 and not under mandatory HACCP. Surveys were conducted based on Good Manufacturing Practices (GMPs), written and implemented Sanitary Standard Operating Procedures (SSOPs), written and implemented HACCP plans, monitoring, verification, validation and recordkeeping. One-hundred percent of the Processors surveyed (small and large) thought that SSOPs would be the most challenging part of HACCP to implement. Eighty percent of the middle sized processors surveyed indicated that they already had written and implemented HACCP plans; at the same time, they indicated that monitoring, verification, validation and record keeping were weak and needed improvement. Ninety percent of the large producers had already implemented HACCP plans; however, these producers indicated that they could still improve validation, verification and record keeping in terms of trend analysis. Egg processors already utilize and comply with GMP guidelines; however, in addition to GMPs, processors will need to develop Sanitary Standard Operating Procedures (SSOPs) to meet the prerequisite requirements of HACCP. From these surveys, we determined that larger producers have implemented HACCP plans using the current regulations; however, smaller processors lack the time and expertise to develop, implement and validate HACCP plans under the current regulations. This information has provided the foundation for the development of HACCP training materials that will be the most effective in helping egg processors and further processors implement complete HACCP plans in their facilities.

T2-05 Level of Adoption of HACCP and ISO 9000 within the Mexican Pork Industry

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Regulatory measures imposed by international and domestic markets on food industry have multiplied and increasingly rigorous systems are required. Throughout the world food companies vary widely in their concern for quality management systems. The Mexican pork industry has gradually become aware of the need to certify the extent of food safety achieved in their plants. This study describes the characteristics of the Mexican pork sector and the adoption status of quality management systems and product destination. Fifty enterprises were surveyed; 96% answered the questionnaire, of which 90% were small to medium-size and 10% large processing plants. Nineteen percent of the enterprises interviewed had totally adopted HACCP, 63% were in the process of implementing and/or planning it, and only 19% had no plans to adopt any new food safety protocol in the near future. ISO 9000 was in full operation in 13% of the enterprises. Of their sales, 34% went to supermarkets, 57% to other retail chains and 9% to exports. Export was mainly to Central America, the United States of America, Asia and to some fast-food chains and restaurants operating in Mexico under international quality and food safety standards. Improvement of processes, efficiency and quality of products were the main reasons to implement HACCP. Customer satisfaction and legal requirements were the external factors related to this adoption. Staff motivation and training were pointed out as the principal problems faced by these enterprises in the process of HACCP implementation. The results suggest that the HACCP implementation is taken as an important component by the Mexican pork industry, as a mean to achieve stability within the domestic market. However, there are enterprises that need some incentive to adopt any food safety protocol.

T2-06 The GAPsNET: Farm Food Safety at Your Fingertips

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For the last eight years, the safety of fresh fruits and vegetables has been of significant concern due to an increase in produce-associated foodborne illnesses. Many educational programs have been created to assist growers with implementation of Good Agricultural Practices (GAPs) to reduce microbial risks in the fresh fruits and vegetables they grow and pack. Finding GAPs information has not always been easy because it is located in many different places (journals, web sites, government documents, etc.) and in many formats. The Good Agricultural Practices Network for Education and Training (GAPsNET) brings all GAPs related information together in one, user friendly web site for growers, farm workers, extension educators, scientists, and state and federal government personnel. GAPsNET was created by the National GAPs Program with support from its collaborators at Land Grant Universities in 25 states across the US. Not only does GAPsNET provide access to educational materials and links to GAPs programs at many institutions, it is an interactive web portal that contains a searchable database as well as other features. It was developed with assistance from Cornell Cooperative Extension web programmers and library professionals at the Cornell University's Mann Library. The GAPsNET searchable citation database is updated through monthly, automated searches of AGRICOLA and PubMed databases. GAPsNET users can search under specific GAPs topics such as water, manure, and worker training or can search the entire database using key words to find the most current information and recommendations. To make the GAPsNET even more valuable to constituents, a navigable Farm Assessment will be available so that growers can work online to directly develop a food safety plan for their farms. This presentation will guide the audience through the interactive features and information available through the GAPsNET at www.gaps.cornell.edu.

T2-07 Microbial Population Dynamics in Hot-drinks Vending Machines

DSC

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Hot-drinks vending machines are an established part of the food industry in the UK. Operation of these machines causes an accretion of drink powders within the mixing bowls and dispensing tubing, which may support growth and contribute to the microbial load in the drinks. This study examined the development of microbial populations in a hot-drinks vending machine and the effect on vended drinks over a period of 4 months normal operation. The machine dispensed only hot chocolate and was cleaned weekly, using a simple, detergent-based protocol. Swab samples were taken before and after cleaning, from the mixing bowl and dispense point. Vended drinks were sampled at 0, 3, 6 and 9 min after vending to examine the microbial load of the drink over time. Drinks powder was also sampled. Within 1 month of operation, levels of bacteria found on the mixing bowl and dispense point increased from 2.5 log CFU/cm^2 to around 3.5 log CFU/cm^2 after cleaning. Levels of bacteria within the drink increased from levels below the detection limit to 3.4 log CFU/ml. The mixing bowl and drink samples showed around a 1 log reduction in numbers after cleaning. The inactivation of the machines self-flushing mechanism lead to a significant increase in levels found at the dispense point and in the drink. The number of organisms present in the powder was around 1 log lower than those in the vended drinks. Further analysis of the drink and powders has shown that the microbial load of the hot chocolate consisted of Gram positive, spore forming rod-shaped bacteria, dispensed into the drink as spores. These have been confirmed by biochemical tests as *Bacillus cereus*. This research shows a simple cleaning protocol can control the level of organisms within vended hot drinks, although the process of vending increased the microbial load in the drink.

T2-08 Thermal Inactivation of *Bacillus anthracis* Spores in Milk

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Milk poses a high probability/high severity risk potential if *Bacillus anthracis* were purposely added to it at the farm, truck delivery or processor level. The thermal inactivation kinetics of spores of three *B. anthracis* strains (two Sterne strains, 7702, ANR-1 lacking the pXO2 plasmid; and strain 9131, a Sterne derivative lacking both pXO1 and pXO2 plasmids needed for infectivity) were

evaluated in milk from 72 to 130°C. Decimal reduction time (D) values were determined at 72, 100, 104, 108 and 112°C using a sealed capillary tube technique. At 72°C, D values ranged from 3.4 to 16.7 h depending on the strain, and at 112°C, they varied from 1.6 to 3.3 s. The Z values were 9.4, 8.7, 11.0°C for strains 7702, ANR-1 and 9131, respectively. The temperature dependent inactivation kinetics of the spores was also modeled according to the Arrhenius equation. For all three strains, the correlation of the natural logarithm of the death rate (k) vs. the inverse absolute temperature (K) had an R2 greater than 0.99. The activation energies (Ea, which is an indicator of temperature sensitivity) ranged from 227.4 to 291.3 KJ /mole, typical of other bacterial endospores. The results indicated that the three *B. anthracis* spores had similar heat resistance ($P > 0.05$). No significant difference ($P < 0.05$) in thermal resistance was found when whole and skim milk were used. Consistent reductions of more than 6 log CFU/ml in the number of viable spores of all three strains were achieved if the capillary tubes containing spores in milk were heated at 120°C for a total of 16 s, which included come-up time (6 s). These results provide baseline data that could be used to develop a thermal process that food processors could use in the event of an intentional addition of *B. anthracis* to milk.

T2-09 Microbial Food Safety Assessment of Cream Cheese

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The FDA's final Risk Assessment for *Listeria monocytogenes* provides a relative risk ranking for 23 different food categories. Cream cheese is in the category of soft unripened cheese (moisture > 50%). On a scale of 1–23 (highest risk–no risk) this category ranked 5th and 8th on a risk per annum basis and per serving basis, respectively, indicating relatively high-risk. This study was designed to evaluate the food safety risk of cream cheese. Three types of cream cheese were evaluated; (1) plain cream cheese spread containing sorbic acid, (2) Salmon cream cheese spread containing sorbic acid and (3) whipped cream cheese without preservative. Each product was challenged with *L. monocytogenes, Salmonella, Staphylococcus aureus, Escherichia coli* O157:H7 and *Clostridium botulinum*. Product was stored at 45°F, 55°F and 75°F and analyzed in triplicate for each organism over time. No growth of vegetative pathogens was observed in any of the products stored at 45°F over 1.3 × shelf life. No growth of pathogens was observed in the plain formulation stored at 55°F through 21 days. The salmon and whipped formulations supported the growth of *S. aureus* (> 3 logs) after 14 days at 55°F, with no growth of *E. coli, Salmonella*, or *Listeria* observed. All formulations supported the growth of *S. aureus* at 75°F; ranging from 72 h to 14 days. *Listeria* growth in the whipped formulation was observed; >1 log after 7 days at 75°F (no *E. coli* or *Salmonella* growth reported). All formulations were negative for *C. botulinum* toxin through 1.3 × shelf life at 55°F and 75°F except for the whipped formulation, which was toxin positive at 8 weeks of storage at 75°F. These data indicate that when properly stored (< 55°F), cream cheese will not support the growth of vegetative pathogens or C. *botulinum*. The FDA risk ranking for this product should be re-assessed.

T2-10 Survival and Growth of Foodborne Microorganisms in Processed and Individually Wrapped Cheese Slices

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Our objectives were to determine growth, survival, or inactivation of selected microorganisms in individually wrapped cheese (IWC) slices stored at 5°C vs. 22°C, and, compare quality indices. IWC slices were spot inoculated with foodborne pathogens (*Listeria monocytogenes, Staphylococcus aureus*, and *Salmonella* spp.), spoilage bacteria (*Pseudomonas* spp. and *Lactobacillus* spp.) and spoilage molds (*Penicillium* spp. and *Cladosporium* spp.). Each bacterium was inoculated at 10^5 cells/g to determine growth, survival, or inactivation. Molds were inoculated at 10^2 spores/g and observed for growth. Half of the inoculated product samples were held at 5°C and half were held at 22°C throughout shelf life. Samples were obtained at days 0, 3, 7, 10, 14, 28 and after 2, 3, 6, 9 months and evaluated for surviving cells (using appropriate selective media), for color (Hunter colorimeter, cheese color guide) and for lipid oxidation (peroxide values). Bacterial inactivation was observed in all conditions. At 14 days, a 5-log reduction was observed for *Listeria monocytogenes* and *Salmonella*, while a 3-log reduction was observed for *Staphylococcus aureus*. For *Pseudomonas* spp. and *Lactobacillus* spp. bacteria, a 2-log reduction was obtained within 3 days with an additional 1-log reduction seen after several months. Molds showed no change during the first several weeks of storage. At 84 days, molds decreased at 5°C, but showed growth at 22°C to approximately 10^5 cells/g. Visual color was evaluated on a 10 pt National Cheese Institute scale. During storage at 5°C or 22°C, color values increased from 4–5 and 4–7, respectively. No significant changes were noted, however, in Hunter color values. Higher peroxide values were also obtained for the samples held at 22°C vs. 5°C. From a microbiological standpoint, pathogenic and spoilage bacteria were unable to grow in this product; however, long term storage at 22°C led to lower product quality and mold growth.

T2-11 Using Photo Novels in Farm Worker Education and Training

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One of the key areas of concern in Good Agricultural Practices (GAPs) is the proper education and training of farm workers. The health and hygiene of individuals working on farms and in packing houses are of paramount importance because microorganisms on hands, clothes and harvest tools can be easily transferred to the fruits and vegetables that they harvest, sort, grade, and pack. To assist farm workers with understanding their importance in reducing microbial risks in fresh fruits and vegetables, three photo novels were developed by the National GAPs Program. Photo novels tell a story through the use of photos and dramatic dialogue. These photonovels specifically address field hygiene and safe food preparation in the family kitchen. These photo novels contain dialogue and themes that are culturally relevant and they are presented at a literacy level that makes the text available to most farm workers. As part of a national program, these photo novels have been distributed to collaborators at 25 land-grant universities throughout the US as well as organizations serving farm workers such as rural health clinics. Recently 360 photo novels were distributed to farm workers at a Migrant Expo in Collier County, FL and initial feedback is extremely positive. This presentation will address the important subject of farm worker education and training using these three different photo novels as the focal point of discussion.

T2-12 Effect of the Refrigerated and Frozen-storage on Microbiological Change in Soft Raw and Pasteurized Milk Goat Cheese

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Microbiological changes of soft raw or pasteurized milk goat cheeses were determined. Three lots of raw (RC) and pasteurized (PC) goat cheeses were subdivided into two groups. One was stored at 4°C as non-frozen and the other was immediately frozen (-20°C) for 3 months, then thawed and stored at 4°C. All groups were evaluated at 0, 14, 28, 42, 56 and 70 days. Microbiological counts (log CFU/g), including lactic acid bacteria (LAB), yeasts and molds, coliforms, *E. coli*, *Salmonella*, *Listeria* spp. and *Campylobacter* were determined using the plate count technique. Refrigeration storage did not affect the LAB count, but in the frozen-stored sample counts decreased during storage time. *Lactobacillus plantarum*, *L. helvaticus*, *Lactoccocus lactis cremoris* and *Leuconostoc mesenteroides* were identified. Yeast and molds counts increased with refrigeration storage. After 3 months of frozen-storage all microbiological populations were dramatically reduced. Coliforms in RC refrigerated at 0 days were 3.1, 4.3 and 1.8 log CFU/g decreasing with extended storage time (< 1, 2.2 and <1, respectively). PC coliforms counts were 1.6 and 4.4. In the first lot, counts decreased (< 1) at 70 days unfrozen stored. However, in the second lot counts increased by 5.34 log CFU/g. Frozen-storage diminished coliforms in RC and PC, but were not completely eliminated (0.6 and 1 log CFU/g). *E. coli* was isolated in both unfrozen and frozen-stored cheeses. *Salmonella*, *Listeria*, and *Campylobacter* were not isolated from any samples. In conclusion, this study confirmed that fresh goat milk cheese contained higher counts of microbiogical populations than the frozen-stored cheeses. Additionally, occurrence of *E. coli* suggests that it is necessary to improve hygienic conditions to control microbiological hazards.

T3-01 Antimicrobial Activities of Plant Compounds against *Escherichia coli* O157: H7 and *Salmonella enterica* Serovar Hadar in Tomato and Vegetable Juices and in a Tomato/Pectin Edible Film Formulation

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The use of fruits and vegetables previously contaminated with foodborne pathogens may result in contaminated tomato and vegetables juices. Previously we found that low levels of plant essential oils and oil compounds induced rapid reduction of pathogenic bacteria in apple juices. To extend these findings, we evaluated the following plant-derived antimicrobials in tomato and vegetable juices and in an experimental tomato/pectin edible film formula in an BA50 antimicrobial assay (% of the sample that induced a decrease in 50% of CFU at 21°C after 60 min): oregano oreganum, oregano Spanish, and thyme plant essential oil and their active constituents carvacrol and thymol. Average BA50 values (n = 4 to 8) of carvacrol in Campbell's Tomato Juice, Campbell's Tomato Organic Juice, tomato/pectin film formula, and V8 Vegetable Juice against *E. coli* O157:H7 ranged from 0.013 to 0.027% and for *S. enterica* serovar Hadar, from 0.0089 to 0.015% (the lower the value the greater the activity). The corresponding values for oregano oils ranged from 0.022 to 0.044% and 0.012 to 0.017%; for thymol, from 0.018 to 0.028% and 0.011 to 0.013%; and for thyme oil, from 0.022 to 0.056% and 0.014 to 0.017%. Susceptibilities of the bacteria to the antimicrobials in the tomato/pectin formulation were similar to those observed with the tomato juices. Juices and tomato/pectin slurries without added antimicrobials were inactive against both pathogens. These results show that antimicrobials are strong inhibitors of the two pathogens in tomato and vegetable juices and in the tomato/pectin formulation and that the activity against *Salmonella* was greater than against *E. coli*. The cited observations provide information about new ways to protect foods against foodborne pathogens.

T3-02 Effect of Microencapsulated *Lactobacillus reuteri* on *Escherichia coli* O157:H7 in Dry Fermented Sausages

DSC

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Escherichia coli O157:H7 is known to survive the fermentation process applied in sausage manufacturing. The effect of adding probiotic *Lactobacillus reuteri* as a co-culture with the meat starter cultures on the viability of *E. coli* O157:H7 in dry fermented sausages was studied. A 5 strain cocktail of *E. coli* O157:H7 was added at 7.4 log CFU/g to the sausage batter and challenged with *Lb. reuteri* before or after they were microencapsulated. Sausages were fermented at ≤ 26°C and 88% relative humidity (RH) followed by drying at 75% RH and 13°C for 25 days. The pH, water activity (a_w), and numbers of all inoculated organisms were monitored during processing. A sensory evaluation was conducted to determine if addition of *Lb. reuteri* as planktonic cells or in microcapsules affected the sensory quality of the dry fermented sausages. The pH and a_w decreased from 5.7 and 0.98 to 4.9 and 0.88 at the end of fermentation and drying, respectively. No significant difference in physiochemical as well as sensory quality was found between control and sausages containing either planktonic or microencapsulated *Lactobacillus reuteri*. Free *Lactobacillus reuteri* cells dropped from 7.1 to 4. 5 log CFU/g, whereas microencapsulated *Lactobacillus reuteri* cells were reduced by ≤ 0.5 log at the end of drying. The processes reduced *E. coli* O157:H7 by 1.7 log CFU/g at the end of drying in the control containing starter cultures alone. Planktonic *Lactobacillus reuteri* with the starter culture was effective in reducing *E. coli* O157:H7 by 3.0 log CFU/g, but microencapsulated *Lactobacillus reuteri*. *Reuteri* did not show any significant reduction compared to control. Thus microencapsulation of probiotics like *Lactobacillus reuteri* may be an option to provide fermented meat products with viable heath-promoting bacteria but their action against pathogens such as *E. coli* O157:H7 is limited.

T3-03 New Primer Set Improves Detection of *Escherichia coli* O157:H7 from Environmental Samples

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There are multiple methods to detect Shiga-toxin producing *Escherichia coli* from environmental sources. Two common approaches amplify regions of *stx*I and *stx*II (high rate of false positives) or the highly conserved point mutation in uidA at +93 (TàG, occasionally generates false positives). Additionally, in some primary environmental enrichments, no or one target gene region is amplified despite known *E. coli* O157:H7 presence. In an attempt to avoid these limitations across numerous platforms, a new primer set was designed and optimized for real-time PCR. This new primer set and published *eae* primers were optimized separately using purified *E. coli* O157:H7 DNA. The product was run on an agarose gel to verify a single band and the amplified product's size. Ten *E. coli* O157:H7, 20 non-pathogenic *E. coli*, 16 other bacterial human pathogens, and 34 common environmental isolates were screened with both primer sets. There were no false positives and no false negatives. Enrichment cultures, with initial *E. coli* O157:H7 inoculated at 0 to 5 log CFU/ml in 100 ml of Tryptic Soy Broth, incubated at 25°C for two hours, amended with novobiocin (20 µg/ml) and additionally incubated for two hours at 42°C. One-and-a-half ml of culture was pelleted and resuspended in 50 µl of 0.1% peptone water. The post-enrichment limit of detection was 101.48 CFU/ml at CT=23 with only one primer set per tube. Both primer sets were combined in one tube and conditions were optimized; the post-enrichment limit of detection was 101.59 CFU/ml at CT=25.5. Creek water, with approximately 10^7 CFU/ml of environmental bacteria, was adjusted to 1 to 4 log CFU/ml of *E. coli* O157:H7, enriched, and tested; the post-enrichment limit of detection was 102.82 CFU/ml at CT=30. These primers offer greater specificity and sensitivity in environmental testing across multiple amplification and detection platforms.

T3-04 Multi-drug Resistance Profiles of Generic *Escherichia coli* from Commercial and Natural (Organic) Bovine Feedlot Lagoon Water

DSC

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The increased use of antimicrobials has contributed to the rapid spread of antimicrobial resistant microorganisms and the emergence of multi-drug resistant microorganisms in humans, food-animals, and the environment. Relatively few studies have evaluated multi-drug resistance of indicator microorganisms from food-animal production environments. The objective of the study is to investigate the multi-drug resistance profiles of generic *Escherichia coli* cultures obtained from commercial (using antimicrobials) and organic (not using antimicrobials) bovine feedlot lagoon waters. 241 and 123 strains of generic *E. coli* cultures were obtained from three commercial feedlots and three organic feedlots, respectively. Cultures were isolated and purified from the 3-M Petrifilm – *E. coli*/ Coliform and identified using the API 20E system (bioMérieux). Twenty-one food animal antimicrobial (such as tetracycline, neomycin, spectinomycin, sulfachloropyridazine, tilmicosin, etc.) resistance profiles of the *E. coli* isolates were determined using the Sensititre automated antimicrobial susceptibility system (TREK Diagnostic Systems, Cleveland, OH). Interpretive standards and MIC breakpoints were based on NCCLS standards. Overall, of the generic *E. coli* cultures from commercial feedlot lagoon water, all 241 exhibited multi-drug resistance, defined as isolates resistant to 3 or more antibiotics (> 3). 74/241 (31%) showed overall greater multi-drug resistance and were resistant to 9 or more of the 21 antibiotics. Of the generic *E. coli* isolates from organic feedlot lagoon water, all 123 demonstrated multi-drug resistance (> 3 antibiotics). Also, 31/123 (25%) showed overall greater multi-drug resistance patterns and were resistant to 9 or more of the 21 antibiotics evaluated. This study indicated that although all cultures from both commercial and natural feedlot lagoon waters exhibited multiple resistance (> 3 antibiotics) more overall multi-drug resistant generic *E. coli* cultures were present in commercial feedlot lagoon water (31% vs. 25%). This research illustrates the importance of further investigation of multi-drug resistant generic *E. coli* in different food-animal production environmental reservoirs.

T3-05 Withdrawn

T3-06 Subtyping of *Salmonella enterica* subsp. *enterica* Serotypes from Human and Cattle Clinical Isolates in New York State Region by Pulsed-field Gel Electrophoresis and Serotyping

DSC

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Salmonellosis is a major cause of bacterial enteric illness in both animals and humans. We investigated the genetic relationships of 167 clinical *Salmonella* isolates from cattle and 185 clinical *Salmonella* isolates from humans by serotyping and pulsed-field gel electrophoresis (PFGE). Human and cattle isolates, collected in the New York State region in 2004, were obtained from the Wadsworth Center, New York State Department of Health and the New York State Animal Health Diagnostic Center, respectively. XbaI PFGE patterns were determined using the standard CDC PulseNet protocol and data were analyzed using the BioNumerics software package. Out of a total of 165 PFGE patterns, 113 patterns were unique to human isolates, whereas 44 were unique to cattle isolates; 8 PFGE patterns were shared by human and cattle isolates. Overall, PFGE provided very high discrimination among human (Simpson's index, D= 0.9918) and cattle (D= 0.9673) isolates; discrimination was considerably higher than that achieved by either serotyping or multilocus sequence typing. Among the human isolates, in 6 instances the same subtypes (i.e., same PFGE and serotype) were observed for two or three specimens collected in the same county in the same or consecutive months, possibly indicating small temporal and geographical case clusters. Most cattle isolates from the same farms shared the same serotypes and PFGE patterns, indicating that these subtypes were endemic to these farms. Among cattle isolates, three PFGE types were identified in multiple farms in adjacent counties, indicating geographical clustering of *Salmonella* subtypes, which may help to trace sources of foodborne outbreaks to agricultural sources in a specific location. We conclude that further development of large subtype databases for human, food, and animal associated *Salmonella*, will help to provide a better understanding of *Salmonella* transmission and facilitate better tracking of outbreaks sources.

T3-07 Population Genetics of Virulence Potential in Environmental Reservoirs of *Vibrio vulnificus*

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Vibrio vulnificus is ubiquitous to estuaries worldwide and causes foodborne infections associated with raw oyster consumption. Fatal septicemia is limited to persons with underlying hepatic or immune dysfunction, but serious wound infections also occur in healthy individuals. Virulence potential of environmental reservoirs is unclear, as oyster isolates are equivalent to clinical strains used in virulence studies with animal models. However, previous studies showed that clinical strains were genetically distinct from oyster isolates by DNA sequence comparison of individual genes or by multilocus DNA sequencing typing. In the present study, PCR was used to screen strains for divergent DNA sequences at three discrete loci. These results confirmed DNA sequence polymorphisms corresponded to either a typical "environmental" or "clinical" profile, comprising haplotypes based on strain origin. Genomic relationships among these strains were evaluated by repetitive extragenic palindromic DNA PCR (rep-PCR) typing. Most oyster isolates (88%), including all strains with the typical environmental haplotype, formed a single rep-PCR cluster. Isolates from clinical origin in this cluster showed either environmental or atypical haplotype. Interestingly, clinical populations with the clinical haplotype were distributed among multiple rep-PCR clusters, demonstrating greater genetic diversity than was evident by specific loci. Wound isolates were genetically distinct from blood isolates by all assays. A single oyster isolate showed the clinical haplotype, and it also clustered with clinical strains by rep-PCR. Strains from an outbreak in Israel (biotype 3) were related by rep-PCR to several US strains, suggesting potential reservoirs of emerging disease. Rep-PCR provided a rapid method for more sensitive discrimination of population genetics and virulence potential in environmental reservoirs of *V. vulnificus*.

T3-08 Quantitative Determination and Toxigenicity of *Bacillus* Species Associated with Raw and Cooked Rice

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Foodborne illness associated with *Bacillus* species, especially that involving starchy foods, is attributed most commonly to *Bacillus cereus*, though other bacilli are known to cause disease. This study determined the numbers and diversity of bacilli associated with rice before and after cooking and their potential and ability to elaborate toxins. Using centrifugation-plating, *Bacillus* species were enumerated and isolated from raw and cooked rice. Representatives of each colony type were selected, confirmed morphologically as bacilli, then identified using biochemical tests and rDNA sequencing. Toxigenic potential was determined using PCR for both diarrheal and emetic genes. Diarrheal and emetic toxin (cereulide) production was determined using commercial and prototype ELISAs, respectively. The centrifugation-plating method proved superior to conventional plating, the former frequently yielding isolates when the latter did not. The major *Bacillus* species isolated from both raw and cooked rice samples using the centrifugation-plating method included *B. cereus/thuringiensis*, *B. mycoides*, *B. subtilis/mojavensis*, *B. pumilus*, *B. licheniformis*, and *B. megaterium* though the proportions of the species among the total bacilli present varied before and after cooking, and populations varied with the type of rice. While 69% of raw rice samples yielded < 100 CFU/10g, 9% yielded >1000 CFU/g. For cooked rice products, the percentages were 79% and 15% respectively. Of the *B. cereus/thuringiensis* isolates, over 80% produced enterotoxin, though only about 50% were capable of producing cereulide, and a number of isolates could produce both toxins. In addition, a high proportion of both *B. subtilis/mojavensis* and *B. pumilus* isolates appeared capable of producing cereulide.

T3-09 Destruction of Bacterial Pathogens in Non-heated Acidified Vegetable Products

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Recently, outbreaks of *Escherichia coli* O157:H7 and *Salmonella enterica* have occurred in acid foods, such as apple cider and orange juice. The US Food and Drug Administration has expressed concern about potential for the survival of acid tolerant pathogens in acidified (non-fermented) vegetable products which are not heat processed. Many of these products cannot be heat processed due to the loss of desirable texture and flavor. The objective of this research was to define the time and temperature conditions that will ensure a 5-log reduction in acid-resistant food pathogens in a product formulation representative of selected acidified vegetable products. Five strain cocktails of *Escherichia coli* O157:H7 were inoculated into 1.3 L jars containing pasteurized brined cucumbers that had an equilibrated pH of 3.3 and a final concentration of 2.4% acetic acid. The bacterial cells were prepared by static growth in the presence of glucose to induce expression of acid resistance genes. Similar experiments were carried out with cocktails containing *Salmonella enterica* or *Listeria monocytogenes* strains. Acid-killing curves were generated by plating samples from the inoculated jars on non-selective media. Data were analyzed with both linear and non-linear (Weibull) models to determine 5-log reduction times, depending on the observed killing kinetics. Independent replication of the experiments was carried out using different lots of cucumbers. At 25°C a 48 h holding time was required to ensure a 5-log reduction in the most acid-resistant pathogen, which was found to be *E. coli* O157:H7. At 10°C more than 5 days was required to ensure a 5-log reduction in *E. coli* O157:H7 cell counts. Results from these experiments may be used to develop methods that ensure the safe production of a variety of pickled vegetable products, including pickled peppers.

T3-10 Role of Biofilm Growth in *Campylobacter jejuni* Oxidative Stress Response

DSC

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Campylobacter jejuni is a foodborne pathogen that causes gastroenteritis and diarrhea. It requires strict microaerophilic conditions for growth. Unlike other pathogens such as *Escherichia coli*, *C. jejuni* possesses very few oxidative stress response genes. However, *C. jejuni* is still the primary cause of foodborne diarrhea in developed countries. This indicates that the organism does have mechanisms for enhanced survival in the presence of oxygen and one possible explanation for this may lie in *C. jejuni*'s ability to grow in biofilms. Therefore, the objective of this study is to evaluate whether *C. jejuni* deals with oxygen through increasing expression of oxidative stress genes during biofilm growth. After designing primers for the Superoxide Dismutase (SodB), Alkyl Hydroperoxide

Reductase (AhpC), Catalase (KatA), and Ferridoxin (FdxA) genes using sequence information from genebank for *C. jejuni* NCTC 11168, gene expression in a *C. jejuni* meat isolate (*C. jejuni* 653) was studied under planktonic and biofilm growth conditions. DNA and RNA were extracted from cells (approx. 10^7 CFU) grown planktonically and in biofilms for 48 h, microaerophilic, using commercial kits. Amplification of DNA with the polymerase chain reaction (PCR) was positive for all four genes. Expression of the genes of interest of *C. jejuni* 653 grown planktonically and in biofilms was assessed with the one tube reverse transcription PCR (RT-PCR) system (Roche). Bands on agarose gels showed that amplification of SodB, AhpC, and FdxA was more efficient in *C. jejuni* biofilms than in planktonic cells. Similarly, semi-quantitative RT-PCR (ABI 7000 real time thermocycler) indicated that SodB and KatA were expressed at higher levels in *C. jejuni* biofilms than in planktonic cells. Our data suggest that biofilm growth contributes to survival of *C. jejuni* in aerobic conditions by up-regulating its oxidative stress response.

T3-11 Effect of Chitosans and Chitoligosaccharides upon Growth of Microorganisms Contaminating Foods

DSC JOÃO C. FERNANDES, Sandra Borges, Tânia Ribas, Freni K. Tavaria, José A. Lopes da Silva, Manuela E. Pintado, and F. Xavier Malcata, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, Porto, Porto, 4200-072, Portugal

Chitosan has been advertised as a food supplement that encompasses a few desirable antibacterial and antifungal properties; these may be useful in preventing food contamination, and thus in extending shelf life. The purpose of this research effort was to evaluate in vitro the antibacterial activity of chitosans with several molecular weights (MW) – termed high, medium and low chitosans—as well as two chitoligosaccharides (5000 and 3000 Da) at various concentrations against a wide spectrum of food pathogens. Such features were evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Salmonella* spp., *Listeria monocytogenes* and *Candida albicans* – via first determining minimum inhibitory concentrations (MIC), followed by assessment of the death rate of each microorganism at either of three inoculum levels – 10^7, 10^5 and 10^3 CFU/mL, for various periods – for 0.5, 1, 2 and 4 h. At the higher inoculum levels, the antimicrobial effect was strongly dependent on both the microorganisms and the MW of chitosan. At the inoculum level of 10^3 CFU/mL, all five compounds showed bactericidal effect, leading to a reduction of more than 99.9% viable cells by 30 min. At the aforementioned levels, *Pseudomonas* sp. was the most resistant microorganism, whereas chitoligosaccharides exhibited the highest effect. The antimicrobial effect on *E. coli* increased with decreasing MW for the compounds tested. In view of the broad spectra of antimicrobial activity of the compounds tested, their application as food additives or packaging coats appears promising.

T3-12 Effects of Seasonality on the Pre-Harvest *Escherichia coli* Prevalence of Fresh Fruits and Vegetables

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The incidence of foodborne outbreaks typically has a seasonal pattern during the year. The number of produce-linked outbreaks is greater during the months of June, July, August and September. This study was conducted to determine the monthly variability of *Escherichia coli* prevalence as indicator of fecal contamination during the summer months in fresh produce grown in the Upper Midwest. During the harvest seasons of 2002, 2003 and 2004, a total of 1,431 produce samples were collected directly from the farm fields. Among these samples, 888 were provided by 19 semi-organic farms (claimed to use only organic inputs but were not certified) and 543 were from 10 organic farms (certified by accredited organic agencies), located in Minnesota and Wisconsin. The produce samples were analyzed for the presence of *E. coli*, *Salmonella* and *E. coli* O157:H7 to indicate potential fecal contamination. Only two semi-organic produce in 2002 had *Salmonella* contamination, and none of the produce in the three years tested positive for *E. coli* O157:H7 contamination at farms. The Generalized Estimating Equation (GEE) technique was used for determining the risks of fecal contamination, using data for *E. coli*. The interaction of the months of harvest with produce type was included in the GEE model, as produce type such as greens (lettuces, leafy greens and cabbages) contributed 80% of the samples in June, while this vegetable type contributed only 20% of the samples collected in September. The risk of fecal contamination was significantly greater in June (OR = 15.6, 95% CI = 2.0 – 118.1, $P = 0.008$) and July (OR = 18.2, 95% CI = 2.5 – 135.1, $P = 0.004$) compared to August and September. The odds of fecal contamination was 2.4 times greater (95% CI = 1.4 – 4.0, $P = 0.001$) in greens than in other produce types. No significant effect on risk of fecal contamination was determined between organic and semi-organic produce.

T4-01 Food Safety and Food Defense Simulation: A Realistic Approach

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The food industry and other food safety defense stakeholders are in need of an effective crisis management approach to counteract intentional or inherent contaminations to our nation's food supply. A computer-based simulation was developed for the food industry, regulatory agencies, and food defense related groups to determine the potential magnitude of economic and public health impacts during a virtual attack on the food supply. The simulation allowed participants to evaluate vulnerabilities in the food supply chain for both intentional and inherently contaminated food. The simulation incorporated product manufacturing and distribution data with epidemiological risk data to provide a real-time impact assessment of decisions made during the simulation. The food industry was modeled to allow for decision-making inputs by bulk ingredient suppliers, processors and retailers, whose roles were filled by various food companies, government agencies and the media. The scenario tested involved a chemical agent that was introduced into the food supply, which resulted in contamination of over 13 million servings of different food products with a dosage high enough to elicit a symptomatic response nationwide. Decisions made and actions taken by the participants (microbiological/toxicological testing, holding/recalling products, and communication up and down the supply chain) resulted in 806,394 cases of foodborne illness. Insight gained and conclusions drawn from this simulation were as follows: communication throughout the food supply chain was vital and economic and public health impacts could be minimized based on one's awareness of information

necessary for critical decision-making. As a result, some companies created and implemented new policies and procedures to take a proactive food bio-defense approach and minimize the potential for an intentional attack. The simulation provided an important vehicle to develop a network of food defense specialists, improve communication, and develop critical thinking skills to minimize public health and economic impacts.

T4-02 On-farm Risk: Prevalence of Zoonotic *Giardia* and *Cryptosporidium* in Adult Dairy Cows in Seven Eastern States

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This study was conducted to determine if adult dairy cattle can harbor zoonotic species or genotypes of *Cryptosporidium* and *Giardia*, and thus serve as potential sources of water or food contamination. Fecal samples were collected from 541 adult dairy cows (>24 months of age) on two farms in each of the following states: VT, NY, PA, MD, VA, NC, and FL. For analysis by PCR, fifteen-gram portions of each fecal sample were mixed with 35 ml of water, passed through a 45-micron screen, and concentrated using a CsCl density gradient. The resulting pellet of material was suspended in 100 microliters of distilled water. Fifty microliters of this suspension was subjected to DNA extraction using a commercial extraction kit. To detect *Giardia* and *Cryptosporidium*, PCR amplification was done for the respective ssu-rRNA genes, using nested protocols. PCR products were sequenced, and gene sequences were compared to each other and to previously sequenced *Giardia* and *Cryptosporidium* isolates. The average prevalence across all farms was 144/541 (27%) for *Giardia* and 31/541 (6%) for *Cryptosporidium*. Of the 144 *Giardia* positive isolates, 94% belonged to the hoofed-livestock genotype, Assemblage E, whereas 6% were the zoonotic genotype, Assemblage A. Of 31 *Cryptosporidium* positive isolates, 6% were zoonotic *C. parvum*, whereas 29% and 65% were host specific *C. bovis* and *C. andersoni*, respectively. These results indicate that although adult cows can harbor these parasites, they are generally not a significant source of the species or genotypes that infect humans.

T4-03 Generic Exposure Assessment Model of *Salmonella* spp. in Poultry

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Salmonella spp. is a significant cause of foodborne illness in the United States and poultry is thought to be one of the major foodborne sources of human exposure to *Salmonella* spp. Although most infections result in mild and self-limiting disease, severe illness commonly occurs in sensitive populations, especially very young, elderly, and immunocompromised individuals. In this study a generic plant-to-table risk assessment model for *Salmonella* spp. in poultry was developed to characterize the degree of the risk associated with the pathogen and to assist in developing risk-based performance standards and risk-mitigation strategies that affect public health. The model was built in Analytica (Luminr Decision System, Inc., CA) consisting of three stages representing the exposure pathway for *Salmonella* spp. in poultry products; (1) processing, (2) retail and storage, and (3) preparation and cooking. The processing stage begins with the entry of birds into the processing facility and examines the impact of the stages of processing on the prevalence and contamination levels on carcasses and products. In particular, this stage addresses the effect of cross contamination at processing and the effect of the chiller on end-stage product. Retail and storage addresses the impact of storage during retail and by the consumer upon the contamination level. The preparation and cooking stages estimates the exposure level of consumer, following cooking and as a result of any cross contamination. Two cross-contamination modules are employed by the model: the fluid-drip model and a generic exposure pathway including the effects of hand and cutting-board washing. Also, the model has been set up to be able to track different *Salmonella* serotypes within different poultry product types (e.g., whole carcass, ground, and parts). Further application of this generic model for specific mitigation scenarios will be developed to address mitigation strategies to reduce the risk of *Salmonella* spp. in poultry products.

T4-04 Predictive Model for Growth of *Salmonella* Typhimurium DT104 in Ground Chicken Breast Meat

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A multiple antibiotic resistant strain of *Salmonella* Typhimurium definitive phage type 104 (DT104) was used to investigate and model growth of this pathogen on ground chicken breast meat portions (1-g) with a competitive micro flora, which ranged in initial density from 3 to 7 log/g. Inoculated breast meat portions were stored at times and temperatures ranging from 186 h at 10°C to 35 h at 40°C. *Salmonella* was enumerated by most probable number and plate count on a selective medium containing 4 antibiotics. Data collected from 5 replicates for each temperature were combined and fit to a primary model to determine maximum specific growth rate (SGR), maximum population density (MPD) and the 95% prediction interval (PI). Non-linear regression was used to obtain secondary models as a function of temperature for SGR, MPD and PI, which ranged from 0.048 to 0.41/h, 1.6 to 9.4 log/g and 1.4 to 2.4 log/g, respectively. Secondary models were combined with the primary model in a computer spreadsheet to create a tertiary model for predicting the variation (95% PI) of pathogen growth among portions of ground chicken breast meat. The criterion for acceptable model performance was that 90% of observed values for pathogen density (PD) at a given time had to be within the 95% PIs predicted by the tertiary model. For data (n = 236) not used in model development but collected using the same methods, 94% of observed PD values were in the predicted 95% PIs of the tertiary model. Thus, the tertiary model was successfully validated for predicting the variation of growth of *S.* Typhimurium DT104 among portions of ground chicken breast meat and from a low initial density.

T4-05 An International Outbreak of *Salmonella* Linked to Pet Treats

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Salmonella Thompson in an Alberta (AB) resident was traced to beef jerky pet treats fed to a companion pet dog. This case shared an indistinguishable pulsed field gel electrophoresis (PFGE) pattern with the pet treats. The treats originated from a Washington (WA) manufacturing plant that were packaged and distributed by a company in British Columbia (BC). Cases were identified

using PulseNet USA and PulseNet Canada to search for PFGE patterns with the same outbreak strain of *S.* Thompson between August 2004 and June 2005. Retrospective case interviews were conducted in BC, AB and WA. Trace-back of pet treats led to inspections of pet food facilities and collection of pet treat samples from cases' homes, pet stores, distributors and manufacturers in BC and WA. Human and animal fecal samples and pet treats were tested for *Salmonella*. Results: Nine cases of *S.* Thompson were identified all with the same PFGE pattern as the pet treat beef jerky (AB:2; BC:4; WA:3). *S.* Thompson was also identified in one asymptomatic companion dog. Interviews of six cases found 75% had exposure to pet treats. Other implicated pet treats were made from raw dehydrated salmon, shrimp and/or beef. *S.* Thompson was found in the salmon, shrimp and beef jerky treats: additional *Salmonella* serotypes identified included *S.* Montevideo, *S.* Newport, *S.* Give, *S.* Meleagridis, *S.* Cerro, and *S.* Muenster. Elevated counts of *Salmonella* in the shrimp pet treats ranged from less than 100 CFU/gram to 80,000 CFU/gram. Although these pet treats were not consumed, handling of pet treats or infected pets caused human illness. These pet treats were not subjected to any heat treatment or kill step, which resulted in high levels of *Salmonella* contamination.

T4-06 A Risk Assessment Model of *Enterobacter sakazakii* in Powdered Infant Formula

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In response to a request from the Codex Committee on Food Hygiene (CCFH), a risk assessment model has been developed for *Enterobacter sakazakii* in powdered infant formula to estimate the relative risks and risk reductions associated with various scenarios and potential control options in the manufacturing, preparation and handling of powdered infant formula. This model was then applied by an expert working group convened as part of the Joint FAO/WHO Expert Meetings on Microbial Risk Assessment (JEMRA). This group was tasked with the provision of scientific advice, to CCFH and member countries, regarding the implications of alternate risk control options. Due to considerable knowledge gaps, the model is limited to calculations of relative risk. Within this approach, different scenarios can be compared to one another, as well as to a common baseline scenario. Differences among scenarios can include those resulting from alternate scientific assumptions including powder contamination patterns, predictive microbiology parameters and dose-response assumptions. The model was employed to study the impact of a considerable number of formula preparation, handling and feeding scenarios. These scenarios spanned diverse practices in both home and hospital care, including situations expected in both developing and developed countries. The individual scenarios were specified by detailed characterization of the processes of rehydration, cooling, holding, rewarming and feeding. This provided clear evidence of relative risks spanning many orders of magnitude among the preparation and handling scenarios. The model also provides the means to calculate the level of risk reduction associated with the application of microbiological criteria in the manufacturing environment for *E. sakazakii* and indicator organisms such as *Enterobacteriaceae*. This presentation will review the model components, provide relative risk calculations for selected scenarios and will discuss their implications.

T4-07 The 'Fermi Solution' – A Potential Tool in Estimating the Number of Victims in Food Poisoning Outbreaks

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The 'Fermi solution' is the art of finding approximate solutions to complicated problems by giving "reasonable" estimates to relevant factors when precise information is unavailable. Where the result is the product of several such factors, the overly high estimates will most probably be offset by low ones, so that the solution will likely be close to the correct value. The method might be used in estimating the number of individuals needing hospitalization after an outbreak of food poisoning, when the number of people that ate the contaminated food, the fraction that ingested enough of the pathogen, the immunity state of those who did, etc., are usually unknown. Rather than using a single guessed value, we specify a likely range for each factor. When a value is chosen at random in each specified range, their product is a "random Fermi solution". If the number of factors is large, the distribution of the "random Fermi solutions" will be approximately lognormal. Simulation results indicate that the lognormal approximation is already reached with as few as 5 or 6 factors. This allows us to use the lognormal distribution's mode as a "best guess" and compute a confidence interval for the solution. An MS Excel® program to do this has been posted as freeware on the web. With it, it is easy to examine the consequences of differences in the number of factors and their ranges.This sensitivity analysis provides for more confidence in the result. Several examples of hypothetical food poisoning scenarios are illustrated.

T4-08 Accounting for Hypothetical Variability (Over-Stratification) Inflates Uncertainty in Risk Assessment: The Case of Analyzing BSE Surveillance in Low Prevalence Countries

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Separating uncertainty and variability has been widely touted as a principle of good risk assessment. Moreover, stratified data analysis can yield more precise risk estimates if the stratification variable(s) account for much of the observed variability in the population. However, without good prior information about the population, selecting stratification variables can be speculative, and over-stratification to account for hypothetical variability may inflate uncertainty in risk estimates. An illustrative example of this effect is provided by the estimation of the prevalence of bovine spongiform encephalopathy (BSE) in countries with low BSE prevalence. The European Union (EU) BSurvE model, for example, was developed to estimate the prevalence of BSE in the standing cattle population of an infected country and provide an analysis of BSE stratified by birth year cohorts. If prevalence truly varies among birth year cohorts, this modeling approach may allow identification of differences in BSE exposure levels over time, potential disease control failures, and appropriate corrective actions. However, in low BSE prevalence countries such as the United States and Canada, the available animal health surveillance data provide no statistical basis for distinguishing BSE prevalence among birth year cohorts. In such cases, accounting for hypothetical variability among birth year cohorts inflates uncertainty in the national BSE prevalence estimate. Defining cohorts by birth year is intuitively appealing, but there is no definitive answer to how narrowly or broadly a cohort should be defined. For example, a cohort could be defined by birth date, market class (e.g., dairy or beef cows), geographic location,

feed source, etc. For a given surveillance level, as cohorts are more narrowly defined, the result is a smaller sample size per cohort. If the variability in prevalence among cohorts is simply hypothetical, the consequence of over-stratification is a more uncertain prevalence estimate for the standing population.

T4-09 Database on Breakdowns in Food Safety

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In September 2001, the UK's Food Standards Agency (FSA) requested a project proposal to examine the nature and extent of breakdowns in food safety which had occurred in the past, throughout the whole food chain. It was considered that a thorough knowledge of past events could direct policy and priorities for the future. This paper will describe the results of a 2-year study which constructed a computer-based database to collect data on, (a) microbiological outbreaks of foodborne illness during the period 1992 – 2003, (b) adverse effects from chemicals found in foods and drinks for the years 1998 – 2003 and (c) information on physical (foreign) bodies in foods referred to Campden and Chorleywood Food Research Association for investigation. Together these three core data sets provided information on a total of 7,650 incidents which populated the main database, detailing a total of 8,505 breakdowns; in several incidents multiple breakdowns occurred. In addition, information was collected from three other sources in order to obtain the broadest view of breakdown types and causes. These were (a) UK FSA Food Hazard Warnings issued between December 2001 and July 2004, (b) Food Safety Information Sheets issued originally by the Ministry of Agriculture, Fisheries and Food and more recently by the FSA and, (c) Pesticide Residues – information on MRL exceedances and non-UK approved uses. An attempt was also made to analyse media coverage of food safety issues. This paper will also describe a generic food chain model which was constructed to facilitate collection of the data. It will highlight key findings of the study and show how the database can be used to illustrate breakdowns in food safety that have occurred and recommend improvements in food safety control measures.

T4-10 Calculation of Lot Rejection Rates and Risk Reduction through the Application of Microbiological Criteria

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Risk reduction through application of sampling plans can occur through a number of mechanisms. The most direct mechanism is lot rejection, where product is diverted from the food chain if the application of the microbiological criteria deems the lot unacceptable. Ideally, though not necessarily, this results in a net reduction in the pathogen burden of the food supply. In this presentation, we present an approach for calculating risk reduction by this pathway. Risk reduction is estimated through explicit simulation of the pattern of lot contamination, the sampling process and the subsequent decision to accept or reject a lot. By simulation, it is possible to estimate the change in the number of pathogens entering the food supply, with and without the application of microbiological criteria. The fate of accepted lots of product can then be simulated as part of a standard risk assessment approach. The proposed calculation methods require specification of the usual components of microbiological criteria. An additional requirement is to separately characterize the variability in contamination levels both between and within lots of product. These calculation methods and the associated results differ from current calculations implemented and published by the ICMSF. One difference results from an error in a spreadsheet calculation of the probability of lot rejection. The ICMSF calculation method fails to include the Poisson process of taking samples (as distinct from measuring concentrations) and misinterprets the meaning of the parameter, m, for sampling plans that rely on qualitative test results (e.g., 'absence in 10 samples of 25 g'). The overall benefits of a simulation-based approach to calculating the performance of sampling plans are described, including the ability to consider the joint impacts of sampling for both pathogens and indicator organisms as well as flexibility in assumptions regarding the shape of the distribution of contamination.

T4-11 Investigation of Using NI-P-PTFE Coating to Minimize Cleaning Time of Tomato Fouling Deposit

DSC

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Formation of fouling inside the food processing plant is more severe than other processing plants. The complexity of the food composition is one reason for this. For hygienic reasons in the food industry, stainless steel is used in fabricating almost all of the process equipment. However, stainless steel surface is hydrophilic, has high surface energy and encourages fouling formation. Modifying the surface properties might solve the problem. Many studies have been done to investigate the effect of surface free energy on fouling build-up, but few studies have been done to investigate the effect of surface free energy on cleaning time. In this work, the effect of surface free energy on cleaning time of tomato fouling deposit was studied. Seven stainless steel disks were coated with Ni-P and Ni-P-PTFE composite coatings. All disks have different surface free energies (20.11mN/m – 37.47mN/m). Tomato deposits on the coated disks and on stainless steel disk were cleaned with water alone at different flow rates and temperatures. Results from cleaning studies were compared with results from micromanipulation technique to relate the effect of adhesive strength of the deposit to cleaning time. Results showed that the response of cleaning time towards different surface energies is not the same as that of fouling build-up. There exists an optimum surface free energy for reducing the cleaning duration (approximately 21-26 mN/m), but this was only observed at cleaning condition of 70°C and 2.3 l/min. The results suggest that the effect of surface free energy on cleaning is not always obvious, due to the influence of other parameters.

T5-01 The Health Belief Model as a Framework for Analyzing Food Safety

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Food safety is a serious issue in America. This makes it important to understand consumers' food safety behaviors and what factors predict those behaviors. The primary objective of this study was to examine food safety within the framework of the Health Belief Model. The sample population was Kentucky households. A total of 1,321 households were contacted by telephone between July and August 2005, and 841 (63%) consumers participated. The sample was obtained using a random-digit dialing procedure to

ensure that every household had an equal probability of selection. The questionnaire was based on a US Food and Drug Administration (FDA) survey. Three sets of variables derived from the Health Belief Model were included in the analysis: readiness variables that motivate action, modifying variables that enable or hinder action, and food safety behaviors. For this study, readiness variables included: confidence in the safety of the nation's food supply, commonality of food poisoning, and location of most food safety problems. Modifying variables included: (a) self efficacy, where respondent was the main food preparer or had stopped eating a food because of a safety concern, (b) health motivation based on satisfaction or dissatisfaction with one's health, and (c) socio-demographics. The food safety behavior variable contained ten items pertaining to practices people employ during preparing, cooking, holding, cooling, and eating food and were summed to create a single behavioral indicator. Multiple regression analysis was used to determine the predictive effect of the independent variables on the dependent variable, food behavior. Self efficacy was found to be significant (P value=.0026; beta coefficient 3.93759), along with some socio-demographic variables. Understanding of real and perceived food safety risks combined with strategies that target specific behaviors may be the most effective approach to improve food handling practices. A better understanding of consumers' food safety risk perceptions and behaviors could lead to more effective food safety education.

T5-02 Exploring the Role of Risk Perception and Sociodemographic Factors on the Use of Thermometers in Food Preparation

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Despite the extolled benefits of using thermometers during food preparation and food handling, there seems to be a rather low usage of thermometers among food preparers and handlers. Public health food safety interventions, including educational campaigns, will be more effective with improved knowledge of the factors that influence the use of thermometers in food preparation. We use survey data to analyze attitudinal, risk perception and sociodemographic factors associated with consumer use of food thermometers. A bivariate probit model of thermometer use for three different food sizes (large roast, chicken, and hamburger) and thermometer ownership was estimated. To analyze the determinants of food safety behavior we employ as a conceptual framework the Health Belief Model (Ajzen and Fishbein, 1980). The model assumes that individuals make rational decisions about health behavior based on awareness of a risk, knowledge of the risk, and judgment about the level of the risk (McIntosh et al., 1994). We model thermometer use as a function of risk perception, attitudes, illness experience, and individual social and demographic variables. In general demographic factors did not significantly influence thermometer use. Individuals who thought thermometer use was costly and a hassle were less likely to use thermometers. Consumers were more likely to use thermometers if they perceived a greater risk for food safety. Education and awareness of the safe handling labels positively predisposed thermometer use in food preparation. The results are also suggestive that educational messages should stress the positive benefits of thermometers in preventing foodborne illness.

T5-03 Foodservice Manager Credentialing: Effects on Food Safety and Health Inspection Scores

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When consumers dine in restaurants, they have the right to expect that the food they consume is healthy, wholesome, and free from contaminants. One of the best ways to protect the public from foodborne illnesses is through the restaurant inspection process. Even though this process can vary from state to state and county to county, the basic elements of the inspection process are usually consistent. Many studies have shown that food safety knowledge and manager certification have translated into better health inspection scores and fewer cases of foodborne illness. Other studies have shown no such improvement, while still others have shown certification leads to improved knowledge but has no effect on health inspection scores. The purpose of this study was to examine the impact of foodservice manager credentialing and knowledge on health inspection scores. The study was conducted by surveying 4,788 foodservice managers from 8 states and 28 counties at the time of the routine health inspection. The questionnaire was divided into three sections; the first gathered information concerning the manager, the second presented 12 scenarios to gauge food safety knowledge, and the third gathered information concerning the foodservice establishment. Data were analyzed to determine if there were any relationships between inspection scores and food safety knowledge scores. Additionally, correlations and regression analysis were used to examine relationships between credentialing, knowledge, training method and certification program. The results of the study showed that certification program and training method do have an impact on the level of food safety knowledge of the foodservice manager. The study showed that managers who were certified were more knowledgeable in food safety practices, but this knowledge did not transfer over to better health inspection scores. This showed that certifying foodservice managers is not enough to keep food safe.

T5-04 Examining the Exam — Food Safety Training and Certification for School Food Service Personnel

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Foodborne illness continues to plague US consumers. Children are one of the most vulnerable populations to suffer severe consequences of unsafe food. The Conference on Food Protection has been proactive in developing standards for the food manager credentialing exam to address critical food safety issues in foodservice operations. States have varied in the extent to which they have adopted and implemented this policy as required by the FDA Food Code. After 15 years conducting certification courses and administering nationally accredited exams, University of Massachusetts (UMass) Extension noted a dramatic decline in exam passing rates, raising questions about both the exam itself and the examinees. In 2001, UMass Extension conducted a study of ten classes in four Massachusetts cities (n=267 school food service personnel), which revealed that passing rates averaged only 59%, compared to previous rates of 85–88%. In 2003, UMass Extension and Department of Nutrition began collaborating with state and national partners on a three-year, three-phase study to examine barriers to successful exam completion. Quantitative and qualitative methods were used to examine learners' needs, exam items, and educational materials. In Phase I, 34% of the 209 participants failed the exam. The mean reading level of those who failed was a grade equivalent of 4.0. In Phase II, 31% of the 214 participants failed; mean reading

level of these individuals was a 4.9 grade equivalent. Participant interviews revealed language and comprehension difficulties, and potential concerns regarding relevance of exams to food service personnel in school settings. Findings from this study will be presented within the context of characteristics of school food service employees who failed the exam. Conference participants will reflect on their personal learning styles, experience a qualitative interview process, and discuss educational and policy implications.

T5-05 DSC Operational and Individual Self-reported Behavior Change among University Employees and Residents in Response to a Norovirus Outbreak

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The US Centers for Disease Control and Prevention (CDC) estimate that 23 million cases of acute gastroenteritis are due to norovirus infection. The mode of transmission by food and person-to-person is well recognized. On January 30, 2006 a cluster of acute gastroenteritis cases (n = 47) was reported to a public health department in southwestern Ontario. By February 8, 2006 the number of confirmed cases hit 155: all university students, most notably from a single residence. In this study, combinations of qualitative and quantitative methods were combined to provide a more complete picture of the phenomenon. This research reports on the results of interviews and focus groups conducted with housekeeping and foodservice staff to explore the impact of the outbreak on clean-up and food-handling procedures, including how effectively these frontline individuals perceived that university administrators dealt with meeting their needs (information, additional staff, supplies). As well, surveys were used to determine how knowledge of, or infection during, the outbreak affected the personal hygiene of student residents. The data obtained from this research can be used to highlight lessons learned, and help to develop plans of action in managing future outbreaks on university campuses.

T5-06 An Evaluation of Inter-auditor Reliability within an Accredited Food Safety Program

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As a result of the escalating demands on food retailers and manufacturers to develop a "due diligence" defense, there are an increasing number of globally recognized food safety accreditation programmes. Each program encompasses a common theme of food safety disciplines, but there is a need to establish a structural comparison of auditor performance. The purpose of this research was to attain an understanding of auditor reliability and of non-conformances identified. The Global Food Safety Initiative recognizes the BRC food safety standard, and it was this program that was used to develop the data for the research. Non-conformance records were evaluated by accredited auditors over 30 separate audits (15 either side of a revision to the BRC Standard). Findings suggest that the average number of non-conformances identified by auditors fall within a consistent range in up to 90% of audits undertaken. The difference in the average number of non-conformances awarded between individual auditors can range up to 10. One-hundred percent of permanently employed auditors awarded higher average numbers of non-conformances than sub-contracted auditors. Despite the number of clauses being raised by 25% during the revision of the Standard, the number of non-conformances issued after the revision increased by 31%.

T5-07 Consumers' Need for and Use of Information on Restaurant Food Safety

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Consumer perceptions of food hygiene can be a key determinant of restaurant choice, although little information is available on how consumers acquire and use hygiene information. One solution – publication of inspection reports – is currently being debated / implemented in many countries. The present investigation was conducted in the UK to determine the public's knowledge of the inspection system and their perception of hygiene standards. A telephone survey of 110 respondents found that 99% who ate out regularly (once a month) claimed the standard of food hygiene was important when deciding where to eat. Assessments of hygiene standards were mainly based on aesthetics (cleanliness of glasses, cutlery, tablecloths). A minority (30%) had concerns/complaints about the hygiene standards of eating places visited. The type of complaints and the action taken varied by age and gender. A quarter knew who was responsible for, and the mechanism of, inspecting food premises. They had unrealistic expectations about the frequency (18% thought monthly) and duration (35% thought 2 h for a small business) of inspections. Ninety percent believed that results of inspections should be available. Half thought that it was difficult to find information on the hygiene standards of eating places and 10% believed they would eat out more if this information was available. The majority (97%) thought that eating places should be required to display evidence that they had passed the hygiene inspection, with a star system preferred, followed by grade, then a score. These findings indicate that consumers would like more information on inspection results, and whatever method of information disclosure is used, the public and media will need to be educated on the inspection and enforcement process. The challenge is to identify creative ways to disseminate up-to-date information that can be readily interpreted by a wide diversity of consumers.

T5-08 Consumer Storage Practices for Refrigerated Ready-to-Eat Foods: Results of a Web-based Survey of Pregnant Women, Seniors, and the Remaining Population

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Listeria monocytogenes can cause serious illness in pregnant women, their unborn fetuses and neonates, seniors, and immunocompromised individuals but causes mild illness in the remainder of the population. Proper storage and handling of refrigerated Ready-to-Eat (RTE) foods can help reduce the risk of listeriosis. To characterize consumer storage practices for refrigerated RTE foods, a nationally representative Web-enabled survey was conducted of pregnant women (n = 249), seniors (n = 946), and the remaining population (n = 865). The survey collected information on refrigerator storage time for bagged salads, pre-cut fresh fruit, pre-cut fresh vegetables, frankfurters, and vacuum-packed luncheon meats. We compared respondents' reported storage times for unopened and opened packages to USDA/FDA storage time guidelines. Most respondents (> 95%) stored unopened packages of these products for the recommended time or less. Most respondents stored opened packages of bagged salads, pre-cut fresh fruit, and

frankfurters for the recommended time or less; however, one-third or more stored pre-cut fresh vegetables and vacuum-packed luncheon meats for longer than the recommended time. The survey also collected information on refrigerator storage time for smoked seafood, cooked crustaceans, soft cheeses, freshly-sliced deli meats, and deli salads. For these products, we compared respondents' total storage time (unopened and opened) to USDA/FDA storage time guidelines. Most respondents stored smoked seafood for the recommended time or less; however, about one-third of respondents stored cooked crustaceans and deli salads for longer than the recommended time and about one-half stored freshly-sliced deli meats and soft cheeses for longer than the recommended time. For some products, pregnant women were more likely to store product outside the storage time guidelines and seniors were more likely to store product within the guidelines. The survey findings suggest the need to educate consumers about recommended storage times for refrigerated RTE foods.

T5-09 Understanding Food Safety Information Needs: Using an Information Service as a Research Tool

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For more than 10 years, the Food Safety Network at the University of Guelph has provided research, commentary, policy evaluation and communications on food safety issues from farm-to-fork. In December 2002, a public information service component was launched, providing food safety information through a number of venues including a national toll-free phone line. The line is staffed by food and health professionals with expertise in home economics, food science, nursing and adult education. The service is unique in Canada, providing not only food safety information on issues across the entire farm-to-fork food chain, but an opportunity for researchers to gain insight into the information needs of those who use the toll-free line. Data on the calls received through the line were collected from January 2003 through December 2005. To facilitate statistical analysis, presenting questions (initial questions posed by the callers) were categorized as one of 22 food safety themes, ranging from agriculture to food preparation. Themes of any additional questions posed during a single phone call were also recorded, along with demographic data. Consumers (80.6%) were the most frequent users of the toll-free line. Callers were more frequently female (79%). Food storage was the most common theme of the presenting question (example: Are canned goods that have been frozen still safe to use?). Callers often posed additional questions after the initial presenting question. The on-going collection of data will continue to provide information on food safety issues of public concern and the people who are actively seeking information on those issues. Analysis of results of this research permits the development of information materials tailored to the specific needs of identified audiences.

T5-10 Music Enhances a Food Service Food Safety Curriculum for High School Students

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A nine-lesson curriculum, entitled "Ready, Set Food Safe" (RSFS), for teaching food-service food safety to high school students was revised to add nine popular songs with food safety lyrics. The songs were selected from those written by Dr. Carl Winter (http://foodsafe.ucdavis.edu/music.html#songsgeneral). Idaho Family and Consumer Sciences high school teachers, experienced in teaching RSFS, were recruited to teach the curriculum and evaluate student food safety knowledge, attitudes and behaviors. The teachers were randomly assigned, after blocking by school size, to teach RSFS either with or without the added songs. Data was obtained from 17 classrooms for 324 students (music-added, 181; control group, 143). Knowledge was assessed using twenty questions, selected from the 50-item certification test, that covered topics reinforced by the song lyrics. Students in the music-added group answered correctly more often on seven questions, while the control group answered three of the questions correctly more often; there was no difference for 10 questions. Attitudes were evaluated by asking students to assess 16 food safety actions of food-service workers in a case study. More of the students in the music-added group provided the same rating as food safety professionals for seven of the behaviors, while more of the control group agreed with food safety experts for 5 behaviors (no difference for four behavior ratings). Teachers recorded their observations of 19 food safety behaviors of 4 students per classroom in the foods lab. Because of the large variation produced by individual observation style, we were unable to draw distinctions between the two groups. Teachers in the music-added group were universally enthusiastic about the addition of the songs and often provided anecdotes of increased student interest. Student opinion of the songs was more mixed, but overall was positive. Our experience with adding music to a food safety curriculum indicates this is a valuable addition in classroom teaching of food safety, providing increased interest and enthusiasm.

T5-11 Coloring Fruit and Vegetable Food Safety Education

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A coloring book entitled the "Fun Fruit and Very Vegetable Tour" was developed for children of farm workers to teach them about produce safety. This story follows three children as they tour the United States produce industry to see how fruits and vegetables are grown, harvested, and sold. Main learning themes include proper food preparation, the importance of hand washing, and the nutritional value of fruit and vegetable consumption. Each book is bilingual (English and Spanish) and appropriate for ages 5–10. These coloring books are packaged with crayons, soap, and a washcloth in a clear vinyl envelope to encourage interactive play and learning. This presentation will discuss the development of the coloring book and present data from focus groups that were conducted in 2005 at summer education programs with teachers and children of farm workers. Focus groups were conducted at five different schools in several classrooms in each school to capture various age groups. A total of 96 children responded to written surveys while a smaller and much younger group of students (ages 5 and under) responded verbally to a limited set of questions. Although the main educational goal of developing these books is to educate children about produce food safety and hand washing, many of the children indicated that they would share these books with their parents who work in the produce industry harvesting and packing fruits and vegetables. It is hoped there will be an education cascade from child to parent. The coloring books are part of a larger farm worker education program created by the National GAPs Program to reduce microbial risks in fruits and vegetables through education and extension programs. Visit www.gaps.cornell.edu for more information and to view available educational materials.

T5-12 Recruiting in the Digital Age: How to Promote Poultry Science and Food Science to Generation Y

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Marketing college curriculums to Generation Y students requires using more involved techniques than those used to attract their parents. Today's students represent the first generation to grow up in a technologically reliant society, resulting in a "gap" between digital immigrants and digital natives. The objective of this ongoing research is to determine innovative ways to recruit students into the processing curriculum of Auburn University's Poultry Science Department by utilizing web-based tools and new technology. An experiment was done to determine if high school students in rural Alabama had internet access, how much they used online services, and if they would be interested in internet-based recruiting methods. Students were asked to rank questions using a 1–10 (lowest to highest or least to most) scale to establish internet access availability, interest in web-based recruiting, interest in Poultry Science, and where they were getting information about colleges and scholarships. Based on the results from this simple study, it was realized that there was a definite need for online curriculums and course material and that more students turned to the internet instead of school counselors or teachers for college information. A recruiting website for the Poultry Science Processing Curriculum would pose the best vehicle to inform students about opportunities in the poultry and food science industry, giving video links to recent Auburn Poultry Science graduates and their job descriptions, and have the most current information about salary ranges and career ladders. This new method will help bridge the digital immigrant-digital native gap, while still embracing the more traditional approach to student recruiting.

T6-01 Effect of Temperature and Storage Time on the Fate of *Listeria monocytogenes* on Inoculated Salami

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Although dry/semi-dry fermented sausages are characterized as low risk, data are lacking relative to the fate of post-processing *Listeria monocytogenes* contamination during storage. This study evaluated the fate of the pathogen during storage of salami. Commercially-produced salami was sliced and inoculated (3.47 to 4.26 log CFU/cm^2) with one of four inocula. The inocula, consisting of a 10-strain *L. monocytogenes* composite, were prepared in (1) tryptic soy broth (containing 0.25% glucose) + 0.6% yeast extract (TSBYE); (2) tryptic soy broth without glucose + 0.6% yeast extract (TSBYE-G); (3) TSBYE-G + 1.0% glucose; or (4) salami homogenate. The first three inocula were cultivated at 30°C (24 h), while for the fourth inoculum, cells were first prepared in TSBYE (30°C, 24 h) and then habituated in salami homogenate at 7°C for 72 h. Inoculated samples were vacuum-packaged (three per inoculum type; two replicates), stored at 4, 12 or 25°C, and analyzed periodically for surviving populations. In general, pathogen levels decreased during storage, and rates of reduction increased with storage temperature. Levels of the acid-adapted inoculum (inoculum iii) decreased more rapidly at 4°C than those of than those of other inocula, but survived longer than other inocula at 25°C. The habituated inoculum (inoculum iv) survived the longest at 4°C, but died faster than other inocula at 25°C. At 12°C, all inocula decreased similarly during storage. In general, all inocula decreased below the detection limit (-0.37 log CFU/cm^2) by 39 days of storage. These results may be useful in quantifying risk of listeriosis via consumption of salami products stored at various temperatures.

T6-02 Effects of Low Equal Molar Concentrations of Three Food Grade Acids on *Listeria monocytogenes* in Bologna

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Research has shown that acetic, lactic, and citric acid are highly inhibitory against *Listeria monocytogenes* in ready-to-eat meat products when applied as 1–2.5% dipping solutions. However, most research has evaluated volume-to-volume (v/v) concentrations, and found acetic acid as the most effective. This study compared the antilisterial effects of low equal molar concentrations (0.083 M) of acetic (0.5% v/v, pH 2.90), lactic (0.75% v/v, pH 2.30) and citric (1.6% v/v, pH 2.05) acid, and their combination (0.17% v/v acetic, 0.25% v/v lactic, 0.53% v/v citric acids, pH 2.24) in bologna. In addition, one acid-resistant and one acid-sensitive strain were compared as individual inocula for antimicrobial activity by these treatments. Bologna slices were inoculated with a ten-strain composite or with each of the two individual strains (3 log CFU/cm^2), and inoculated slices were dipped in each treatment solution. Slices were vacuum packaged and incubated at 7°C for 50 days. Three samples of each treatment were plated on PALCAM and tryptic soy agar with 0.6% yeast extract during storage. Control (undipped) samples with the ten-strain inoculum reached 6 log cycles within 9 days, while citric acid was the most effective, suppressing growth below 6 log units until day-33. The acid treatments inhibited growth in increasing ($P < 0.05$) order as follows: citric>combination>lactic=acetic. Individual strains, even though selected for extreme resistance or sensitivity to acid in broth, showed no difference ($P < 0.05$) in growth rates on bologna, indicating that acid sensitivity in pure culture may not be associated with inhibitory activity of acids in food. In general, the acids showed antimicrobial activity even at low molar concentrations. Citric acid, which had the lowest pH, caused the most inhibition, while the acid combination, which had a pH similar to lactic acid, was second in activity. The interactive effects of pH, acid type, and concentration need further study.

T6-03 Modeling to Predict the Growth/No Growth and Selected Growth Limit Boundaries of *Listeria monocytogenes* in Ready-to-Eat Products as a Function of Lactic Acid Concentration, Dipping Time, and Storage Temperature

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This study developed models for the prediction of lactic acid concentration, dipping time, and storage temperature combinations determining growth/no growth interface boundaries, preventing growth, or allowing selected levels of growth of *Listeria monocytogenes* in Ready-to-Eat (RTE) meat products. *L. monocytogenes* (10 strain-composite) was inoculated (2 log CFU/cm^2) on frank-

furters and ham slices, and a total of 75 treatment combinations (lactic acid [0, 1, 2, 3, or 4%] ↔ dipping time [0, 1, 2, 3, or 4 min] × storage temperature [4, 7, or 10°C]) were tested. Samples were stored in vacuum packages for up to 60 days, depending on product type and storage temperature and analyzed periodically. Bacterial populations on samples were enumerated on tryptic soy agar plus 0.6% yeast extract and PALCAM agar. Treatment combinations allowing significant ($P < 0.05$) increases in *L. monocytogenes* populations (PALCAM agar) at a given storage time were scored as 1 (growth), while combinations showing significant ($P < 0.05$) decreases or non-significant ($P \leq 0.05$) increases were scored as 0 (no growth). These binary growth response data were fitted with logistic regression to develop a model predicting growth/no growth boundaries. Conditions allowing selected growth levels (e.g., 1 log cycle) were predicted by a quadratic formula using estimates of coefficients selected ($P < 0.1$) from linear model analysis fitted with the data of differences in *L. monocytogenes* populations between time-zero and the end-point of storage. The models were developed with data from real foods and their validation was done with published *L. monocytogenes* data. The results showed successful prediction of the response of the pathogen in 90% of the tested cases. Therefore, the models developed may be useful in selecting appropriate lactic acid concentrations and dipping times to control *L. monocytogenes* in RTE meat products. In addition, the procedures developed may be useful in the development of models for other products, conditions or pathogens.

T6-04 DSC Control of *Listeria monocytogenes* on Frankfurters Formulated without Lactate by Dipping in Sodium Lactate before and after Inoculation

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Control of *Listeria monocytogenes* is an important food safety concern, especially in vacuum packaged cold-stored (4°C) Ready-to-Eat (RTE) meat and poultry products. The purpose of this study was to evaluate non-lactate formulated frankfurters for the effectiveness of sodium lactate (12% v/v) as an antimicrobial dip when used prior to and after inoculation of *L. monocytogenes*. For treatment one, five frankfurters were inoculated with a five-strain cocktail of *L. monocytogenes* for 10 min, stabilized for 30 min, then dipped in sodium lactate for 30 s. Treatment two frankfurters were dipped in sodium lactate for 30 s, stabilized for 30 min and then dip inoculated for 10 min in *Listeria monocytogenes*. Controls were prepared by dip inoculating frankfurters. One frankfurter from each treatment was sampled immediately. The remaining frankfurters were vacuum packaged, stored at 4°C and sampled after 30, 60, 90, and 120 days. On each sampling day one frankfurter from each treatment was stomached and serial dilutions were plated on tryptic soy agar (TSA) for viable cell counts, and Modified Oxford Medium (MOX) for *L. monocytogenes* counts. Plates were incubated at 35°C for 48 h. Results showed that frankfurters that were first inoculated and then dipped in sodium lactate had lower total counts than the control up to day 120, and did not steadily increase in total counts until day 90. Frankfurters that were first dipped in sodium lactate and then inoculated had significantly lower total counts than the control on days 30) ($P < 0.0001$), 60 ($P \leq 0.0011$), and 90 ($P \leq 0.0266$). *Listeria* counts were also significantly lower than the control on days 10 ($P \leq 0.0285$, 30) ($P < 0.0001$), 60 ($P \leq 0.0001$), and 90 ($P \leq .0521$). This research indicates that sodium lactate, when applied to frankfurters before *L. monocytogenes* contamination, could significantly reduce the levels of the organism in RTE frankfurters.

T6-05 DSC Effects and Interactions of Sodium Lactate, Sodium Diacetate, and Pediocin on the Thermal Inactivation of Starved Cells of *Listeria monocytogenes* on the Surface of Bologna

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The effects and interactions of sodium lactate, sodium diacetate, and pediocin on the thermal inactivation of starved cells of *Listeria monocytogenes* on the surface of bologna were investigated. The heating temperatures used in the study were 56.3 to 60°C and the antimicrobials were: sodium lactate (SL), 0.0 to 4.8 % w/w; sodium diacetate (SDA), 0.0 to 0.25% w/w; and pediocin (P), 0.0 to 10000AU. Thermal treatment of bologna was done in vacuum packaged bags submerged in a circulating water bath set at the specified temperatures. The samples were plated on the modified Oxford formulation and brain heart infusion agars. Decimal reduction times (D-values) were calculated using linear regression. The observed D-values ranged from 2.8 min at 60°C to 24.61 min at 56.3°C. Injury ranged from 9.1 to 76%, and it was seen in all experiments under all conditions studied. The observed D-values were analyzed using second order response surface regression for temperature, SL, SDA, and pediocin and a multiple second order regression equation was developed. The predicted D-values were calculated, and they showed a good correlation with the observed D-values. A predictive model was developed. Temperature had the most significant effect on the inactivation of starved cells of *L. monocytogenes* on the surface of bologna. The combination of temperature with all the antimicrobials (SL, SDA and pediocin) showed significant effect in inactivating the starved cells of *L. monocytogenes*. The combinations between pediocin and SL, and pediocin and SDA showed significance also. The combination of SL and SDA rendered *L. monocytogenes* on the surface of bologna less resistant to heat. The model can predict D-values for any combinations of temperature, SL, SDA and pediocin within the tested range of factors.

T6-06 Use of Octanoic Acid as a Post-lethality Treatment to Reduce *Listeria monocytogenes* on Ready-to-Eat Meat and Poultry Products

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Antilisterial efficacy and organoleptic impact of an octanoic acid-based treatment (OA) for ready-to-eat (RTE) meat and poultry products were investigated. Whole-muscle and comminuted RTE products were inoculated with a five-strain mixture of *Listeria monocytogenes*. Octanoic acid treatments were applied to the surface of RTE products by dispensing a specific volume of solution directly into the final package prior to vacuum sealing. Once sealed, the vacuum packaged RTE products containing OA were immersed in water heated to 93.3°C (200°F) for 2 s to affect adequate package film shrinkage. Extending the time at which the packaged, treated RTE products were exposed to 93.3°C water was also evaluated, using a commercial cascading shrink tunnel fitted with a modified drip pan. Once treated, RTE products were examined for survivor populations of *L. monocytogenes* after 24-h storage at 5°C. Sensory evaluation was conducted, using a 60-member trained panel, on 11 treated RTE products. Octanoic acid treatment of RTE products reduced *L. monocytogenes* numbers between 0.85 log CFU/product (oil-browned turkey) and 2.89 log CFU/product

(cured ham) when compared to controls. Antilisterial activity of OA was improved by increasing the duration of the heat shrink exposure. Log reductions ranged from 1.46 CFU/product (oil-browned turkey) to 3.34 CFU/product (cured ham). Results from the sensory evaluation demonstrated that 10 of the 11 treated RTE products were not perceived as different ($P \leq 0.05$) from the nontreated controls. Panelists detected reduced ($P \leq 0.05$) smoke flavor intensity with treated mesquite turkey although the treated product was viewed as acceptable. Results demonstrate the effectiveness of octanoic acid as a post-lethality treatment meeting FSIS regulatory guidelines for RTE meat and poultry products with minimal impact on sensory quality.

T6-07 Impact of Nitrite on Detection of *Listeria monocytogenes* in Selected Ready-to-Eat Meat and Seafood Products

DSC

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This study was conducted to determine the impact of nitrite ($NaNO_2$) in smoked salmon, smoked ham and bologna on viability and detection/recovery of *Listeria monocytogenes*. Nitrite-containing (NC-200 ppm $NaNO_2$) or Nitrite-free (NF) foods were inoculated with a 5 strain cocktail of *L. monocytogenes* at ca. 2.5×10^2 CFU/g, vacuum-packed, and stored at 5°C. Samples were analyzed weekly for presence of *L. monocytogenes,* using modified University of Vermont broth (UVM), *Listeria* enrichment broth (LEB), *Listeria* repair broth (LRB), a combination of UVM and LRB (dual enrichment), and the PCR-based BAX System during 4–8 weeks of storage. Residual $NaNO_2$, total aerobic count, and % injury were also determined at each sampling. *L. monocytogenes* were directly enumerated on Modified Oxford agar (MOX) and CHROMagar. By the end of week 1, plating on CHROMagar and MOX resulted in 1×10^2 CFU and $< 1 \times 10^2$ CFU *Listeria*/g, respectively, for NC foods. This decrease in *L. monocytogenes* is attributed to $NaNO_2$-induced injury. $NaNO_2$ decreased from 200 ppm at week 0 to < 20 ppm at week 4 and thereafter. The BAX system and the dual enrichment detected 100 and 92.4% of positive samples, respectively, in all products throughout the storage period. While LRB recovered 43/48 *Listeria*-positive samples and UVM recovered 39/48 in NC smoked salmon, both media recovered *Listeria* in NF salmon-positive samples 100% of the time. As for smoked ham and bologna, UVM recovered 72/72 and 53/72 of NF and NC positive samples, respectively, while LRB recovered 45/72 and 40/72. Overall, more positive samples escaped detection at the earlier sampling times, indicating that high $NaNO_2$ masked detection of positive samples. These results provide evidence that $NaNO_2$-injury masks detection/recovery of *L. monocytogenes* in NC Ready-to-Eat meat and seafood products, in which the organism can repair and grow to high levels over extended refrigerated storage periods.

T6-08 Interaction of *Pseudomonas putida* and *Listeria monocytogenes* in Mixed Culture Biofilms

DSC

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Listeria monocytogenes is a foodborne pathogen that causes problems in many food processing plants because it produces biofilms and thus is difficult to control by regular cleaning and sanitizing procedures. It has been stated that the growth of *L. monocytogenes* is enhanced in mixed culture biofilms, but little information is available that provides a mechanistic explanation for this. Mixed culture biofilms with *Pseudomonas putida* (labeled with green fluorescent protein) and *L. monocytogenes* EGD were examined to determine whether one organism would enhance growth of the other. Mono and mixed culture biofilms were grown on glass cover slips, in M9 1× minimal salt medium supplemented with 1mM glucose at 22°C for 24 h using a flow cell. Images were taken using a scanning electron microscope and with a confocal scanning laser microscope, after staining *Listeria* cells with Texas Red-X conjugate of wheat germ agglutinin. Confocal images captured at inlet, middle and outlet of the flow cell were analyzed with the novel biofilm program PHLIP for total biovolume and mean thickness. At the inlet, almost all biofilms produced highest total biovolume and mean thickness; this was significant for *P. putida* ($P < 0.05$). In mixed culture biofilms at the inlet of the flow cell, biovolume contributed by *L. monocytogenes* cells was much higher than in monoculture *L. monocytogenes* biofilms (532% increase). In contrast, in mixed biofilms in the middle section and at the outlet, biovolume contributed by *P. putida* cells was much lower than in monoculture *P. putida* biofilms, with reductions of 183% (middle) and 793% (outlet) Further investigation will be required to examine whether distribution of extracellular polymeric substances and/or oxygen levels throughout the flow cell play a significant role in affecting the biovolume and thickness of these biofilms.

T6-09 *CtsR* and Its Interaction with Sigma B are Required for Heat Tolerance, Motility, and Host Cell Invasion in *Listeria monocytogenes*

DSC

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The *L. monocytogenes*' alternative sigma factor B positively regulates transcription of class II stress response genes while *CtsR* negatively regulates class III stress response genes. To characterize interactions between these two stress response systems, we generated *L. monocytogenes* D*ctsR* and D*ctsR*D*sigB* mutants as well as a D*ctsR* strain with an IPTG inducible *CtsR*. In combination with the wild type parent strain and a D*sigB* strain, the newly created mutant strains were examined using phenotypic assays (i.e., motility, heat resistance, invasion of human intestinal epithelial cells) as well as TaqMan qRT-PCR. Electron microscopy revealed that the D*ctsR* and D*ctsR*D*sigB* strains are morphologically distinct from the wild type strain: the mutant strains are elongated and have fewer flagella. The D*ctsR* and D*ctsR*D*sigB* strains both showed reduced swarming and significantly higher thermotolerance than the wild type *L. monocytogenes* strain. To illustrate, after 4 s at 72°C, D*ctsR* and D*ctsR*D*sigB* viable numbers were reduced 3.1 and 3.5 logs, respectively, compared to a 6.5 log reduction for the parent strain. Full heat sensitivity was restored to the mutant strains when ctsR was expressed in *trans*. qRT-PCR showed increased expression of the *clpC* heat shock gene in the D*ctsR* and D*ctsR*D*sigB* strains, suggesting that enhanced stress resistance results from increased expression of stress response genes in the mutant strains. While the D*ctsR* strain did not show reduced ability to infect human intestinal cells, the D*ctsR*D*sigB* strain had considerably lower invasion efficiency (10.3%) than either the parent strain (100%) or the D*sigB* strain (44.8%). In conjunction with qRT-PCR results showing much lower inlA expression in D*ctsR*D*sigB* than in the parent strain, the data presented here indicate that interactions between the CtsR and the sigma B stress response systems are essential for both stress response and virulence, including survival of heat treatments used to eliminate *L. monocytogenes* in Ready-to-Eat foods.

T6-10 A Method to Detect Significant Clusters in Phylogenies Shows That *Listeria monocytogenes* Contains Clonal Groups with Distinct Ecological Preferences

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While phylogenetic analysis has been widely used to define closely related groups of isolates within a bacterial species, identification of phylogenetic clades that are associated with different environmental niches or host species remains a challenge. We used *Listeria monocytogenes*, which causes disease in many host species and can be isolated from a number of environments, as a model organism to develop and evaluate a novel two-step statistical approach to identify phylogenetic clades that are significantly associated with distinct sources (i.e., humans, animals, and food). Specifically, if the null hypothesis that the genetic distance between isolates within and between sources is identical can be rejected using the SourceCluster test, then clades within a phylogeny that are characterized by significant overrepresentation of isolates from a particular source can be identified via the TreeStats test. Sequence data from 120 *L. monocytogenes* isolates from human and animal clinical listeriosis cases and food samples were used to build phylogenies from two virulence genes (actA and inlA) and a concatenated housekeeping and stress response gene sequence. SourceCluster analysis revealed that *L. monocytogenes* isolates from the same source are more genetically related than isolates from different sources based on actA ($P = 0.02$) and inlA ($P = 0.07$) sequences, respectively. TreeStats results identified 9 clades with significant ($P < 0.05$) or marginal ($P < 0.10$) source associations in actA and inlA-derived phylogenies. Human-, animal-, and food-associated clades were observed in all phylogenies, supporting niche adaptation within *L. monocytogenes*. Epidemiological and virulence phenotype data for the 120 *L. monocytogenes* isolates studied here supported the power of this novel statistical approach to identify biologically meaningful clonal groups. In summary, our data show that (i) the SourceCluster and TreeStats tests can identify significant same source clades in phylogenetic trees and (ii) *L. monocytogenes* includes both host- and environmentally-adapted clonal groups.

T6-11 Sensitivity and Inclusivity of a *Listeria* Genus PCR Detection Assay Using a Novel Bacteriophage Derived Cell Binding Domain and Phage Endolysin Lysis

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In food processing plants, *Listeria* sp. environmental sample monitoring is important since it can be an indicator of possible sources of *L. monocytogenes* contamination. Sensitive detection and inclusivity are critical since small numbers of *Listeria* are often present in environmental samples and false negatives could result in release of contaminated product. While sensitive and specific, traditional monitoring methods which rely on culture and biochemical detection are time-consuming and labor intensive. Molecular methods of detection are typically faster than traditional culture methods, but may suffer from reduced sensitivity, and specificity problems may arise if primer design is flawed. We hypothesized that using cell capture and concentration with a *Listeria* specific phage derived cell binding domain (CBD) and phage endolysin lysis would allow for improved sensitivity compared to non-captured cells. Moreover, the specificity of these reagents would allow for most *Listeria* genus to be captured and lysed. The CBD and magnetic particles were used to capture and concentrate *Listeria* sp. from enrichments that had been serially diluted to extinction. In addition, a *Listeria* specific endolysin derived from phage was employed to lyse the cells prior to PCR. Using melt curve analysis of real time PCR products from 1-ml capture reactions, we have shown lower limits of detection for *L. innocua*, *L. monocytogenes*, *L. welshimeri*, *L. ivanovii*, and *L. seeligeri* of between 1.2×10^1 and 8.8×10^2 CFU/ml. In contrast, non-captured cells of the same species detected and lysed using a commercially available *Listeria* PCR detection kit required between 10^3 and 10^5 CFU/ml. The use of bacteriophage derived capture and/or lysing reagents allowed for an improvement of 2–4 logs in overall assay sensitivity. Additionally, all of the *Listeria* genera tested were captured, lysed, and detected using PCR. Bacteriophage derived product technology may be amenable to other food pathogens in the future.

T7-01 Factors Affecting Attachment of *Escherichia coli* O157:H7 to Apple Tissues

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Attachment of *Escherichia coli* O157:H7 and fluorescent microspheres to the stem, calyx sepals, russet and discontinuities on the skin of Golden Delicious apples was investigated. Attachment of the *E. coli* cells to the stems resulted in their removal from the inoculum solution over time where the cells were found within crevices spanning the length of the stem. Treatment of the stems with 5% trisodium phosphate, following immersion in inoculum solution for 30 s, resulted in only a 1-log reduction. Concentration of *E. coli* cells that attached to stems following immersion in inoculum were significantly higher than cells attached to stems that were pre-hydrated in sterile water prior to inoculation ($P < 0.05$). SEM imaging showed preferential attachment of *E. coli* to what appeared to be mold mycelia on the calyx sepals. Attachment to the russets was often observed within the cork cells. Cells were also found within discontinuities on the skin, especially at cell junctions. Confocal Scanning Laser Microscope imaging showed that regardless of their surface charge, hydrophobic microspheres attached more regularly than hydrophilic ones to various apple surfaces. Attachment of hydrophobic/negatively charged microspheres to similar surfaces suggests that attachment of *E. coli* to these regions is at least partially governed by hydrophobic interactions. Understanding bacterial interactions with produce surfaces may aid development of more effective methods to improve the safety of produce.

T7-02 Compost Tea from a Food Safety Perspective

DSC

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In simplest terms, compost tea is a brewed water extract made from composted material. The use of compost tea in horticulture production is gaining popularity as a biologically-based disease suppressant because it contains soluble nutrients and has a diverse range of bacteria, fungi and protozoa that theoretically create an environment inhospitable to pathogens. The process of composting

doesn't guarantee the eradication of pathogens that may be found in manure. Therefore, the use of compost tea, depending on the quality of ingredients and the method of production, has the potential to contaminate crops. This study examined the practices of two Ontario producers who actively use compost and compost tea in their operation, in order to evaluate the possibility of compost tea being a potential vehicle in the transfer of human pathogens to produce. Compost, compost tea, tomato leaf (control and sprayed) and tomatoes (control and sprayed) were sampled and analyzed for coliforms, *E. coli*, *E. coli* O157:H7, *Salmonella* spp., *Cryptosporidium*, *Pseudomonas*, yeasts, molds and lactic acid bacteria. Results suggests that the age and type of compost as well as the brewing time impacted the micro flora population in the tea. Low levels of *Cryptosporidium* were detected in all sample types analyzed, but *E. coli* O157:H7 and *Salmonella* spp. were not. Twenty-two month old cattle compost produced tea with significantly lower levels of *E. coli* (ND – 25 CFU/g) compared to 3-month-old cattle (10 – 3700 CFU/g) and 3-month-old horse (3300 – 23000 CFU/g) compost tea. Compost tea that was brewed for less than 19 h had significantly higher levels of *E. coli* compared to compost tea that was brewed for 20 h or longer. Food safety practices need to be diligently implemented during compost and compost tea production and use. It is recommended that compost tea be used only on crops that are not consumed raw.

T7-03 Water Pressure Effectively Reduces *Salmonella* Enteritidis on the Surface of Raw Almonds

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Since 2001, two large outbreaks of salmonellosis have been linked to consumption of raw almonds. These outbreaks collectively caused 223 cases of salmonellosis across North America. As a result of both outbreaks, the Almond Board of California's Food Quality and Safety Committee approved an action plan to ensure that raw almonds entering commerce are treated to reduce microbial contamination. Several methods have been developed to reduce the microbial load on almonds, including chemical and steam based methods. The objective of this study was to investigate the use of high hydrostatic pressure (HHP) as a method to reduce contamination of raw almonds. The effects of continuous (60,000 psi and 70,000 psi with a holding time of 6 min) HHP treatments on the viability of two *Salmonella* Enteritidis strains (PT9c and PT30) inoculated onto raw almonds were evaluated at 25°C. Complete inactivation of both *S.* Enteritidis isolates was achieved when the almonds were directly suspended in water, pressurized at 60,000 psi and 25°C for 6 min, and then dried at 115°C for 25 min. When the almonds were pressurized at 60,000 psi, followed by a dry step at 55°C for 5 min, a log reduction of greater than 3.37 was achieved for both isolates. Increasing the drying temperature to 65°C resulted in a log reduction of greater than 4.13 for both isolates. Increasing the water pressure to 70,000 psi further decreased the *S.* Enteritidis concentration on the surface of the raw almonds. When the almonds were water pressurized at 60,000 psi, the decimal reduction times were determined to be less than 30 s for both *Salmonella* isolates. HHP of certain dry foods appears to be feasible if the food is directly suspended in the pressurizing medium (water).

T7-04 The Effect of Ozone and 'Open Air Factor' against Aerosolized and Surface Attached *Micrococcus luteus*

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With increased concerns over failures in cleaning and the environmental persistence of pathogens, evaluating the efficacy of novel decontaminants is increasingly important in the food industry. Traditionally used antimicrobials and fumigants can be toxic and corrosive. The bactericidal properties of Open Air Factor (OAF) were identified in the 1970s, however, the potential practical applications of artificially generated OAF have only recently been considered. This study investigated the effects of OAF derived from monocyclic monoterpene against *Micrococcus luteus*. OAF was generated and distributed into a bioaerosol test chamber by the delivery of the monoterpene into ozonated air (0.1 ppm) at concentrations of 2.0 (high), 0.75 (medium) or 0.3 (low) mgm-3h-1. *M. luteus* was aerosolized and tested after 2, 5, 10, 20 and 60 min, or attached to stainless steel surfaces (60-minute exposure) that were horizontal, vertical or inverted. Survivors were enumerated by aerobic plate count (aerosolized) or direct overlay with TSA (surface attached). Data were analysed for statistical significance, using a t-test comparing mean net log reductions (MNLR) of test and control data. When aerosolized bacteria were exposed to monoterpene alone, there were no statistically significant differences between test and control. With OAF (high and medium rates), there were significant differences after 2 min (MNLR 4.2 (high) and 3.5 (medium) after 60 min). When low rate OAF was used, a significant reduction was seen after 20 min (MNLR 1.2 after 60 min). These reductions were significantly greater than ozone alone at 0.1 ppm. When the bacteria were attached to stainless steel, OAF was effective at all concentrations (MNLR 1.6). OAF is potentially an effective terminal sanitizer, which can further reduce the microbial load in air and on surfaces. As the technology uses reaction compounds naturally found in the environment, the risks to health may be lower than with ozone or other gaseous treatments.

T7-05 Growth of *Listeria monocytogenes* and a Sigma B Mutant in Soil and on Radishes Grown in Contaminated Soil

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The ability of *Listeria monocytogenes* to grow in soil and on radishes grown in contaminated soil was monitored over the course of radish gestation from seed to mature tuber. Radish seeds were sown in soil that had been autoclaved and then inoculated with *L. monocytogenes* 1 day previously. Levels of a *L. monocytogenes* wild type and an isogenic *sigB*D strain in the soil were monitored for 4 weeks. Sigma B is the alternative sigma factor important for adaptation to stresses in *L. monocytogenes*. Upon radish harvest, levels of *L. monocytogenes* were determined in the soil, on radishes wiped clean of soil with a paper towel, on radishes rinsed free of soil with water, and on radishes that were ethanol-sterilized and stomached. The wild type strain was able to survive in soil over the 4 weeks of the experiment at levels of 4–7 log CFU/g soil. The *sigB* mutant strain also survived in soil, but at levels at least 1 log reduced from the wild type. Upon harvest of the radishes, *L. monocytogenes* wild type was detectable on radishes wiped and rinsed free of soil at approximately 3–4 log CFU/g radish tissue. Similar to the results in soil, the *sigB* mutant was at least 1 log reduced. On radishes that were ethanol-sterilized and stomached, *L. monocytogenes* wild type was detected at low levels on the radish tissue from 0–2 log CFU/g radish tissue; however, no *sigB* mutant was detected on radishes treated this way. Therefore, *L. monocytogenes* was able to survive in soil to colonize mature radishes in a fashion not easily removed from the radish surface, and potentially colonized inside the radish tissue. Sigma B was important for both soil and radish colonization.

T7-06 Evaluation of *Citrobacter youngae* as an Environmental Surrogate for Enteric Bacterial Pathogens on Produce

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Assessments of the fate of pathogens in field and pilot processing studies are facilitated or only permissible with validated surrogates. *Citrobacter youngae* is commonly recovered within preharvest and postharvest environmental and produce samples. Our study objective was to determine if *C. youngae* could qualify as a surrogate for enteric bacterial pathogens, with primary emphasis on *Salmonella*, on leaf surfaces, using a model parsley system. Trifoliate leaves (ntrifoliate=3; nplant=15; ncondition=3) were inoculated with 20 µl of either sterile water or 6.0 log CFU/ml per isolate. *C. youngae* TVS230 is similar to pathogens in vitro and lacks PCR virulence markers for *Salmonella*, *Shigella*, and *E. coli* O157:H7. After inoculation, plants were exposed to conditions of extended free moisture (HH), acute desiccation followed by relative humidity (RH) > 98% (DH), or acute desiccation followed by low RH (DL). All plants were placed under 24-h light at 15°C, either in containment chambers per condition (n = 3) or individual containment units (n = 45). Populations were enumerated at 3, 6, and 9 days after inoculation. Relative attachment was compared by sequential wash recovery. On day 15, plants were exposed to 15-h desiccation before enumeration. The epiphytic behavior of TVS230 under HH and DH was similar to all pathogens tested, though more reflective of *Salmonella*. Under DL, pathogens maintained populations to day 6 while TVS230 decreased by 1 log. From HH and DH, following 15-h desiccation, TVS230 behaved most similarly to *E. coli*. At or near epiphytic carrying-capacity, for all conditions, the log difference between first and second washes was 0.5 to 1.4; the exception was *E. coli* under HH for which more bacteria were liberated in the second wash at day 9. Cross contamination to non-inoculated leaves was detected under all conditions; HH>DH>DL. Individual chambers under DL had the lowest cross contamination. TVS230 is suggested as a surrogate; however, direct comparisons under specific model conditions remain essential to meaningful, applied interpretations.

T7-07 Persistence of Indicator Bacteria in Agricultural Soils following Winter Flooding Events

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Flooding of surface water sources, runoff conveyances or wastewater lagoons can introduce pathogens and other hazardous substances onto agricultural land. This may represent a potential food safety risk to crop production, with special concern being raised for lettuce and leafy greens. As part of a broader effort to evaluate the fate of *E. coli* O157:H7 following a flooding event, this sub-objective investigated the survival of presumptive indicators [*E. coli* and thermo-tolerant coliform (TTC)] in a vegetable production field (SVC) exposed to annual flooding from a creek containing urban and agricultural runoff. Soil was collected from SVC during 2004 and 2005, both following three-month periods of winter flooding and continuing through pre-plant field preparations. In 2004, SVC soil sampling also included areas where lettuce, spinach, and broccoli were planted. Floodwater samples were monitored in 2005. *E. coli* O157 was not detected in soil or water samples. In 2004, *E. coli* and TTC populations recovered from soil (n =142) were higher following flooding at SVC (0.70 and 1.38 log CFU/g, respectively) than non-flooded production soils (0.67 and 1.04 log CFU/g). *E. coli* and TTC populations at SVC were below the limit of detection (0.56 log CFU/g) following pre-seeding preparations. Planting crops in the previously-flooded field did not stimulate the re-growth of indicator bacteria populations in the rhizosphere. In 2005, *E. coli* and TTC populations in the floodwater were 1.95 log CFU/100 ml and 3.85 log CFU/100 ml, respectively; immediately after floodwater receded, indicator populations across the field averaged 1.19 log CFU/g and 2.34 log CFU/g, respectively. Levels declined an additional 0.33 log CFU/g and 0.47 log CFU/g, respectively, after drying and soil tillage. These results suggest that presumptive indicators of fecal contamination decline following natural drying and pre-plant soil management; however, the risk associated with each flooding situation should be evaluated individually.

P1-01 Analysis of Beauvericin and Unusual Enniatins Co-produced by *Fusarium oxysporum* FB1501

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Beauvericins and enniatins are mycotoxins exhibiting various biological activities on animal systems including human. *Fusarium oxysporum* FB1501 producing four different mycotoxins was isolated from soil in Korea and their structures were elucidated by HPLC, MS, IR and NMR analysis. The molecular weights of compound 1, 2, 3, and 4 were determined to be 654.5, 784.5, 668.6, and 682.5, respectively, on the basis of ESI-MS measurements. IR spectra of all compounds exhibited absorptions for the ester (v 1733~1743cm-1) and amide (v 1649~1655cm-1) bonds. The results of IR and NMR analysis (1H, 13C, 135-DEPT, COSY, HMQC and HMBC; in CDCl3) revealed that compound 1, 2, 3 and 4 were enniatin H, beauvericin, enniatin I, and enniatin MK1688, respectively. This study is the first report related to co-production of beauvercin with other unusual enniatins e.g. enniatin H, enniatin I, and enniatin MK1688 from *Fusarium* species.

P1-02 Effect of Coffee Cherries Storage after Harvest before the Beginning of Drying on Contamination by Fungi and the Relationship to Ochratoxin A Production

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The main mycotoxin found in coffee is ochratoxin A produced by molds of the genera *Aspergillus* in tropical regions and by molds of the genera *Penicillium* in moderately cold regions. This mycotoxin has been classified as a possible human carcinogen. Its occurrence can be due to environmental conditions and badly controlled post-harvesting. Thus, the present study was carried out to evaluate the influence of the storage of coffee cherries after harvest before the beginning of the drying on fungal growth and kinetics of ochratoxin A production in order to contribute to the identification of good post-harvest practices to prevent development of this mycotoxin. In this study, Robusta (*Coffea canephora*) coffee cherries from Cóte D'Ivoire were used. The results obtained show that for coffee cherries where drying was started the day of harvest, only 5% of the samples analyzed contained infected beans while, for those that drying was started the fourth and the sixth day after harvest, all the samples analyzed contained infected beans. The identification of fungi isolated shows that the most common species found was *A. niger* (42.19–100%). The test for ochratoxin A production by isolated fungi shows that only strains of *A. niger*, *A. carbonarius* and *A. ochraceus* are ochratoxin A producers. The evaluation of ochratoxin A content shows that coffee cherries more contaminated by this mycotoxin are those when drying was started the sixth day after harvest with levels ranging from 51.27 to 1517.12 µg/kg of green coffee. The storage of coffee cherries after harvest before the beginning of the drying is most likely one of the main parameters contributing to ochratoxin A production. Thus, the prevention and the reduction of ochratoxin A production in coffee can be achieved by starting the drying the day of harvest.

P1-03 Histamine Contents of Fermented Fish Products in Taiwan and Isolation of Histamine-forming Bacteria

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Twenty-seven imported fermented fish products from Southeast Asian countries and sold in the supermarkets in Taiwan, including fish sauce, fish paste and shrimp paste, were tested to determine the occurrence of histamine and histamine-forming bacteria. The levels of pH, salt content, TVBN (total volatile basic nitrogen), trimethylamine (TMA), and aerobic plate count (APC) in all samples ranged from 4.8 to 6.5, 16.2 to 45.3%, 51 to 275 mg/100 g, 5.4 to 53.9 mg/100 g and 1.0 to 4.2 log CFU/g, respectively. The average content for each of eight different biogenic amines in all samples was less than 90 ppm, except for histamine which has an average content of 394 ppm in fish sauce, 263 ppm in fish paste, and 382 ppm in shrimp paste. Most of the tested fermented fish products (92.6%) had histamine levels greater than the FDA guideline of 50 ppm, while seven of them (25.9%) contained > 500 ppm of histamine. Although *Bacillus coagulans* and *B. megaterium* were identified as the two histamine-producing bacteria capable of producing 13.7 ppm and 8.1 ppm of histamine respectively in trypticase soy broth (TSB) broth supplemented with 1.0% L-histidine, they were not determined to be the main contributors to histamine accumulation in these fermented fish products.

P1-04 The Exploratory Data on Furan Content in Canned Food Products and Coffee in the Korean Local Market

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The presence of furan in canned and jarred food is considered as a potential carcinogen and a new endocrine disrupter to humans. In spite of furan's low leveled existence in food product and exposure in human diet, furan could be a carcinogen of major concern for humans. Since they are exposed to canned food products and coffee.The objective of this study was to provide quantified information about furan and its potential toxicity to humans by performing exploratory work on furan levels and by evaluating the amount of exposure in canned food products containing coffee to furan in diet. Fifty canned food products and 21 coffees were purchased from local markets in Korea. A 1–10 gram test portion of liquid, semi-solid, or solid foods were diluted with water, fortified with internal standard (d4-furan), and sealed in headspace vials. Using SPME (Solid Phase Micro Extraction) headspace sampling followed by gas chromatography/mass spectrometry (GC/MS) analysis was used to detect furan and d4-furan in the sim mode. The furan levels in canned food and coffee samples ranged from non-detectable (ND) to approximately 2552.7 parts per billion (canned

fruit foods 0.6–3.5 ppb, canned cone and bean based foods 0.6–22.8 ppb, canned vegetable based foods 0.7–25.0 ppb, canned meat-based foods ND–44.5 ppb, canned marine product based foods 0.4–199.5 ppb, instant coffee 22.6–224.5 ppb, coffee 267.1–2552.7 ppb). Coffee had the highest amount of furan of the foods in this study. This study may provide data for constructing a database for the furan levels in canned foods as well as coffee, which eventually may prevent the potential danger of furan.

P1-05 Trial of the Quality Control in Mercury Contents by Using Tail Meat of Full-cycle Cultured Bluefin Tuna

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Generally there are high levels of mercury in tuna meat, and some fish contain over 10 times the safety standard value. It is desirable that mercury content for each fish be determined. However, this may not be cost effective, since tunas are expensive fish. In this study, we tried to build a low cost checking system of mercury content of tuna meat by using the muscle of the tail part which tends to be disposed. The samples used in this experiment were bluefin tunas, cultured in the Fisheries Laboratory of Kinki University (Oshima Experimental Station, Wakayama, Japan). They were raised from eggs spawned in 2002. Ninety-eight fish were selected between December 2004 and November 2005 (22.3 – 61.6 kg). After catching, each fish was immediately killed by high-electric shock treatment and spinal break-down. After bleeding, they were immersed in iced water. After one day, the tail was cut before shipping. Each sample was kept at -25°C until its use. In three fish, whole body (seven parts including tail) was used for comparison with the t ail. The total mercury level was measured using the reduction vaporizing atomic absorption method after acid digestion (Munns and Holland, 1971). As a result of mercury determination for the parts of meat, except for an abdominal front part where less mercury was contained (0.45 ppm), tail and other parts contained almost the same amount of mercury (0.60 ppm). Therefore, it became clear that the quantity of mercury in bluefin tuna meat could be estimated from the contents in tail part. On the basis of these results, we determined the quantity of mercury for full-cycle cultured bluefin tunas that were shipped after one year. As a result, the mercury level was lower than 0.6 ppm in all fish, irrespective of the increased body weight.

P1-06 Quantification of Amygdalin in Various Seeds and Nuts Using ELISA

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Amygdalin is a cyanogenic glycoside compound commonly found in the pits of many fruits and raw nuts and in other plants. Cyanide reversibly inhibits cellular oxidizing enzymes which contain ferric iron such as cytochrome oxidase and induces the uncoupling of oxidative phosphorylation. HPLC has been used to detect amygdalin, but it takes a long time and requires expertise. There is a need to develop a rapid analytical method to quantify amygdalin. The objective of this study was to produce an antibody against amygdalin after immunization and develop an ELISA to quantify amygdalin in various seeds and nuts. We synthesized hapten for amygdalin and immunized New Zealand White rabbits with Amygdalin-KLH conjugate. We successfully raised sera reactive to amygdalin and purified specific antibody reacts to coated antigen and amygdalin standard solution competitively. Apricot stone, kernel of peach, crataegus, pumpkin seed, almond, and Japanese madlar leaf, in which amygdalin is rich, were analyzed. Soxhlet apparatus was used to extract amygdalin in crushed samples. Ion-exchange column was used to reduce inhibitors for ELISA. We prepared amygdalin standard solutions from 1 ppb to 100 ppb and quantified amygdalin in seeds and nuts samples in the range of 1 ppb to 50 ppb using ELISA. It is 10 times more sensitive than the detection limit of amygdalin using HPLC method. This is the first time amygdalin has been quantified using competitive indirect ELISA.

P1-07 Development of Immunoassay-based Test for the Detection of Hazelnut Residue in Food Products

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Allergy to hazelnut proteins is one of food allergies that account for about 3% of American population. We developed a 30-min quantitative Sandwich Enzyme-Linked Immunosorbent assay (S-ELISA) for determination of hazelnut contamination in food products. Polyclonal antibodies against hazelnut specific proteins were produced and used as capture and detector antibodies. Samples were extracted by shaking 5 g of ground samples with 125 ml of PBS in a hot water bath. Extracts were filtered and filtrates were used directly for ELISA analyses. Extracts added to antibody-coated wells where proteins bind to the capture antibody during a 10-min incubation period. Any unbound protein is washed away and anti-hazelnut horseradish peroxidase-labeled antibody (detector antibody) is added. The detector antibody binds to the hazelnut protein during a 10 min incubation period. Unbound enzyme-labeled antibody is washed away and a one step substrate is added. Color develops as a result of the presence of bound-labeled antibody during a final 10-min incubation period. Absorbance readings of samples are compared with those of the standards and the concentrations in parts per million (ppm) are calculated. For quantitative analyses a standard curve of hazelnut ranging from 0 to 25 ppm was used. The minimum detection limit for hazelnut was determined to be 0.04 µg/ml (1 ppm). Recovery of hazelnut from various spiked commodities varied from 78 to 109% with a mean recovery of 93%. The test is specific for hazelnut. No cross-reactivity was observed with any grains, legumes or other tree nuts (except walnut). A simple version of this assay was developed to analyze swabs of machinery and equipment to insure proper cleaning after each processing to eliminate cross contamination.

P1-08 Detection of Allergens: Considerations for Selecting the Method of Analysis

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Currently no legal controls exist in the EU regarding labeling of food containing allergens due to cross contamination, but manufacturers have a duty of care to minimize the chance of cross contamination or to warn consumers if products may contain allergens. Where HACCP plans identify cleaning as a critical control to ensure adequate removal of allergens, validation of cleaning regimes is required. Widely used methods to verify cleanliness in food factories, in terms of microbial and general food residue

contamination, are based on ATP and protein tests. However, whether these methods are appropriate for cleaning validation with specific reference to allergens is open to question. This work aimed to demonstrate how different methods of analysis can affect the conclusions reached with respect to cleaning. Commercially available hygiene tests (ATP and protein, Biotrace), ELISA kits (Neogen, R-Biopharm, Tepnel and ELISA Systems) and a general protein assay (Pierce) were used in laboratory trials and factory case studies. Laboratory trials tested simulated rinse waters (whole milk powder diluted in water to various levels, 1 – 50 mg/l) and surface swabs taken from stainless-steel plates soiled with whole milk powder solutions (0 – 2000 mg/l). Factory case studies tested rinse waters and surface swabs taken at sites with potential for cross contamination of crustacea. For milk powders, methods based on ELISA were most sensitive, being able to detect 1 and 5mg/l whole milk powder in simulated rinse water. However, hygiene methods classified rinse waters containing up to 50 mg/l whole milk powder as clean, suggesting that these tests may be unsuitable when following milk allergen residues. In contrast, hygiene tests could indicate when the crustacea allergen, as detected by the ELISA method, was present. This work demonstrates how limits of quantification and varied specificities of different methods can affect conclusions reached about cleaning regimes, and highlights the importance of method selection.

P1-09 Simultaneous Detection Immunochromatography Using Two Colloidal Gold-antibody Probe for the Detection of Aflatoxin B1 and Ochratoxin A in Grain and Feed Samples

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The objective of this study was development of immunochromatography (ICG) for simultaneous detection of aflatoxin B1 (AFB1) and ochratoxin A (OTA) in grain and feed samples. The simultaneous detection ICG composed of three pads (sample pad, conjugate pad, absorbance pad) and one nitrocellulose membrane. Two monoclonal antibodies (MAb) against AFB1 and OTA were produced from two hybridoma cell lines (AF-78, OTA-3), which were developed by cell fusion. For development of simultaneous detection ICG, two antibodies were conjugated with each gold particle and sprayed on one conjugate pad, and AFB1-BSA, OTA-BSA conjugate and rabbit anti-mouse IgG were drawn on the AFB1 test line, OTA test line and control line in the nitrocellulose membrane. The detection limit of simultaneous detection ICG for AFB1 and OTA was 0.5 ppb and 10 ppb, respectively. The spiked rice, barley, and feed samples with various concentrations of AFB1 (0.5, 1, 5, 10 ppb) and OTA (10, 30, 50 ppb) were assayed by this method and the detection limit of this method for three samples was 1 ppb and 20 ppb, respectively. We verified that the simultaneous detection ICG was able to detect AFB1 and OTA at one time in food, grain, and feed. Also, this method could be completed in less than 10 min. The simultaneous detection ICG was sufficiently sensitive and accurate to be useful for the rapid detection of AFB1 and OTA in various food, grain and feed samples.

P1-10 Immunochromatography Using Colloidal Gold-antibody Probe for the Detection of Aflatoxin B1 in Grain and Feed Samples

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The objective of this study was the development of immunochromatography (ICG) for rapid detection of aflatoxin B1(AFB1) in grain and feed samples. A monoclonal antibody (MAb) against AFB1 was produced from the hybridoma cell line (AT-1-M3), which was obtained by the fusion of myeloma cells (V653) and spleen cells isolated from a BALB/c mouse immunized with AFB1-BSA conjugate. This antibody was specific for AFB1 but also showed cross-reactivity with aflatoxin B2(AFB2), aflatoxin G1(AFG1), and aflatoxin G2(AFG2) at 21%, 48%, and 22%, respectively. The specific MAb was used to develop an ICG with a sensitivity of 0.5 ng/mL. This method also can detect below 1 ng/mL of other aflatoxins. Spiked rice, barley, and feed samples with various concentrations of each aflatoxin were assayed by the ICG and the detection limit was 0.5, 1, 0.5 and 1 ng/mL to AFB1, AFB2, AFG1 and AFG2, respectively. The ICG was sufficiently sensitive and accurate to be useful for the rapid detection of total aflatoxins in various food, grain and feed samples.

P1-11 Detection and Quantification of Genetically Modified Soya Using the Warnex™ Real-time PCR System

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In recent years, much attention has been directed toward the safety of Genetically Modified Organisms (GMOs) and has led to increased interest in the detection of GMOs in food. The detection and quantification of GMOs has also become an important subject in the international trade of agricultural products. The aim of the study was to develop a real-time molecular beacon-based polymerase chain reaction assay to quickly detect and quantify the presence of genetically modified soya by targeting the 35S CaMV promoter element in a variety of food types and raw grain material. Genetically modified Certified Reference Material from Institute for Reference Materials and Measurements (IRMM) was used to evaluate the performance of the system. Internal and external validation studies showed that the Warnex assay was found to be precise and accurate (< 30% error) for the quantification of varied levels of Certified Reference Material. The Warnex quantification assay on proficiency samples from the Food Analysis Performance Assessment Scheme (FAPAS) showed good performances as well, with < 30% deviation to the true value. In addition, no PCR inhibition was observed when testing for different processed food types such as soya flour, tofu, crackers and soya milk. The Warnex™ Real-Time PCR System is highly specific and sensitive for GM-material detection and is suitable to be used as a routine control assay to evaluate the presence or quantify the GM-soya in food.

P1-12 Safety Assessment of Herbicide-resistance Genetically Modified Red Pepper (*Capsicum annuum*) and Perilla Seeds (*Perilla frutesncens*) in Mice

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Genetically modified (GM) foods are the change of hereditary material by placing properties of one organism (microorganism, plant or animal) into another organism. This biotechnology makes it possible to produce foods with higher resistance to herbicide, greater nutritional value, longer shelf life, better appearance and taste. Despite benefits of GM food, the safety of the food derived from GM crops is a primary consideration. The safety assessment of GM food is based on establishing that the food is substantially equivalent to its non-GM food. However, this concept has not detected the inserted gene designed to kill insects or protect from disease. For more safety related on GM food, safety assessments include specific toxicity tests, allergenicity tests and reproductive studies. This study has been designed to assess the acute toxicity effects of herbicide-resistance GM red pepper (*Capsicum annuum*) and perilla seeds (*Perilla frutesncens*) in male and female mice with non-GM red pepper and perilla seeds. Male and female ICR mice were 5 weeks of age at the start of the study. Mice were divided into four groups fed GM crops 2000, 1000 ppm, non GM 2000 ppm and control, each consisting of 5 male and 5 female. Mice were orally treated with a single dose per day at one time and sacrificed after 7 days. During the experimental period, mice in every group grew well, without significant differences in body weight and body weight gains. Statistically no significant differences were also observed in hematological parameter and serum biochemical indices. There were also no toxicological difference in organ weights and histological appearances of liver, kidney, spleen and testis/uterus. Judging from these results, the novel herbicide-resistance GM red pepper and perilla seeds are comparable to the non-GM counterparts in terms of food safety.

P1-13 Withdrawn

P1-14 Synchronous Comparison of Risk Perceptions Concerning Food Safety of European and United States Consumers

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During the final months of 2005, surveys were conducted in the 25 member states of the European Union and the United States on consumers' perceptions of risks associated with food. Although face-to-face interviews were conducted in Europe and telephone interviews were conducted in the US, survey research methodology suggests that the cultural appropriateness of these two methods produces equally reliable data. The two studies thus make possible a synchronous comparison of European and United States beliefs about the safety of the food supply. It may come as a surprise to some people that levels of concern about the safety of food are not very different between Europe as a whole and the US as a whole; slightly less than half of the respondents in each survey believe that the food they eat can make them sick. In both surveys, microbial contamination and chemical contamination are the most frequent bases of concern. In both surveys consumers are much more concerned about the factors outside their direct control (pesticide residues, antibiotic residues, food additives) than about hazards due to their own handling and preparation of food. In both surveys a large majority of respondents were aware of government activity in support of food safety, and in both surveys slightly less than half of the respondents felt that government food safety activity was adequate. In both surveys, gender and education significantly influenced risk perceptions with respect to food safety.

P1-15 Consumer Perceptions of Food Safety and the Effectiveness of the Food Safety System

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Food safety studies have consistently shown that consumers have relatively high levels of concern, but that concern has not translated into safe food hygiene practices. A lack of education about food safety and cognitive factors, such as optimistic bias, are often cited as reasons for the discrepancy between concern and food hygiene practices. We argue, however, that consumers may conceptualize food safety as a system rather than an individual act. A plausible hypothesis is that consumers have unsafe food hygiene practices because they believe that the foods they buy are safe. This research examines how consumers perceive food safety and the effectiveness of the food safety system, using data gathered from a national telephone survey of 1000 randomly selected US consumers. The goals of this research are two-fold. First, we seek to determine how consumers perceive food safety within the food system, and whom they think should be responsible for food safety. Second, we examine whether consumers believe the levels of foodborne illness are acceptable, and evaluate whether consumers are willing to spend more on food to insure a greater degree of safety. A majority of consumers believed that the government, food processors, farmers, grocery stores, restaurants, and average Americans are doing a good job, and are competent and committed to food safety, although the government should devote more resources to insure foods are safe. Respondents indicated that the federal government should be most responsible for food safety, followed by food processors, and individual consumers.The majority of respondents indicated that the current level of foodborne illness is unacceptable and suggested that they would be willing to pay more to insure that the foods they eat are safe. In addition, they stated that imported foods should be inspected more thoroughly and labels should contain information related to food safety.

P1-16 FightBAC!® Food Handler Training for In-home Child Care Providers Using a Self-study Format

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Time and distance are often barriers to in-home child care providers receiving training. The lack of training and seriousness of foodborne illness in young children make food safety training a critical need. This study examined food handling experience, knowledge and training needs of in-home providers; assessed food handling conditions; designed a self-study curriculum; and evaluated the outcomes in terms of knowledge gained and practices improved. Focus groups with providers and written surveys with child care

surveyors identified topics for video scenarios, each having a wrong and a right segment. Providers completed an activity sheet to identify food handling mistakes in a "wrong" segment, watched the "right" segment, checked answers using answer sheets provided and reviewed "Tips to be Learned" for each scenario. Fact sheets for providers and parents were included. Extension Agents were trained to implement and evaluate the curriculum. Providers (n = 77) completed pre- and post-course behavior checklists using a 5-point Likert scale and pre- and post-course knowledge tests. Project personnel completed pre- and post-course kitchen inspections in the homes. Eighty-eight percent (88%) of caregivers improved overall knowledge of safe food handling ($P < 0.001$), with 69% improving knowledge related to cleaning ($P < 0.001$), 51% related to separating ($P < 0.001$), 74% related to cooking ($P < 0.001$), and 57% related to chilling ($P < 0.001$). Overall, 90% of caregivers improved self-reported food handling behaviors ($P < 0.001$): 72% related to cleaning, 47% related to separating, 68% related to cooking, and 73% related to chilling ($P < 0.001$, respectively). Based on home kitchen inspections, 96% of providers improved actual or observable food handling behaviors. The percentages improving behaviors were 69% for cleaning, 54% for separating, 63% for cooking and 96% for chilling ($P < 0.001$, respectively). This indicates that the self-study is effective, and in this population, gaining knowledge of safe food handling results in actual improvements.

P1-17 Local Provision of Consumer Food Safety Education in the UK

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In the UK, > 1.7 million cases of foodborne disease are estimated per year. Inadequate implementation of food safety practices in the home is known to contribute to this incidence and effective food safety education concerning risks and correct domestic food-handling behaviors is essential. Research has shown that Environmental Health/Health Promotion departments (EH/HP depts.), are cited as the most frequent disseminators of consumer food safety information in the UK. Therefore the extent, sources, diversity, formats and content of food safety educational interventions for consumers provided by UK EH/HP depts. has been investigated. A postal survey was administered to all UK EH/HP depts. in 2004 (n = 461). The survey included 20 segmented questions which evaluated the extent of the EH/HP depts. provision of consumer food safety advice, types, formats and content of interventions, rationale for information provision, methods used for design and delivery of information and participation in national initiatives (response rate 63%). Results indicated a considerable quantity of food safety advice is provided to UK consumers and overall, 95% of UK EH/HP depts. reported current provision of consumer food safety advice. The most common intervention format used for provision of consumer hygiene information was found to be leaflets (93% of EH/HP depts.) and data indicated that this is likely to continue. Hand-washing (87%), cross contamination (85%) and cooking (77%) were the most common issues reportedly addressed in hygiene initiatives. Less than a third of EH/HP depts. reported evaluating the effectiveness of food hygiene advice. Evaluation was more prevalent in Northern Ireland (58%) than in England, Wales and Scotland (11–30%). Nearly all of the evaluation methods reported included an assessment of self-reported practices. Research findings will be discussed in terms of reported changes in provision of food safety advice over time and apparent national differences. Cumulative results from this study will be discussed in the context of generic recommendations for strategy development of consumer education applicable for use in different countries.

P1-18 Consumer Experience of Food Safety Interventions in the UK: Potential for Behavioral Change

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Internationally, it is recognized that effective consumer food safety education is required to reduce the risk of food poisoning. Research has indicated that a substantial amount of food safety information is available to UK consumers however, relatively little is known about consumer experience of food safety advice in terms of exposure, attitudes and perceived effectiveness. This information is valuable for the development of more effective communication strategies in the future. This research investigates consumer perceptions, recall and exposure to general and specific food safety education interventions in the UK. Quantitative and qualitative research methods were used to evaluate consumer awareness, exposure, recall and experience of food hygiene interventions. In-home face-to-face interviews (n = 2014) and focus groups (n = 19) were conducted across the UK. Findings indicated that ~75% of consumers recalled encountering some food hygiene information in the past 12 months, most commonly from television sources, namely adverts, cooking programs and documentaries/news. Results indicated differences in recall according to consumer demographics. Interview and focus group data supported the finding that few respondents (< 31%) were aware of national food safety initiatives, similarly few respondents (9–11%) recalled seeing any of the most commonly distributed leaflets in the UK. Such data suggests that placement, promotion and distribution strategies to ensure intervention reach to UK consumers may need reviewing. However, qualitative and quantitative findings indicated that a large number of respondents (61%) remembered encountering a specific food safety TV advertisement, images of which appeared to have a lasting impact. Of the consumers who recalled encountering specific food safety information in the UK, up to 34% self-reported behavioral change as a result. Examples of quantitative and qualitative findings that complemented each other in this study will be discussed in the context of targeted strategy development. Research outcomes will also help to identify valuable information to inform the development or modification of effective future initiatives, as well as advise upon potential opportunities for placement of food safety advice for food manufacturers.

P1-19 Withdrawn

P1-20 Identification of Products Showing Detectable Differences in Microbial Indicator Counts in Low Socioeconomic Status (LSES) Markets Versus High Socioeconomic Status (HSES) Markets

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It is known that socioeconomic factors contribute to food insecurity and limitations in types of products available to individuals of low socioeconomic status. This study represents the first step in an effort to determine if there is a difference in the food quality, as defined by microbial loads, of milk and produce available to individuals of LSES versus HSES. The goal of this study was to identify

produce products consistently available in both HSES and LSES markets which were most likely to show differences in microbial counts and eliminate products where the microbial flora made differences difficult to detect. Testing compared the APC, coliform and Y&M counts of broccoli, cucumbers, lettuce, strawberries, watermelon, grapes, and 2% milk from two supermarkets in LSES areas and two supermarkets in HSES areas. Each store was sampled four times for each product. Each time a product was sampled at a store, three sub samples were obtained. A total of 336 products were sampled (168 from LSES store and 168 from HSES stores) with 3 analyses performed on each sample. Analyses were performed utilizing FDA B.A.M. methods. Results indicated that broccoli showed a statistical difference in APC, Y&M ($P < 0.0001$ for both) and coliforms ($P = 0.01$) between LSES and HSES stores. Lettuce and cucumbers showed a statistical difference, ($P = 0.04$) and ($P = 0.01$) respectively, in APC but not Y&M or coliforms, between LSES and HSES stores and strawberries showed a statistical difference ($P < 0.0001$) in Y&M counts only. Milk showed higher APC counts in LSES stores than HSES stores, but the difference was not statistically significant. Based on the data obtained in this study, broccoli, lettuce, strawberries, cucumbers and milk will be utilized in a broader, more comprehensive study to compare the difference in food quality and safety in markets in HSES vs. LSES areas.

P1-21 Congruence of Own-checking System Evaluations Performed by Food Safety Authorities

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It is important that the in-house control systems of food-processing plants are of high quality and evaluated in a commensurable manner in order to ensure food safety and congruence in food control. Comparing evaluations from 2002, performed by municipal food safety authorities on the in-house control systems of food-processing plants, has revealed differences in the quality of in-house control systems between different regions and types of plants in Finland. Whether the observed differences are true differences in the quality of the in-house control systems or due to incongruence in the municipal inspections, or both, could not be deduced from that material. In this study, in-house control system evaluations performed by municipal and provincial food safety authorities were compared. The in-house control evaluations on those food-processing plants where both the local and provincial food safety authorities had performed an inspection during 2002 were included in the study. Differences in the assessments of the in-house control systems could be observed between local and provincial authorities. It appears that the local authorities in many cases assess the in-house control systems to be of higher quality than the provincial authorities do. The results indicate that the previously detected differences in the in-house control systems can, at least partly, be explained by differing assessment. A more extensive study is needed in order to find out whether the suggested incongruence influences food safety.

P1-22 Security of Food in United States' Child Nutrition Program Settings: Survey Results

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Information was gathered through anonymous surveys distributed to child nutrition personnel by program directors and returned to the researchers by participants. The sampling unit was the area defined by a public school district and included all child nutrition programs within the geographic range. Sample stratification was based on percentage of school lunches within the US Department of Agriculture (USDA) Food & Nutrition Service (FNS) Region. Responses were received from 1174 participants from 121 districts in 33 states, representing every USDA region. Participants self-reported working in Child and Adult Care Food Programs (75), Summer Food Service Programs (110), and/or the National School Lunch/School Breakfast Programs (1096). Measures of security included monitoring of self-serve areas, unsupervised access to food areas, and food delivery. Over half of respondents (612 respondents/55.7%) reported that their facilities had self-serve areas. These facilities were more likely to be monitored continuously than to be left unmonitored during service ($P = .000$) or monitored sporadically ($P = .000$). Only nine percent (91 respondents) of the respondents reported that no one was allowed in the food service area unsupervised. Respondents reported that food deliveries were usually made during working hours, although 7.8% (87 respondents) reported that food is left unattended "always" or "most times," and 8.4% (94 respondents) reported that food is "usually" or "sometimes" left unattended before employees arrive at work. Approximately 27% (300 respondents) reported that the delivery person takes food into the storage area before it is checked, and 64% (718 respondents) reported that the delivery person takes food into the storage area after employees check it. Custody of food issues require review and should be addressed in any HACCP plans and in food safety training, since tampering is more likely when food is left unsecured or when access to food is poorly monitored.

P1-23 To Open Date or Not to Open Date – What is Industry Doing and Why?

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Product open dating, a date stamp on a product's package that helps determine how long to display a product for sale or the time limit to purchase or use the product, is a potentially powerful tool for preventing foodborne illness, as it provides information to the consumer about product freshness and safety. At present, there is no uniform system of food product open dating in the US. Despite the lack of such a system, many food products currently sold in the US have some type of open dating. There is, however, no comprehensive data (public or private) that can be used to generate estimates of the share of food products that use some form of open dating. To fill that gap, we first conducted a total of five supermarket surveys in which our staff visually inspected and recorded the type of dates and other product characteristics for 1,982 name brands and 987 products of five private labels. An analysis of the data shows that the prevalence of open dates and the type of dates vary significantly between brand name and private label products sampled. Fifty-three percent (± 1.1 percent) of name brands and 21 percent of private label products do not have any open dates [a]. Among those name-brand products that are open dated, over 25 (± 0.4 percent) percent do not have an explanatory phrase, such as "best if used by" or "sell by," for the date. This use of unspecified dates is even higher (44 percent) among open-dated private label products. Next, we conducted a total of seven case studies to gain a better understanding of the manufacturer's decision-making process and to qualify our survey findings. Due to the lack of a standardized open dating system, manufacturer responses varied

widely. The majority of manufacturers, however, concurred on a few key factors that influence their decision to open date: shelf-life duration, existing regulations, and marketing considerations. Further, manufacturers indicated that addition or modification of an open date may require (1) changes in inventory control practices, (2) purchase or modification of in-line printing equipment, (3) changes to label and/or package design, and/or (4) additional shelf-life testing for validation purposes depending on the specific requirements of any future standard.

[a] Because the private label product survey utilized convenience sampling, confidence intervals for the estimates are purposefully omitted.

P1-24 Consumer Knowledge and Use of Dates on Product Packaging: Results of a Web-based Survey

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Consumers are increasingly relying on Ready-to-Eat (RTE) foods because they are convenient, quick, and easy. RTE foods have long, refrigerated shelf lives and are usually consumed without reheating. Open dates help consumers know by when to purchase or use RTE foods for best quality. To characterize consumer knowledge and use of product dates for specific refrigerated RTE foods (smoked seafood, cooked crustaceans, bagged salads, pre-washed, cut produce, soft cheeses, frankfurters, deli meats, fermented sausages, and deli salads), we conducted a nationally representative Web-enabled survey (n = 2,060). Prior to purchasing RTE foods, 48% to 68% of respondents check product dates all or most of the time. Before preparing RTE foods, 43% to 64% of respondents check product dates all or most of the time. Sixty-two percent of respondents reported their senses (taste, smell) were the most important factors in deciding whether to eat a refrigerated food, which is an unsafe practice. About 32% of respondents reported the product date is the most important factor in deciding whether to eat a refrigerated food; however, many respondents do not understand the meanings of the different types of dates. Less than 20% of respondents correctly defined the "use-by" date; more than 50% have the misconception that the "use-by" date indicates the last date recommended for safe consumption of a product. About 45% percent of respondents correctly defined the "sell-by" date. About 50% of respondents think the most useful open date is one that provides information on the last date recommended for safe consumption of the product. These findings suggest consumers could benefit from education regarding open dating. If open dating is to be useful to consumers, they must understand what information is to be gleaned from open dates and how to use this information to make informed decisions regarding length of product storage.

P1-25 Microbial Quality of Treated and Untreated Apple Cider Produced in New Jersey Following the FDA Juice HACCP Rule

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Unpasteurized apple cider was implicated in several foodborne disease outbreaks prior to the promulgation of the FDA Juice HACCP regulations. The objective of the study was to evaluate the microbial quality of New Jersey apple cider produced following the US FDA Juice HACCP rule. Seventy-five samples of apple cider were collected from 16 New Jersey farms throughout the fall and winter cider seasons over a 3-year time period (2003–2006) and analyzed for the presence of total aerobic plate count, coliforms and *E. coli*. Since cider is a seasonal business in NJ, and samples could be collected only when cider mills were in operation, most (29) samples were collected in October with fewer (16 and 15) samples collected in November and December. Very few samples (15) were collected in January and February combined. Cider samples were collected on-site, and transported in an insulated container to the testing location. Samples were held at 4°C until tested, generally within 24 h. Test methods followed the FDA Bacteriological Analytical Manual protocols. A total of 43 untreated and 32 treated samples were collected. Of the treated samples, 7 were treated with heat, while 25 were treated with UV light. Total aerobic plate counts (TAC) exceeded 100,000 CFU/ml in three of the untreated samples and zero of the treated samples. The average TAC count was 3.3 log CFU/ml for untreated and 2.6 log CFU/ml for treated samples. Coliforms were detected in 2 of the untreated samples and zero of the treated samples. *E. coli* were detected in 5 of the untreated samples and one of the treated samples. These results show that the microbiological quality of treated cider in NJ is generally acceptable, and that treatment can reduce total aerobic plate count. Treatment also reduces (but does not eliminate) the chance of cider contamination with coliforms or *E. coli*.

P1-26 Hazard Analysis for Foods and Environments in Korean-style Restaurants

DSC

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The purpose of this study was to analyze microbiological hazards of foods and environments in large Korean-style restaurants. Microbiological tests included total plate count, coliforms, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli* O157:H7, *Vibrio parahaemolyticus*, and *Bacillus cereus*. The samples consisted of heated foods, non-heated foods, and heated/non-heated mixing processes. Temperatures and times were measured to evaluate the degree of hazard control. Microbiological tests were also conducted on the knives, cutting boards, kitchen towels, food handlers' hands, drinking water, cooking water, and air. The result showed that heated and reheated foods such as Sooyuk (steamed pork) and Japchae (pan-fried vegetables, noodles and meat) were safe. However, when served after being heated, cooled, and refrigerated without reheating, the total plate counts and coliforms exceeded standards. *Staphylococcus aureus* was not found in any heated or reheated foods, but it was detected in some salads and raw fish with seasoned vegetables. Food processing equipment, at almost all of the restaurants studied, exceeded the standards for total plate count and coliforms. Tests also indicated the presence of *E. coli* and *S. aureus* on knives and cutting boards at some restaurants and on the hands of some food handlers. The total plate counts and fungi in the air were high around the worktables, inside the refrigerator, and in the kitchen. In conclusion, another study should be done to see if food safety can be improved by following HACCP procedures in Korean-style restaurants.

P1-27 Field Assessment of Sanitation Management Practices in School Foodservice Operations in Seoul, Korea

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Effective and systematic sanitation management programs are necessary to prevent foodborne disease outbreaks in school foodservice operations. The purpose of this study was to identify the elements to improve in order to ensure the safety of school foodservice by evaluating sanitation management practices implemented under HACCP-based programs. The survey was designed to assess the level of hygiene practices of school foodservice by using an inspection checklist of food hygiene and safety. Fifty-four school foodservice establishments considered as poor sanitation practice groups from two-year inspections by Seoul Metropolitan Office of Education were surveyed from September to December in 2005. Inspection checklists consisted of seven categories with 50 check-points; sanitation control of facilities and equipment, personal hygiene, food materials management, sanitary management in food product flows, environmental sanitation and maintenance, HACCP system management, and accident prevention and crisis management. Surveyed schools scored 68.0 ± 12.42 points out of 100 on average. The average score (% of compliance) of each field was 10.7/20 (53.3%) for sanitation control of facilities and equipment, 7.4/11 (67.2%) for personal hygiene, 7.4/11 (74.1%) for food materials management, 22.4/32 (69.9%) for sanitary management in food product flows, 8.9/12 (73.8%) for environmental sanitation and maintenance, 4.2/7 (69.7%) for HACCP systems management, and 7.1/8 (89.0%) for accident prevention and crisis management, respectively. The field to be improved first was the sanitation control of facilities and equipment. The elements to improve this category were unprofessional consultation for kitchen layout, improper compartment of kitchen area, lacks of pest control, inadequate water supply, poor ventilation hood system, and insufficient hand-washing facilities. To elevate the overall performance level of sanitation management, prerequisite programs prior to HACCP plan implementation should be stressed on the school officials for the integration of the system.

P1-28 Incidence of *Listeria monocytogenes* in Minimally Processed Fruits and Vegetables from the City of Campinas–São Paulo, Brazil

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The market of minimally processed fruits and vegetables is in constant growth due to changes in consumer alimentary habits, meaning buying products that brings together practicality and health. The search for healthier, less caloric and more practical food makes this type of product more common in the population's day to day eating habits. Although they are expensive and their sale in Brazil is concentrated in the large urban centers, there is a tendency to reach all social classes and other regions of the country. Because the majority of minimally processed products are Ready-to-Eat and do not go through any kind of treatment before being ingested, rigorous microbiological control is necessary, in order to assure the final consumer's safety in regard to pathogenic microorganisms. *Listeria monocytogenes*, for example, is a psychrotrophic bacterium widely distributed in the environment, capable of growing in refrigerated products. It can cause severe diseases, in spite of not being common, with a high mortality rate among the risk population (children, pregnant women and imunocompromised individuals) representing moderate microbiological risk and high severity, in agreement with ICMSF. The aim of this study was to determine occurence *Listeria monocytogenes* in minimally processed fruits and vegetables from the retail and street markets of Campinas (Brazil). For this assay, 180 samples (155 vegetables and 25 fruits) were acquired between July and December 2005, with pathogen determination performed by the PCR technique, DuPont Qualicon's BAX® System. The results showed absence of *Listeria monocytogenes* in all samples, with a contamination estimate below 0.01MPN/g in minimally processed fruits and vegetables. It can be concluded that the incidence of *Listeria monocytogenes* is low and the consumer is safe from contamination of this specific psychrotrophic bacterium in minimally processed foods commercialized in Campinas.

P1-29 Evaluation of the Transfer of *Listeria monocytogenes* from Surfaces to Foods

DSC

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Listeria monocytogenes on food surfaces can potentially lead to contamination of food products during and after processing. The objective was to study parameters involved in transfer of *L. monocytogenes* from surfaces to foods. We evaluated the influence of surfaces (stainless steel and plastic), inoculation methods (biofilm growth and adherent film), liquid levels (visibly dry and wet) and foods (bologna and American cheese). Each experiment included all 16 combinations and was repeated 11 times. A four strain cocktail was used to inoculate surfaces by growing biofilms or adhesive films. Slides were placed on a universal testing machine and were brought into contact with foods at a constant pressure (45KPa) and time (30 s). Foods were blended and transferred cell levels were determined by plating. To eliminate daily fluctuations in cell numbers, the efficiency of transfer (EOT) was calculated by dividing the number transferred by the number on the control surfaces. The results were analyzed using SAS statistical software. Our results strongly suggest that stainless steel surfaces transferred more *L. monocytogenes* to foods than plastic ($P = 0.05$). More cells transferred from biofilms (EOT = 0.57) than adhesive films (EOT = 0.16). Among foods, more *L. monocytogenes* were transferred to bologna than cheese ($P < 0.05$). This was especially significant for biofilms, where the EOT for bologna was 0.72 and for cheese 0.25. The EOT for adhesive films was similar for bologna and cheese (0.16). The impact of liquid level was only significant for dried biofilms when transferred to bologna ($P < 0.05$). Although not significant similar trend was seen for dried adhesive films when compared to wet. In the adhesive films, cell-to-surface was the predominant interaction, while biofilms had both cell-to-surface and weaker cell-to-cell interactions. The cell-to-cell interactions may have contributed to the higher transfer levels of biofilms. These results indicate bacterial transfer may be related to cell-to-surface, cell-to-cell, and cell-to-food interactions.

P1-30 Fate of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in Soudjouk-style Fermented Semi-dry Sausage

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All-beef (20% fat) raw Soudjouk batter containing dextrose (0.25 or 0.60%), spices, and a commercial pediococcal starter culture was inoculated with a five-strain mixture of *Listeria monocytogenes* or a three-strain mixture of *Escherichia coli* O157:H7 to level of approximately 6.0 log CFU/g. Sausages were transferred to an environmentally-controlled incubator with an air flow of 1.0 to 1.5 m/s,

hung vertically, and fermented at 24°C with a relative humidity (RH) of 90–95% for 72 h and then dried at 22°C for an additional 72 h with 80–85% RH. After the fermentation and drying steps, sausages were vacuum-packaged in bags and stored at 4, 10, or 21°C for up 30 days. The addition of 0.25% or 0.60% of dextrose to the batter decreased the pH values from approximately pH 5.8 to pH 5.2 and pH 4.8, respectively, after fermentation and drying. Fermentation and drying alone reduced the numbers of *L. monocytogenes* by 0.07 and 1.03 log CFU/g for sausages fermented to pH 5.2 and pH 4.8, respectively, whereas the numbers of *E. coli* O157:H7 decreased by 0.27 and 1.11 log CFU/g, respectively. When sausages fermented to pH 5.2 or 4.8 were stored at 4 , 10 or 21°C for up 30 days, the numbers of *L. monocytogenes* decreased by 0.2, 0.7 and 0.2, and 0.7, 0.1 and 1.3 log CFU/g, respectively. When sausages fermented to pH 5.2 or 4.8 were stored at 4 , 10 or 21°C for up 30 days, the numbers of *E. coli* O157:H7 decreased by 1.0, 0.6, and 3.0, and 1.8, 2.2 and 3.2 log CFU/g, respectively. Fermentation to pH 4.8 and storage at 21°C was the most effective process for reducing the numbers of both pathogens. Thus, these findings may be useful for the development of intervention strategies to control *L. monocytogenes* and/or *E. coli* O157:H7 in Soudjouk-style sausage.

P1-31 The Survivability of *Listeria monocytogenes* and Spoilage Microorganisms during Processing and Storage of Wara, a Southwestern Nigerian Soft Cheese

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This study evaluated the survivability of *Listeria monocytogenes* and spoilage microorganisms during the processing and storage of wara, a southwestern Nigerian un-ripened soft cheese. Pasteurized milk inoculated with *L. monocytogenes* at 10^2 or 10^4 CFU/ml was used to process the cheese. Samples were taken along the processing steps and throughout a five-day storage period at 15 and 28°C and analyzed for populations of *L. monocytogenes*, total aerobes, *Enterobacteriaceae*, and psychrotrophs. On the fourth storage day, portions of the control cheese (not inoculated with *L. monocytogenes*) were fried in vegetable oil, mimicking the practice of southwestern Nigerian cheese processors. The results indicated that *L. monocytogenes*, at both inoculation levels, did not survive the processing of wara. In samples initially inoculated with 10^2 CFU/ml of *L. monocytogenes*, the psychrotrophic bacteria counts increased from undetectable levels on the first day of storage to 6.30 log CFU/g at 28°C and 7.63 log CFU/g at 15°C by the 5th day of storage. Aerobic plate counts increased from 5.13 log CFU/g on the first day of storage to 7.81 and 6.70 log CFU/g, respectively, at the end of storage at 28 or 15°C. The *Enterobacteriaceae* counts increased from 5.21 log CFU/g on the first day of storage to 8.02 log CFU/g at 28°C and 8.20 log CFU/g at 15°C after storage for 5 days. In samples with an initial *Listeria* inoculation level of 10^4 CFU/ml, the populations of the three types of microorganisms increased with the extension of storage time. Overall, the survival of the three types of microorganisms responded to storage temperatures with a few exceptions. Frying does not seem to significantly change the populations of total aerobes, psychrotrophs, or *Enterobacteriaceae*. The study suggests that wara can serve as a vehicle to transmit illness if its microbial quality is not appropriately managed.

P1-32 Survival of Healthy and Stressed *Listeria monocytogenes* on Stainless Steel after Desiccation

DSC

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Listeria monocytogenes can reportedly persist for months or years within biofilms in food processing facilities. Despite the best sanitation practices, *Listeria* can also survive on difficult-to-clean stainless steel surfaces such as delicatessen slicers, with small numbers of cells potentially transferable to Ready-to-Eat products. Consequently, the objective of this study was to assess the viability of healthy, chlorine-injured and cold-shocked cells of *L. monocytogenes* that could persist on difficult-to-clean stainless steel surfaces after routine cleaning and sanitizing. In this work, six previously identified strong and weak biofilm-forming strains of *L. monocytogenes* were grown at 37°C/18–24 h on trypticase soy agar containing 0.6% yeast extract, harvested in 0.1% peptone and then combined into two 3-strain cocktails. The cocktails were resuspended in turkey slurry with or without prior cold shock (4°C/2 h) or chlorine injury (100 ppm/1 min) and then used to inoculate flame-sterilized grade 304 electropolished stainless steel microscope slides. After incubation at 22°C/~78% relative humidity for 1, 6 and 24 h, followed by viability staining (LIVE/DEAD® BacLight™ Bacterial Viability Kit, Molecular Probes, Inc., Eugene, OR), 5 fields of view/treatment were examined by confocal scanning laser microscopy (Zeiss LSM 5 Pascal; Carl Zeiss, Inc., Thornwood, NY) and then manually counted. Overall, significantly fewer dead cells were observed for the cold-shocked (44.22 ± 20.92% dead) compared to the chlorine-injured (60.74 ± 21.72%) and healthy (59.93 ± 28.19%) cocktails. Cell viability was also significantly lower for the weak (61.29 ± 24.93%) as opposed to the strong (48.64 ± 23.19%) biofilm-forming cocktail. However, nearly half of the population remained viable after 24 h, with these cells likely to pose a public health concern if transferred to Ready-to-Eat foods that permit growth of *Listeria*.

P1-33 Response of *Listeria* spp. to Pulsed-UV Light Sterilization and Starvation in Physiological Saline

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Pulsed UV light is a novel technology used to inactivate pathogenic and spoilage microorganisms in a short time. The efficacy of pulsed UV light (5.6 J/cm² per pulse) for the inactivation of *Listeria* spp. alone and in combination with cells starved in physiological saline was investigated. To determine the efficacy of UV light treatment or starvation on microbial growth of *Listeria*, tryptic soy broth or physiological saline containing exponentially growth cell suspensions (approximately 10^9 CFU/ml) of *L. monocytogenes* Scott A, *L. innocua* or *L. monocytogenes* hly- were treated under pulsed UV light (3 pulses per s) for 10 s or starved in physiological saline and stored up to 8 days at 25°C. After treatment, 0.1 ml sample was surface plated onto tryptic soy agar supplemented with yeast extract and incubated at 37°C for 24 h to determine log reductions. Also, 0.1 ml of cell suspensions in physiological saline and unstarved *Listeria* spp. cell suspensions, untreated to serve as controls, were surface plated onto TSAYE plates and incubated in the conditions described above. Bacterial counts were deterimined every 48 h. Results indicated a 1.32- to 2.05-log CFU/ ml reduction was observed for *L. monocytogenes* (hly-), *L. innocua* and *L. monocytogenes* Scott A stored for 8 days after pulsed light and starvation treatments,

respectively. A comparison of pulsed light treated and starved cells to the control resulted in significantly ($P < 0.05$) lower bacterial counts, compared to non-treated cells. Our results clearly indicate that pulsed UV technology in combination with starvation has potential for inactivating pathogenic microorganisms.

P1-34 Model Drain System for Biofilm Formation by *Listeria monocytogenes* and Resident Microorganism from a Seafood Processing Plant

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Listeria monocytogenes has the ability to grow and persist within mixed strain biofilms within food processing plants, although the nature of the relationships between this pathogen and other organisms is unknown. A CDC biofilm reactor was to allow full drainage of the liquid and to simulate surface growth under wet but not fully submerged conditions imitating a drain. The behavior of a *Listeria monocytogenes* strain (repeatedly isolated from a food processing plant) was evaluated in the reactor under mixed culture conditions and detected by selective plate counts (Oxford Agar) and enrichment MPN. In monoculture, the persistent *L. monocytogenes* could grow and produce biofilms under intermittent nutrient and water (1/30-TSB added intermittently over 16 h, followed by tap water for 8 h at 20°C). An undefined bacterial mixture (*L. monocytogenes*-free), originally isolated from a floor drain was used as the mixed strain inoculums. When *L. monocytogenes* was inoculated prior to or with the mixed strain, *L. monocytogenes* increased on stainless steel surfaces during all experiments (12 to 20 days). When the mixed strain was inoculated first and allowed to establish for two days prior to *L. monocytogenes* addition, *L. monocytogenes* became established in the mixed biofilm and was detected for the remainder of the experiment. However, when the mixed strains formed biofilms 5 and 10 days prior to *L. monocytogenes* addition, *L. monocytogenes* was undetectable by MPN within the mixed biofilm. The established mixed biofilm appeared to inhibit *L. monocytogenes* through a "competitive exclusion" mechanism. We believe that is an indication that *L. monocytogenes* is the primary colonizing bacteria, requiring access to the solid substrate for establishment within a biofilm. These results indicated that *L. monocytogenes* were enhanced biofilm formers in suitable environments and that mature mixed biofilms were able to inhibit the colonization of this pathogen.

P1-35 DSC Effect of Growth Temperature and Growth Phase on the Inactivation of *Listeria monocytogenes* in Whole Milk by High Pressure Processing

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The aim of this study was to explore the effect of a wide range of growth temperatures, growth phase and plating media on the rate of inactivation of *Listeria monocytogenes* by high pressure processing (HPP). *Listeria monocytogenes* was grown to mid-stationary phase at 4, 15, 25, 35 or 43°C, inoculated into whole UHT milk and HP processed at 400 MPa. Additionally, cells were grown to mid-exponential stage at 15 and 43°C and processed in the same way. Following HPP milk was plated on Tryptic Soya yeast extract agar (TSYEA) and Modified Oxford agar (MOX) to investigate the effect of injury on the rate of inactivation. The results demonstrated that growth temperature had a significant effect on the rate of inactivation in stationary phase *L. monocytogenes*; when cells were grown at higher temperatures the rate of inactivation by HPP was lower ($P < 0.05$). Viable counts were lower on selective agar for all growth temperatures and the difference increased as processing time increased. Rate of inactivation decreased significantly as growth phase changed from exponential to stationary ($P < 0.05$). It was postulated that stationary phase proteins and stress proteins may contribute to pressure resistance. Further work will investigate the role of stresses, such as heat, cold and pressure shock, on the resistance of *L. monocytogenes* to HPP.

P1-36 Role of the uvrA Gene in the Growth and Survival of *Listeria monocytogenes* under UV Irradiation and Acid and Bile Stress

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Listeria monocytogenes encounters numerous stresses both in the food environment and during infection of the host. The ability to survive and tolerate low pH and bile, which are two major stresses, is of particular importance for survival within the host. The uvrA gene has been shown to be involved in the repair of acid-induced DNA damage and adaptation to low pH in other bacteria. Thus, a uvrA gene in-frame deletion mutant was constructed with an internal 1,335-bp (446-amino acid) deletion by gene splicing by overlap extension (SOE) PCR to identify the role of uvrA in the growth and survival of *L. monocytogenes* in various environmental conditions. The survival of uvrA mutant was determined after 45 s of UV irradiation and the mutant was highly sensitive to UV irradiation. In addition, growth under normal laboratory conditions was impaired during exponential phase and the time to reach the exponential phase growth, TVmax, was significantly delayed ($P < 0.05$). Growth of the uvrA mutant in acidic medium (pH 5) was slightly impaired and the TVmax was significantly delayed ($P < 0.05$). Growth and the TVmax of the mutant in the presence of 0.3% bile salts were also significantly impaired ($P < 0.05$). These results suggest that uvrA is needed for optimal growth and survival of *L. monocytogenes* in various environmental stresses.

P1-37 Effect of Sanitizer Stress Response on the Growth Kinetics of *Listeria monocytogenes* on Imitation Crabmeat and in Broth as a Function of Temperature

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Adaptation of *Listeria monocytogenes* to sublethal stresses has been demonstrated in the food processing environment and received much attention due to its impact on the safety of various foods. Limited information is available about the effect of stress response on survival/growth of *L. monocytogenes* in foods. We studied how sanitizer-stressed and unstressed *L. monocytogenes* differ in

their growth kinetics on imitation crabmeat and in broth during storage at 4, 10, or 25°C. Two different *L. monocytogenes* strains were stressed with 25 ppm or 75 ppm sodium hypochlorite sanitizers for 5 min. Unstressed or sanitizer-stressed cells were inoculated in broth or on crabmeat (approximately at 100 CFU/ml or g). Growth curves of unstressed and stressed *L. monocytogenes* were fit to two or three-phase linear models and lag time (LT) and specific growth rate (SGR) of each growth curve were compared. Exposure to 75 ppm sanitizer injured *L. monocytogenes* cells ATCC 15313 and those isolated from pork bulgogi over 90% and 60%, respectively. In comparison to the growth curve of stressed *L. monocytogenes* ATCC 15313, a shorter LT and accelerated SGR were observed in the growth curve of stressed *L. monocytogenes* from pork bulgogi regardless of the storage temperature. Overall, the effect of stress response on the growth kinetics was more obvious at lower temperatures. At 4°C, LT of stressed *L. monocytogenes* with 75 ppm sanitizer was extended 4 times longer than that of unstressed, indicating that injured *L. monocytogenes* cells need time to recover from their injuries for growth. Although sanitizer stress was found to affect mainly LT in the growth curve, the overall growth rate of unstressed and stressed *L. monocytogenes* decreased with the increase of temperature from 4°C to 25°C. These results indicate that the magnitude of stress response varied according to the strength of sanitizer and the strain variation of *Listeria*.

P1-38 DSC Determination of *Enterobacter sakazakii* in Powdered Infant Formula, Reconstituted and Utensils Used in Baby's Bottle Preparation

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Enterobacter sakazakii is a pathogenic bacteria considered as an emergent public health issue, representing risk of death or chronic long-term sequelae. The population group risk is particularly infants under 1 year, especially premature and low-birth-weight neonates. Powdered infant formula has been identified as the most important vehicle and source of infection. This current study was carried out to report the *Enterobacter sakazakii* occurrence in hospitals' environmental facilities located in the Campinas (SP) region, analyzing powdered infant formula samples and evaluating hygienical lapses by microbiological analysis (current Brazilian legislation RDC 12/2001) besides *E. sakazakii*. During the period of May 2004 to October 2005, 99 samples were analyzed, from both manufactures and suppliers, of which 42 were powdered infant formula, 25 reconstituted infant formula (baby's bottle), 27 utensils used in baby's bottle preparation, and 5 cow's milk with starch. The BAX system DuPont Qualicon detection methodology adapted for Most Probable Number quantification analysis, single dilution test (5 aliquots of 100 g or 6 of 110 g) was used. *Enterobacter sakazakii* was present in 12 of 42 powered formula samples (28;57%), and absence for the others. The most frequent count found in powdered formula was 0.51 MPN/100 g with an average of 0.54 MPN/100g. Analysis of *Salmonella*, *B. cereus*, *S. aureus* and thermotolerant coliforms, showed that powdered infant formula was in accordance with current Brazilian legislation. Although *E. sakazakii* occurrence in powdered infant formula have been low, we can conclude that its control depends basically on the application of rigorous hygienical conditions, including time/temperature control during preparation, handling and storage of the reconstituted product and also the continuous control at all production levels since commercially sterile product cannot be available with the same nutritional source.

P1-39 DSC Survival of *Enterobacter sakazakii* in Powdered Infant Formula as Affected by Water Activity and Temperature

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A study was done to determine survival characteristics of *Enterobacter sakazakii* in milk-based and soybean-based powdered infant formulas. A ten-strain mixture of *E. sakazakii* isolates from infected infants (five strains), foods (four strains), and the environment (one strain) was spray-inoculated at low (log 0.86 CFU/g) and high (log 4.81) populations into six infant formulas to give a_w values of 0.25–0.30, 0.31–0.33, and 0.43–0.50. Inoculated formulas were stored at 4, 21, and 30°C for up to 6 months. After storage for 6 months at 4°C, populations decreased significantly ($P \leq 0.05$), although by less than 1 log CFU/g, in all formulas initially containing 4.81 log CFU/g. Populations decreased significantly in all formulas stored for 1 month at 21 or 30°C, followed by little change at 3 and 6 months. At all storage temperatures, overall reductions in populations were greater in formulas at a_w 0.43–0.50 than at a_w 0.25–0.30. *E. sakazakii*, initially at 0.86 log CFU/g, was detected in all formulas at one or more a_w after storage for 6 months at 4, 21, or 30°C. Strain 2855, which produces mucoid colonies, and strain 3396, which does not produce mucoid colonies on violet red bile glucose agar supplemented with pyruvate, were spray-inoculated into a milk-based and a soybean-based powdered infant formula at a_w ranging from 0.43 to 0.86 and stored at 4, 21 and 30°C. Populations of both strains decreased significantly in both formulas within 30 days. Rates of death increased with increased storage temperature, but the effect of a_w on inactivation is not clear. Strain 3396 survived at significantly higher populations than strain 2855 at 4 and 21°C, indicating that the ability of *E. sakazakii* to form extracellular mucoidal materials does not necessarily correlate with protection against death caused by desiccation.

P1-40 DSC Effect of Metabolic Stress on the Resistance of *Enterobacter sakazakii* to Chlorine Sanitizers

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Enterobacter sakazakii has been recognized as a new foodborne pathogen implicated in a severe form of neonatal meningitis. It is recognized that in some cases the same strain of *E. sakazakii* may produce colonies with two distinct morphologies when plated in different media. Typical colonies plated on trypticase soy agar (TSA) produce a smooth and creamy colony with a yellow pigment. Colonies plated in violet red bile glucose agar (VRBGA) are dry, crenated and rubbery. The objective of this study was to determine if morphological changes induced by metabolic stress could increase the resistance of *Enterobacter sakazakii* to chlorine sanitizers. We were also interested in determining the minimum chlorine level and exposure time required for inactivation of *E. sakazakii*. *Enterobacter sakazakii* strains ATCC 29004 and ATCC 29544 were cultivated in brain heart infusion broth BHI (Difco) and violet red bile glucose broth VRBGB (Difco ingredients). Cells were exposed to different chlorine concentrations (2 ppm, 4 ppm and 6 ppm) for periods ranging from three to ten min. Survival of cells was quantified by direct plating and log reductions were calculated. Statistical

analyses (SAS) were conducted to determine if the medium used for recovery of *E. sakazakii* (VRBB, BHI) had an effect on cell survival. Cells exposed to VRBGB prior to treatment with chlorine solutions showed a one log or greater resistance to inactivation with chlorine compared to cells grown in BHI. Changes in survival may be indicative of production of heteropolysaccharides that could provide protection against sanitizer or other inhibitors. Results also showed that exposure to 2 ppm chlorine solutions for 3 min produced 1 to 2 log reduction, exposure to 4 ppm chlorine solutions for 3 min produced 2 to 3 log reduction and exposure to 6 ppm for 3 min produced 3 to 5 log reduction in population. Exposure to chlorine solutions for time periods between 3 min and 10 min did not cause additional increase in the log reduction. These data show that metabolic stress of *E. sakazakii* may increase resistance to chlorine sanitizers.

P1-41 Biofilm Formation among Isolates of *Enterobacter sakazakii*

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The objective of this study was to compare the biofilm forming ability of 72 strains of *E. sakazakii* and to determine which component of bovine milk contributes most to biofilm formation. Six treatments were tested: undiluted skim milk, 1:8 diluted skim milk, and 1:8 diluted skim milk plus lactose, whey protein concentrate 80, or casein, added at such a level to restore their concentrations to that in undiluted skim milk. Each preparation was placed into 12 wells of a 96 well polystyrene plate and inoculated with cultures grown for 24 h at 37°C in TSB. A plate containing uninoculated preparations served as the negative control. Following inoculation, plates were incubated 24 h at 37°C. Plates were washed three times with sterile water, dried, stained with 1% aqueous crystal violet for 15 min, washed again, dried, and the adsorbed crystal violet brought into solution by addition of 33% (v/v) glacial acetic acid. The optical density of each well was measured at 570 nm (OD570) using an automated plate reading spectrophotometer. The mean OD570 of all strains was significantly higher for undiluted skim milk than for 1:8 diluted skim milk ($P < 0.05$). Overall, the densest biofilms were formed in undiluted skim, followed by 1:8 plus whey, 1:8 plus casein, 1:8 plus lactose, and 1:8 diluted skim milk with no additives. The mean OD570 of all strains for 1:8 plus whey was not significantly different than that for 1:8 plus casein, but both were significantly higher than for 1:8 plus lactose. Thus, an ample nitrogen source seems to be a more important determinant for biofilm formation in *E. sakazakii.*

P1-42 Resistance Characteristics of *Enterobacter sakazakii*

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The heat resistance and the ability to grow at low pH and low water activity (a_w) of 72 strains of *Enterobacter sakazakii* were investigated. For heat resistance, log reduction after 10 min at 60°C was determined by growing cultures 24 h at 37°C and diluting into preheated phosphate buffer in a hot water bath. After heat treatment, samples were placed on ice, plated onto tryptic soy agar, and incubated 24 h at 37°C to enumerate survivors. Log reductions ranged from 1.88 to 5.22. To determine ability to grow at low pH, 12 h cultures were diluted in tryptic soy broth adjusted to pH 4.5, 4.3, 4.1, and 3.9. Samples were placed into 96 well plates and incubated at 37°C; and optical density at 600 nm was measured after 24 and 48 h. A similar procedure, using tryptic soy broth adjusted to a_w 0.96, 0.94, and 0.92 with sodium chloride and sucrose, was used to determine growth at low aw. For pH and a_w experiments, growth was reported as negative if the change in optical density was less than 0.010, and positive if the change was greater than 0.010. All 72 strains were able to grow at pH 4.5, 98.6% were able to grow at pH 4.3, 95.8% were able to grow at pH 4.1, and 79.2% were able to grow at pH 3.9. For a_w 0.96, 97.2% were able to grow when the solute was sucrose, and 98.6% were able to grow when the solute was sodium chloride. For a_w 0.94, 83.3% were able to grow when the solute was sucrose but only 30.5% were able to grow when the solute was sodium chloride. Information about the heat resistance and ability of *E. sakazakii* to grow under adverse conditions will enable design of better control strategies for this organism.

P1-43 Desiccation Resistance of *Enterobacter sakazakii*

DSC

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Enterobacter sakazakii (ES) has emerged as an important foodborne human pathogen, especially among premature and young infants as the primary susceptible population. Its presence in dry infant products like infant formulae, dry foods and/or the food plant environment suggests this bacterium's ability to survive under desiccated conditions. In this study, we investigated desiccation survival of nine strains of ES using a simple microscope coverslip technique. Aliquots of each overnight culture of ES (3 clinical, 3 food, and 3 environment isolates) were air dried directly on coverslips under a laminar flow hood after growth in tryptic soy broth in the first set of experiments; in the second set of experiments, three strains of ES were suspended in buffered peptone water (BPW) then dried on coverslips. Coverslips were stored in desiccators at 5 and 25°C in presence of desiccant (Drierite). Survivors were determined by plating on Tryptic Soy Agar (TSA) and Violet Red Bile Glucose agar (VRBG). The difference in counts between TSA and VRBG represents the number of injured cells. Cells dried in TSB survived for longer periods (90+ days) while cells dried in BPW survived shorter periods. Microscopic examination of ES (stained with Indian ink) suggested the presence of capsular material. Cells that were resuspended from BPW dried coverslips survived for shorter periods (5.0–6.0 logs reduction) compared to approximately 2.5–3.0 log reduction of TSB dried ES coverslip samples. As determined by differential counts on TSA and VRBG, 50–60% of the population was injured, with more injured cells on coverslips stored at room temperature on TSB coverslip sample. These results indicate that *E. sakazakii* can survive the desiccated state for long periods of time in a food plant environment and could contaminate food that comes in contact with the bacterium on equipment in the plant.

P1-44 Efficacy of UV-C Light for the Inactivation of Some Microorganisms on the Surface of Fresh Pear

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Raw fruit and vegetables may be vehicles of microorganisms capable of causing human illness. The use of UV-C radiation was proposed as an emergent non-thermal process technology for disinfection of fruits and vegetables. However more research is needed to optimize UV-C treatments, since radiation penetrates differently depending on the type and composition of food and microorganism responses vary with the species and the strain. This study was aimed to investigate and to model the effect of UV-C light at various exposure times on the inactivation of *Listeria innocua* CIP 8011, *L. innocua* ATCC, *L. welshimeri*, *Zygosaccharomyces bailii*, *Z. rouxii* and *Debaryomyces hansenii* in pear surface. Pears were washed and sliced into approximately 3 mm thick discs (3 cm in diameter) under aseptic conditions. Single- and mixed-strain suspensions of *Listeria* (~10^8 CFU/ml) and yeasts (~10^6 CFU/ml) were inoculated on to the peel of the pear discs to mimic mid/post processing contamination. The inoculated discs were placed at 10 cm distance from two UV-C lamps (254 nm) inside a wooden box covered with aluminum foil. The UV-C intensity was measured by the iodide-iodate chemical actinometer. The UV-C radiation dose was varied between 0 and 2.7 kJ/m^2 by altering the exposure time up to 20 mins. Four discs were analyzed for microbial counts at each treatment time following standard enumeration methods. Survival curves were characterized using the Fermi dose-response model. UV-C treatments were effective in reducing microbial populations. Inactivation curves were well represented by the Fermi dose response model. No significant differences were found between mixed and single microorganism responses but the required dose to inactivate half the population of yeasts was almost double (0.9 – 1.0 kJ/m^2) that corresponding to *Listeria* (0.5 kJ/m^2). This study gives useful quantitative information on the effectiveness of UV-C light against some possible contaminant microorganisms in pear fruit.

P1-45 Lethality of Chlorine, Chlorine Dioxide, and a Commercial Produce Sanitizer to *Bacillus cereus* and *Pseudomonas* in a Liquid Detergent, on Stainless Steel, and in Biofilm

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Little is known about the effectiveness of sanitizer treatments in eliminating pathogens and spoilage microorganisms that may remain in food or detergent residues on food-contact surfaces. A study was done to determine the sensitivities of *Pseudomonas* cells and *Bacillus cereus* cells and spores suspended in a liquid dishwashing detergent and on the surface of stainless steel to treatment with chlorine, chlorine dioxide, and a commercial produce sanitizer (Fit™). Cells and spores were incubated in a liquid dishwashing detergent for 16–18 h before treatment with sanitizers. At 50 µg/ml, chlorine dioxide killed a significantly higher number of *Pseudomonas* (3.82 log CFU/ml) compared to a reduction of 1.34 log CFU/ml caused by treatment with 50 µg/ml chlorine. Stainless steel coupons were spot-inoculated with *Pseudomonas* cells and *B. cereus* cells and spores, using water and 5% horse serum as carriers. Chlorine was more effective than chlorine dioxide in killing cells and spores of *B. cereus* suspended in horse serum. Treatment of *B. cereus* biofilm on the surface of stainless steel with chlorine dioxide or chlorine at 200 µg/ml caused reductions in total populations (vegetative cells plus spores) of > 4.42 log CFU/coupon; reductions in the number of spores were > 3.80 log CFU/coupon. Fit (0.5%) was ineffective in killing *B. cereus* in spot inocula and in biofilm but treatment with mixtures of Fit and chlorine dioxide caused greater reductions than treatment with chlorine dioxide alone. In contrast, when chlorine was combined with Fit, the lethality of chlorine was completely lost. This study provides information on the survival and sanitizer sensitivity of *Pseudomonas* and *B. cereus* in a liquid dishwashing detergent, on the surface of stainless steel, and in biofilm. Observations provide insights to developing more effective strategies for cleaning and sanitizing contact surfaces in food preparation and processing environments.

P1-46 Effect of Temperature and Nutrient Status on Adherence of Clinical and Environmental *Listeria monocytogenes* Strains to Food Grade Stainless Steel Coupons

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Adherence of pathogenic bacteria to food processing surfaces poses many problems for food safety. The objective of this study was to determine if clinical and environmental strains of *L. monocytogenes* differed in adherence to stainless steel coupons in response to different environmental conditions. Adherence of ten *L. monocytogenes* strains, five clinical and five environmental, to food grade stainless steel was investigated after prior growth at 4 or 27°C in brain heart infusion broth (BHI) or peptone water (PW), representing a nutrient rich and poor environment respectively. Washed cells from each treatment were resuspended in PW and allowed to adhere to stainless steel coupons for 10 days at 4°C or 24 h at 27°C to simulate refrigeration and room temperatures. Coupons were stained with Live/Dead stain and observed under fluorescence microscopy to enumerate total number of adhered, live and dead/damaged cells. Plate counts of adhered cells were also performed. Environmental strains adhered in significantly ($P < 0.05$) larger numbers than the clinical strains in most treatments. Low adherence temperature decreased numbers of total cells adhering (average 5.67 × 10^6 cells/mm^2) while an average of 7.55 × 10^6 cells/mm^2 adhered at 27°C. Significantly ($P < 0.05$) more dead or damaged cells were observed on 4°C coupons than on 27°C coupons; however, plate counts showed most damaged cells were not dead. Prior growth in BHI resulted in significantly ($P < 0.05$) higher numbers of adhered cells and dead/damaged cells compared to adherence after prior growth in PW. Measurements of the expression of selected stress genes in adhering cells are in progress. In conclusion, origin, nutrient status and prior growth temperature significantly impacted the ability of *L. monocytogenes* to adhere to stainless steel. These results advance our understanding of how *L. monocytogenes* colonizes surfaces in the food processing environment.

P1-47 Characterization of Shiga Toxin-producing *Escherichia coli* Strains Isolated from Swine Feces

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Shiga toxin-producing *E. coli* (STEC) belonging to different serogroups were isolated from swine feces and were characterized to determine the presence of *E. coli* virulence genes by PCR, antibiotic resistance profiles, and acid tolerance. Twenty-nine out of 219 (13%) of the isolates harbored the gene for *stx*1, 14 (6%) *stx*2, 179 (80%) *stx*2e, 46 (21%) STa, 14 (6.4%) STb, 10 (4.6%) F18, 94 (42.9%) EAST1, 192 (87.7%) Cdt-II, 1 (0.46%) Cdt-III, and 25 (11.4%) Hly. None of the strains harbored the genes for LT, BFP, F4, F5, F6, F41, CNF1, CNF2, EaeA, Cdt-I, and Cdt-IV. The strains were also examined for antimicrobial susceptibility profiles, using 16 antibiotics. The STEC isolates displayed resistance most often to tetracycline (95.4%), sulfamethoxazole (53.4%), kanamycin (38.4%), streptomycin (34.7%), and chloramphenicol (22.4%). An *E. coli* serotype O20:H42 strain, which was positive for *stx*2e, astA, and cdtII, was resistant to all of the antibiotics tested, except for amikacin. In addition, 52 of the swine isolates were examined for their ability to withstand acid-challenge by three types of acid resistance (AR) pathways, AR1 (rpoS -dependent), AR2 (glutamate-dependent) and AR3 (arginine-dependent). None of the strains was defective in the AR1 resistance pathway, while one strain was defective in the AR2 pathway under aerobic growth conditions, but was fully functional under anaerobic growth conditions. However, 8 out of 52 strains were defective in the AR3 pathway. The strain that was defective in AR2 was fully functional in the AR3 pathway. Since acid resistance plays a vital role in the survival and virulence of these strains, differences among the isolates to induce AR pathways may play a significant role in determining their infective dose. This study demonstrates that swine STEC are a heterogeneous group, and the possession of virulence genes, including genes for the shiga toxins, ST enterotoxins, hemolysin, and cytolethal distending toxins, indicate that many swine STEC can potentially cause human illness.

P1-48 Inactivation of *Salmonella* in Manure-based Composts with Varying C:N Ratios

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Composting is a process whereby organic matter is decomposed by microorganisms to generate a stable amendment that improves soil quality and fertility. To date, the primary criteria for ensuring the microbiological safety of composts have been adherence to narrowly defined time-temperature conditions. To expand the guidelines whereby inactivation of pathogens could be assured, this study sought to determine if the carbon:nitrogen (C:N) ratio or the presence of ammonium sulfate affects the inactivation of *Salmonella* spp. in cow manure-based compost mixtures. Evaluation of compost conditions on pathogen inactivation was conducted using a bioreactor system. The days to achieve non-detection of *Salmonella* spp. by enrichment culture was used as the endpoint. In addition to pathogen levels, pH and temperature were monitored at 4 locations within the bioreactor. Location within the bioreactor was not a significant variable affecting pathogen inactivation. Compost preparations with an initial C:N ratio of 20:1 required a maximum of 4 days of storage before *Salmonella* was not detected whereas 30:1 and 40:1 C:N preparations required up to 7 days of storage. Both 20:1 and 30:1 C:N preparations were characterized by a decrease in pH to 5.5–5.7 before pH values increased to > 8. In contrast, pH values of 40:1 C:N preparations increased immediately to > 8, generally within the first day of storage. Maximum temperatures encountered in 20:1 C:N preparations for inactivation of pathogens were less than 50°C. Consequently, the cumulative heat exposure required for pathogen inactivation in 20:1 C:N preparations was five-fold less than in 40:1 C:N preparations. Temperatures within preparations supplemented with 0.08% ammonium sulfate were higher than unsupplemented preparations during the first 2 days of storage; however, these higher temperatures did not consistently translate into more rapid rates of pathogen inactivation.

P1-49 Effects of Low-Dose Irradiation on Survival of *Escherichia coli* O157:H7, *Salmonella*, and MS2 Bacteriophage on Fresh Mint (*Mentha piperita* L.)

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Fresh herbs were vehicles of several foodborne illness outbreaks in recent years, and disinfectants at concentrations currently approved for use on herbs cannot eliminate pathogens completely. This study evaluated low-dose irradiation as a sanitation alternative. Commercially packed fresh mint samples were inoculated with a cocktail suspension of a viral indicator MS2 bacteriophage, and nalidixic acid-resistant strains of *E. coli* O157:H7 and *Salmonella*. Following the irradiation treatment at dosages of 0.25 to 2.0 kGy, the microbial populations (log CFU or PFU/g) and visual quality were monitored at 1, 3, 6, 9, and 12 days of storage at 4°C. Bacterial populations decreased ($P < 0.0001$) with increasing irradiation doses. A day after the treatment, reductions for both *E. coli* O157:H7 and *Salmonella* at 0.25, 0.60, and 1.0 kGy were approximately 2.0, 3.5, and 5.8 log CFU/g, respectively. Both bacteria were completely eliminated at 2.0 kGy. *E. coli* O157:H7 was not recovered (by surface plating method) on samples irradiated at 0.60 kGy after 6 days of storage and thereafter, while *Salmonella* remained at 0.97 log CFU/g on day 6 and reduced to 0.27 log CFU/g on day 12, suggesting that *E. coli* O157:H7 was more susceptible to irradiation than *Salmonella*. Although reductions were statistically significant, no irradiation dose reduced MS2 bacteriophage populations by more than 0.60 log PFU/g at day 3, and the numbers persisted throughout the storage period. The visual quality of irradiated samples (2.0 kGy) was rated as excellent through day 9 of storage and remained fair on day 12. The results demonstrated that low-dose irradiation (1.0 – 2.0 kGy) can be a potential means of effectively inactivating selected pathogens in fresh herbs commonly consumed raw as garnish.

P1-50 Utilization of Chlorine Dioxide for Microbial Control of Minimally Processed Cheiro Verde

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In Brazil sodium hypochloride is used to reduce microbial counts and minimize the deterioration of minimally processed vegetables. Although it is efficient, its use has been questioned because it may be a precursor of compounds with high carcinogenic potential. Chlorine dioxide has been proposed as one of its substitutes for vegetable disinfection. Therefore, the aim of this research was to evaluate, through microbial analyses, the efficiency of chlorine dioxide (10, 25 and 50 ppm for 2, 5 and 10 min) in comparison

to sodium hypochloride (120 ppm/15 min standard treatment) in the control of natural microbiota of minimally processed cheiro verde. The raw materials, (parsley and Welsh onion) of cheiro verde were submitted to the action of sanitizer solutions according to times and concentrations above. The control sample was submitted only to a washing step with tap water. The treatments were repeated 3 times at four-month intervals. From each treatment, 3 samples were collected for microbial analysis of pathogenic *Salmonella* sp., total and fecal coliforms and total count of molds and yeasts. Among the treatments carried out with chlorine dioxide, the most efficient was the one made at the 50 ppm concentration for 10 min of contact. It reduced the microbial population by 3.0; 2.7; >2.0 logarithm cycles for molds and yeasts; total and fecal coliforms respectively, when compared with the control (washing with tap water). On the other hand there was no difference in the reduction of the microbial population when compared with sodium hypochloride treatment (population at the same level: log 2 CFU/g). Based on these results, when using chlorine dioxide the addition of no less than 50 ppm for at least 10 min is advisable.

P1-51 Utilization of Chitosan for Microbial Control in Minimally Processed "Cheiro Verde"

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Minimally processed foods ready for consumption need rigorous control of hygienical and sanitation conditions. Sodium hypochloride is the only sanitizer agent allowed by the Brazilian legislation and it has been associated with the appearance of several types of cancer. Therefore there is an intensive search for other non-chemical agents with good antimicrobial action guaranteeing the food microbiological safety as well as maintaining product quality. Chitosan belongs to this group. It is a non-toxic, biodegradable and biocompatible biopolymer, obtained through the processing of chitin, a polymer found in crustacean shells. Chitosan can be dissolved in aqueous solutions of organic and inorganic acids resulting in viscous solutions that can be used as coating for microbiological protection in fruits and vegetables. Therefore, the aim of this work was to evaluate the antimicrobial action of chitosan by the number of decimal reductions of microbial population (total and fecal coliforms, molds and yeasts) and elimination of *Salmonella* in minimally processed cheiro verde (parsley and welsh onion mixture) at concentrations of 5% and 10% and with 5, 10 and 15 min of contact. The results showed the absence of *Salmonella* and fecal coliforms in all samples. For total coliforms a reduction of 2 logarithm cycles was observed for the samples treated with chitosan comparing to the sample treated with tap water. When compared to the sample treated with sodium hypochloride (120 ppm for 15 min) the results were close to each other. In relation to molds and yeasts, the treatment with chitosan as well as with sodium hypochloride inhibited completely the growth of these microorganisms. The conclusion is that chitosan can be used as sanitizer agent, replacing chlorine for the treatment of minimally processed cheiro verde.

P1-52 Retail Ready-to-Eat Luncheon Meats Packages as a Potential Source of Foodborne Pathogens

DSC

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This study examined the exterior of 544 Ready-to-Eat (RTE) deli meat packages sealed under USDA inspection for contamination with *Listeria*, *E. coli*, and coliforms. Packages from poultry (n = 268), pork (n = 96), beef (n = 141) and meat bologna (n = 39) were randomly collected from 41 retail grocery stores that include 26 major A and 15 small B stores. Samples were collected weekly for 4 months from FoodNet counties in Tennessee. A total of 16 counties were visited to collect samples. All stores and counties were visited only once. *Listeria* and *E. coli* were not detected on any of the packages examined. Approximately 9% of the packages were positive for coliforms. The exterior of pork (14%) and meat bologna (13%) packages positive for coliforms were significantly higher ($P < 0.05$) than poultry (7%) and beef (9%) packages. Very high levels (>738 coliform/package) of coliforms were detected on 9 of the 48 coliform positive packages. Most (75%) of the counties and 39% of the stores visited had coliform-contaminated packages. On average, the smaller retail grocery stores had a significantly higher ($P < 0.05$) coliform contamination rates (14%) than the larger retail grocery stores (7%). This study revealed that 544 retail luncheon meats packages were not contaminated with *Listeria* or *E. coli*; however, the contamination of RTE deli meat packages with high levels of coliforms suggest that RTE packages are exposed to environmental contamination at the retail level.

P1-53 Attachment of *Pseudomonas fluorescens* AH2 to Stainless Steel Surfaces is Reduced by Conditioning with Fractions of Fish Extract

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Antifouling coatings that can reduce microbial adhesion and biofilm formation on surfaces are being studied to prevent or reduce hygiene problems in the food industry. Many coatings, however, rely on antimicrobial substances, which may cause development of microbial resistance or be of environmental concern. We have recently discovered that aqueous extracts made from fish reduce bacterial adhesion to stainless steel surfaces. The extract is non-toxic and does not inhibit growth of the bacteria, indicating that microbial resistance would not develop. The purpose of this study was to determine if particular substance(s) in the fish extract caused its antifouling effect, using *Ps. fluorescens* AH2 as target organism. The composition of amino acids (mol %) that bound to stainless steel was very similar to the composition found in aqueous fish extract (except for lacking of hydroprolin and beta-alanin). This indicates that most of the peptides or proteins in the fish extract bind to the steel surface and could be responsible for the antifouling effect. The fish extract conditioning layers were visualised using atomic force microscopy. The molecular weight of the fish extract components was determined using MALDI-ToF-MS. The adsorbed fish extract components were characterized using MALDI in the surface-mode and the amount of adsorbed fish extracts estimated using x-ray photoelectron spectroscopy (XPS). The non-fractionated fish extract caused a reduction in bacterial attachment of 1-3 log units per square cm as compared to surfaces conditioned in laboratory substrate. The effect lasted at least 24 h even when submerged in buffer. Attachment of *Ps. fluorescens* to steel coupons conditioned in fractions of fish juice indicated that more than one fraction had a significant antifouling effect. Together with previous results, our data indicate that coating stainless steel with antifouling, non-inhibitory compounds may be a strategy to reduce biofilm formation in the food industry.

P1-54 Growth of Heated *Bacillus cereus* in Nutrient Broth and Food Extracts

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Bacillus cereus is recognized as a cause of foodborne disease and is frequently isolated from raw and processed food. Its spores are very heat resistant and the heat treatment cannot kill them without compromising the nutritional value and organoleptic properties of food. Exposure to heat during thermal processing may injure spores, and they only germinate and grow if the damage is repaired. The capacity of heat damage repair depends on conditions of recovery medium. A nutrient rich medium is required for repair. The objective of this study was to investigate the effect of different media on kinetics growth of heated spores of *B. cereus*. *Bacillus cereus* strain ATCC 7004 was used. A colony isolated from nutrient agar was inoculated into 1 mL of nutrient broth, chicken meat, rice, bean and trout extracts and skimmed milk. Heat treatment was performed in a stirred water bath at 90°C for 10 min. 0.3 mL of the heat-treated spores mixture were transferred to 150 mL of each one of the culture media. Flasks were incubated at 37°C for 250 h. At appropriate intervals, samples of the cultures (1 ml) were taken from each flask and decimally diluted in sterile 0.1% (w/v) peptone water. Appropriate dilutions were plated on nutrient agar. Plates were incubated at 37°C for 24 h, and the numbers of colonies (CFU/mL) were enumerated. Growth curves were generated by fitting the Gompertz equation and growth parameters were calculated. ATCC 7004 strain survived processing at 90°C for 10 min and was able to germinate and grow after heating under all the conditions in study. Heated *B. cereus* is able to grow in substrate of very different nature; however bean substrate was more stressful than other media. Chicken meat and trout extracts appeared to have a stimulating effect on the growth of *B. cereus*.

P1-55 DSC Comparison of Barosensitive and Baroresistant Strains of *Lactobacillus plantarum* and *Lactobacillus fermentum* by Investigating the Impact of Dose Response and Kinetic Parameters, Buffer Composition, and Buffer pH

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High pressure processing (HPP) is an emerging technology for inactivating microorganisms without adversely impacting the fresh qualities of food. HPP studies have primarily focused on the inactivation of foodborne pathogens, but use of the technology to target spoilage microorganisms is gaining interest. Studies addressing the behavior of spoilage microorganisms (i.e., *Lactobacillus* spp.) during HPP are limited. Previous experiments have shown HPP sensitivity to vary among strains of *Lactobacillus plantarum* and *L. fermentum*. The objective of this investigation was to determine the impact of various treatment conditions on survival of lactobacilli differing in HPP sensitivity. Two strains of *L. plantarum* and *L. fermentum* were studied (one pressure-resistant and one pressure-sensitive). Inactivation kinetic parameters and dose-response patterns were investigated using stationary-phase cells suspended in PBS. Cell suspension media [phosphate buffered saline (PBS), peptone water (1%), or citrate-phosphate buffer (CP)] at pH 7.0–7.4 were compared for their protective effect against pressure treatments. The pH of CP was tested in the range of 3.0–7.0. Survivors were enumerated on MRS after incubation at 30°C for 72 h. Experiments were performed in triplicate. Kinetic and dose studies showed that *L. plantarum* MDOS-32 was consistently the most resistant strain. *Lactobacillus plantarum* OSY-104 was consistently the most sensitive, showing increased sensitivity to the come-up period of the treatment. Inactivation at 550 MPa was linear during the first 3 min of treatment while tailing was apparent with extended treatment times. *Lactobacillus fermentum* strains were similar in their resistance to each other, except with extreme pressure treatments. Buffer studies indicated that CP (pH 7.0) and PBS (pH 7.4) were similar in their protective ability during pressure treatment; peptone water was significantly less protective. As pH of the suspension media decreased, the protective ability decreased for all strains. *Lactobacillus fermentum* strains were more resistant to pH-pressure combinations than *L. plantarum* strains.

P1-56 DSC Intrinsic and Extrinsic Effects of Sporulation Conditions on Heat Resistance of *Clostridium sporogenes* PA3679

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The relatively high heat resistance of spores and the toxin produced by *Clostridium botulinum* in heat processed foods make this organism a considerable concern to the food industry. *Clostridium sporogenes* PA 3679, a surrogate of *C. botulinum*, is commonly used in laboratory practices to analyze the resistances of *C. botulinum*. The purpose of this study was to investigate the effect of factors during sporulation on the heat resistance of *C. sporogenes* PA3679 spores. *Clostridium sporogenes* PA 3679 was grown in TPGY media and transferred to sporulation media which was prepared using combinations of four different factors, including water activity (a_w), pH value of the sporulation media and mineral supplements (intrinsic factors) and different incubation temperatures (extrinsic factor). Water activity was from 0.94 to 0.99, pH value was from 4.5 to 8.0 and calcium chloride was added into sporulation media at 0.1%, 0.5% and a combination of 0.1% calcium chloride and 0.1% manganese chloride. Incubation temperature was at 10, 20, 32, 37 and 43°C. Samples were taken each week for up to six weeks. Spore counts were determined by heat treatment at 90°C for 10 min and heat resistance of spores from each group was evaluated by exposure to 121.1°C for 1 min. The results indicated that PA 3679 vegetative cells incubated in sporulation media at 32°C with low pH value (5.0), low water activity (0.96) and a supplement of 0.5% calcium chloride resulted in spores with stronger heat resistance. These findings can be applied to further research involving the thermostability of *Clostridium* spp. spores.

P1-57 Quantifying the Distribution of Sub-lethal Injury in Thermally Heated *Salmonella* Population

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Exposure of bacterial pathogens to sub-lethal treatments can result in injury and a subsequent increase in thermal resistance. Injury traditionally has been described in a binary manner (i.e., the bacteria are either injured or not injured). However, it is assumed that a bacterial population that is subjected to a lethal treatment would include a continuous distribution across a spectrum of injury. Quantifying this distribution would help improve models for thermal inactivation of sub-lethally injured populations. Therefore, the

objective of this project was to experimentally quantify the changing distribution of injury in *Salmonella* subjected to heat treatment. An eight-strain *Salmonella* cocktail was subjected to heat shock (30 min/ 54°C) and harvested by centrifugation, before being subjected to isothermal heating at 57°C. Five different ratios of selective (XLD) and non-selective (TSA) media were combined in overlay (0:1, 1:2, 1:1, 2:1, and 1:0), as a means to quantify the spectrum of injury within the surviving *Salmonella* population. The derivatives of those spectra were normalized to generate frequency distribution curves for the degree of injury at each sampling time during heating. Results indicated significant differences ($P < 0.05$) in the frequency distribution curves, and a shift in the "mean injury" was observed with increasing heating time. These results are the first direct illustration and quantitative evaluation of the thermally induced shift that occurs in the bacterial population from healthy to partially-injured to severely-injured to non-viable cells.

P1-58 Pathogenic *Enterobacteriaceae* and Aerobic Bacteria Isolates from Domestic Refrigerators

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A significant proportion of foodborne illnesses are due to cross contamination in the domestic kitchen environment. Pathogens have been identified at a wide array of kitchen sites including dishcloths, sponges, oven door handles, counter-tops and draining boards. While possible cross contamination through most of these avenues has been researched, there is a paucity of information on potential pathogens in the domestic refrigerator. The goal of this study was to evaluate the prevalence and levels of pathogenic *Enterobacteriaceae* and aerobic bacteria in domestic refrigerators. Meat and vegetable bins as well as the top shelf of each refrigerator were swabbed to collect samples. Butterfield's Phosphate Buffer was added (1:10) to the samples, which were homogenized and then serially diluted (0, 10–1, 10–2, and 10–3) for subsequent plating onto 3M Petri film *Enterobacteriaceae* and plate count agar (PCA). The 3M Petri film *Enterobacteriaceae* and PCA plates were incubated at 36°C for 24 h and 35°C for 48 h, respectively. Api20E was used for identification of *Enterobacteriaceae*. The populations for *Enterobacteriaceae* and aerobic plate counts ranged from 0.6 – 6.95 log CFU/100 cm^2 and 1.04 – 7.13 log CFU/100 cm^2, respectively. *Klebsiella pneumoniae* spp. *pneumoniae* (25.5%), *Klebsiella oxytoca* (8.2%), *Yersia enterocolitica* (1%), and *Enterobacter sakazaki* (2%) were among the most prevalent pathogenic bacteria isolated from the domestic refrigerators. The high populations for *Enterobacteriaceae* and aerobic bacteria in refrigerators suggested poor sanitation. The results indicate that kitchen refrigerators can be potential reservoirs for pathogenic bacteria and therefore present a hazard for contaminating prepared foods and other parts of the domestic kitchen.

P1-59 Factors Related to Food Worker Hand Hygiene Practices

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Proper handwashing and glove use are critical to the prevention of contamination of food by food worker hands. However, research indicates that food workers often fail to implement these hand hygiene practices. Researchers and practitioners contend that a range of factors influence food worker practices and that to successfully change worker practices, these factors need to be identified and addressed. The Environmental Health Specialists Network (EHS-Net), a network of environmental health specialists affiliated with several federal and state agencies, conducted this study to identify factors related to worker hand hygiene practices. To collect data on hand hygiene practices, EHS-Net specialists conducted hour-long observations of 321 workers in randomly selected restaurants in six EHS-Net states. The specialists recorded when workers engaged in any of seven activities that required handwashing (e.g., preparing raw animal product). They also recorded any handwashing and glove use behaviors that occurred in conjunction with these activities. To collect data on factors thought to be related to hand hygiene practices, such as restaurant demographics, policies, and training, the social and physical environment, and worker business, EHS-Net specialists conducted observations of the kitchen environment and interviews with restaurant managers. Analyses indicated that handwashing was more likely to occur in restaurants where handwashing was taught and where there was more than one hand sink, a hand sink in the observed worker's sight, and management encouragement of handwashing ($P < .05$). Handwashing was less likely to occur when gloves were worn and workers were busy ($P < .05$). Glove use was more likely to occur in chain restaurants and in restaurants with glove policies and glove supplies ($P < .05$). These findings suggest that in order to be successful, hand hygiene improvement programs must address the multiple factors related to hand hygiene behavior.

P1-60 Hazard Analysis for Raw Food Materials of School Foodservices through Supply Chains

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School foodservice operations are considered to be the main place of foodborne disease outbreaks in Korea. One of the causes is the purchase of food materials from unsafe sources. This study aimed to analyze the hazards of potentially hazardous foods through the food supply chains in order to determine the safety of school foodservice. The hazards and factors affecting food safety were investigated at each supply step, from manufacturer to receiving at school. Nineteen foods including Ready-to-Eat vegetables, tofu and frozen processed foods and were collected from eight different enterprises. The hazards considered were temperatures of food materials and environments, and times for delivery and storage. Microbial analysis included total plate counts (TPC), coliforms, *E. coli*, yeasts and molds, *S. aureus*, *B. cereus*, and *Salmonella* spp. The temperatures of trucks and refrigerators were over the limit (5/-18°C) in many cases as were the inside temperatures of some foods. The levels of TPC (1.46×10^6 - 2.60×10^7 CFU/g) and coliforms (9.60×10^3 – 1.49×10^5 CFU/g) in blanched bracken were unsatisfactory. The levels of coliforms and *S. aureus* in sliced burdock were potentially hazardous while there were unacceptable levels of TPC and coliforms in sliced bellflowers. Tofu at receiving was unacceptable due to high levels of TPC and coliforms. Microbiological quality of frozen hotdogs, deep-fried pork covered with sour and sweet starchy sauce and tofuball was unacceptable for TPC and *S. aureus*. These results showed that the temperatures of foods and facilities were not properly controlled and thus potentially hazardous foods were exposed to the possible growth of contaminating microbes after manufacture. Therefore, sanitary practices in processing level and the strict control of time/temperature through the supply chain should be managed systematically to secure safety of food materials.

P1-61 Bacterial Occurrence on Tabletops and in Dishcloths Used to Wipe Down Tabletops in Public Restaurants and Bars

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To determine the occurrence of bacteria in dishcloths used in restaurants and bars, 37 cloths were collected (23 restaurants, 14 bars). Samples were also taken directly from tabletops before and after cleaning. Coliforms were found in 89.2% of dishcloths (geometric mean = 7.6 – 10^5 CFU/cloth) and on 70% of tabletops (gm = 49.8 CFU/156 cm^2). *E. coli* was found in 54.1% of dishcloths (gm = 1.9 – 10^3 CFU/cloth) and on 20% of tabletops (geometric mean= 5.2 CFU/156 cm^2). The numbers of heterotrophic plate count (HPC) bacteria and total coliforms were significantly ($P < 0.05$) higher in bars than in restaurants. The numbers of HPC bacteria found in dishcloths were 25-fold lower than those found in homes in previous studies. The level of total coliforms was 50- to 120-fold lower and the number of *E. coli* was also lower than the number of fecal coliforms. The numbers of HPC bacteria, total coliforms and *E. coli* found on restaurant tabletops were lower than those found on household kitchen countertops. The mandatory use of sanitizers in restaurants and bars may explain these discrepancies between household kitchen and food service environments. The values found for HPC bacteria on tabletops after cleaning were 45-fold greater than those taken prior to cleaning. There were also 19 times more coliforms and twice as many *E. coli*. This may implicate dishcloths in contamination and suggest that current monitoring of linen sanitization solutions is inadequate. *Pseudomonas*, *Klebsiella*, *Listeria*, and *Enterobacter* were the most commonly isolated genera from dishcloths. These differ slightly from the most commonly isolated genera found in homes (*Pseudomonas*, *Enterobacter*, *Salmonella*, *Staphylococcus aureus* and *Aeromonas hydrophila*). Therefore, the mandatory use of sanitizers may not only have reduced contamination levels, but also caused a shift in the microbial populations present in food service establishments.

P1-62 Food Workers' Awareness of and Performance in Sanitation and Customers' Satisfaction with Sanitation at Large Restaurants in Korea

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The purpose of this study was to investigate sanitation management practices, workers' awareness of and performance in sanitation, and customers' satisfaction with sanitation at large restaurants in Korea. Sanitation inspections were carried out in 200 large Korean, Western, Chinese, and Japanese restaurants and in buffet-style restaurants in Daegu and Gyeongbuk province. Sanitation awareness of food workers and customers' satisfaction with sanitation were investigated by interviewing 317 food workers and 205 customers. The result of the inspection of foodservice establishments showed low performance in food handling, employee hygiene practices, and in cleaning food processing equipment. Scores of the food workers' awareness in Chinese restaurants were significantly lower than scores of workers in western restaurants. Food workers had low awareness of sanitation procedures used for food storage and cleaning of equipment in Korean, Chinese, and Japanese restaurants; of cleaning of equipment, food testing, and food distribution in western restaurants; and of cleaning of equipment and food handling in buffet-style restaurants. Food workers at all restaurants had the lowest performance in terms of HACCP. This shows that HACCP application and recording are not yet properly carried out at restaurants in Korea. Food workers had low performance scores in food handling, especially vegetable disinfection, and disinfection after hand washing. Research on customers' satisfaction with sanitation revealed a low rating of the kitchens and food workers at all restaurants. Customers had low satisfaction with servers and kitchen environments in Korean restaurants; with food, tableware, utensils and servers in western restaurants; with food and kitchen environments in Chinese restaurants; with servers, tableware and utensils in Japanese restaurants; and with kitchen environments and servers in buffet-style restaurants. Therefore, cleanliness of kitchen facilities and equipment and hygienic food handling by workers in restaurants are urgently needed.

P1-63 Microbiological Survey of Ready-to-Eat Prepared Foods, Preparation Utensils, and Food Contact Surfaces in Retail Delicatessens

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Microbiological quality and safety of 77 Ready-to-Eat (RTE) foods prepared and displayed in four retail delicatessens in Johannesburg, South Africa were determined. The bacteriological status of food preparation surfaces (n = 66), selected utensils (n = 58) and hands (n = 34) of food handlers was assessed. Foods included filled baguettes (n = 14), salads (n = 15), sliced processed meats (n = 34) and hot meals (n = 14). The highest aerobic plate counts (APC) were found in filled baguettes (6.2 log CFU/g) followed by sliced processed meats (6.1 log CFU/g) and salads (3.7 log CFU/g). Coliform counts (CC) were only determined for filled baguettes (2.8 log CFU/g) and salads (1.8 log CFU/g). *Staphylococcus aureus* (S.) and *Bacillus cereus* (B.) were isolated from filled baguettes (35, 43%), salad (13, 40%) and sliced processed meat (8, 12%) samples, respectively. *Salmonella* spp. and *Listeria monocytogenes* (L.) were detected in salad (7, 7%) and sliced processed meat (6, 6%), samples. In addition *L. monocytogenes* was isolated from filled baguettes (14%). *Clostridium perfringens* was not detected. No foodborne pathogens were found in hot meal samples, indicating that adequate cooking temperatures were reached. Preparation utensils showed the highest APC (2.2 log CFU/cm^2) followed by surfaces (1.7 log CFU/cm^2). Coliform counts were similar, approximately 1.1 log CFU/cm^2 for preparation surfaces and utensils whilst *Escherichia coli* (E.) (1.1 log CFU/cm^2) was found on knives. Bacterial counts of approximately 1 log CFU/cm^2 were associated with foodhandlers' hands. *Salmonella* spp. and *L. monocytogenes* in salads and filled baguettes may pose foodborne illness potential, and *S. aureus* and *B. cereus* in filled baguettes and sliced processed meats may pose intoxication potential as such foods are not further processed before consumption. Furthermore, the presence of coliforms and *E. coli* on preparation surfaces and utensils may indicate improper hygiene.

P1-64 Bacterial Counts and Scanning Electron Microscopy of Cleaning Tools and Gloves Associated with Ready-to-Eat Food Preparation Environments

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Microbial populations associated with cleaning tools and disposable gloves used within retail delicatessens are of interest in the context of food hygiene. It has been speculated that bacterial foodborne pathogens may be transmitted from cleaning tools to food contact surfaces and subsequently to Ready-to-Eat (RTE) foods prepared on these surfaces. This study determined the microbiological status of selected cleaning tools, such as floor mops and cleaning cloths, as well as disposable gloves used by food handlers during RTE food preparation, in selected retail delicatessens in Johannesburg, South Africa. Cleaning tools were aseptically sampled in three replicate surveys of four delicatessens. One gram quantities of each sample were prepared for bacteriological analysis [Aerobic plate counts (APC), coliform counts (CC) and *Escherichia coli* (*E. coli*) counts (EC)], while duplicate samples were prepared for scanning electron microscopy (SEM) by standard methods. Aerobic plate counts were highest on the floor mops (n = 9) (5.3 log CFU/g) followed by the cleaning cloths (n = 13) (4.1 log CFU/g) and the disposable gloves (n = 8) (4.0 log CFU/g). Coliform counts were similar between all three sample types (approximately 1.5 – 2.5 log CFU/g), and *E. coli* counts were similar between mops and cleaning cloths (approximately 1.5 log CFU/g). *E. coli* counts for the disposable glove samples were below the lower detection limit. Scanning electron micrographs showed rod- and coccoid-shaped bacteria associated with all sample types. Results showed that cleaning tools and disposable gloves used within RTE food preparation environments may be potential sources for bacterial contamination.

P1-65 Microbiological Characterization of Water and Ice Used by Provincially Regulated Abattoirs in Ontario

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Operators of meat plants licensed under the provincial meat regulation of the Food Safety and Quality Act are required to supply the plant with potable running water that is protected against contamination. During the calendar year of 2004, meat hygiene officers collected approximately eight thousand water and ice samples for the monitoring and verification of the plant's water safety program. Each aseptically collected sample was analyzed for heterotrophic plate count (HPC), total coliforms (TCC), *E. coli* (ECC) and background colony counts (BCC). Bacterial contamination incidences of water samples were 1.16%, 3.30%, 0.32%, and 3.80% for HPC, TCC, ECC and BCC, respectively. Higher TCC (6.11%) and ECC (2.29%) contamination levels were associated with the ice samples. The microbial quality of the samples was influenced significantly ($P < .001$) by season. As hypothesized, highest contamination levels were encountered during the spring season. The explanatory variables of geographical location of plant (regional or watershed affiliation), water source and water treatment method affected significantly ($P < .05$) the microbial quality of the samples. Data will be used for regulatory action, such as a mandatory requirement for chronic violators to install an effective water disinfection system and an increased monitoring frequency due to seasonal variations and/or incidences of adverse results.

P1-66 Microbiological Survey of a Fish Processing Factory in Johannesburg, South Africa

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Bacterial populations associated with surfaces, product and water in a fish factory processing sub-tropical (e.g., butterfish) and North Atlantic (e.g., salmon) fish were investigated. Duplicate "mock" stainless steel surfaces were attached to 3 processing surfaces: (1) defrosting, gutting, scaling and head removal (DGSH), (2) deboning/slicing (DS), and (3) packaging (P). Attached bacterial populations were dislodged and counted after 7, 14 and 21 days on Tryptone Soya Agar - aerobic plate counts (APC), MRS agar-lactic acid bacterial counts (LABC), and Violet Red Bile Glucose Agar – gram-negative counts (GNC). The presence of *Listeria monocytogenes* (Bio-Rad) was tested. Salmon product counts, i.e., raw (R), after brining/smoking (BS) and after packaging (PK), were determined in parallel. Defrosting water (DW), curing/brining solutions (CBS) and air-conditioner cooling water (ACW) were also tested. Predominant populations from APC plates were characterized using a dichotomous key. Counts ranged from 5 to 6 log $CFUcm^{-2}$ on DGSH, DS, and P surfaces in decreasing order. Similarly, counts ranged from 5 to 6 log $CFUg^{-1}$ on product samples BS, PK and R in decreasing order. APCs of approximately 4 log $CFUml^{-1}$were obtained from DW and CBS samples, 1.5 log $CFUml^{-1}$ from ACW. *Listeria monocytogenes* was not detected. Predominant bacterial populations from APCs of processing surfaces, and DW and CBS samples were *Micrococcaceae* (37 to 66%), of which 7 to 47% were *Staphylococcus*. By contrast, predominant populations on raw product comprised Gram-negative rods (56%), while *Micrococcaceae* (67%) dominated smoked and packaged products. *Micrococcaceae* are reportedly natural flora associated with raw sub-tropical fish, which may have contaminated DGSH surfaces and subsequently raw salmon product. Brining increased *Micrococcaceae* populations on raw product prior to smoking, resulting in contamination of DS and P surfaces and of final product. Furthermore, 11% of predominant populations on packaged salmon products were *Staphylococcus* strains, posing potential foodborne illness implications.

P1-67 *Salmonella* Status of Beef Cattle after Grazing on Hog Manure Treated Pasture

DSC

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Hog manure can contain pathogenic bacteria such as *Salmonella* that may increase the risk of human illness if manure is used as fertilizer. The objective of this work was to investigate whether it could be demonstrated that *Salmonella*, naturally present in hog manure, can be transferred to cattle grazing fields previously fertilized with manure. Trials were conducted over two summer seasons. Hog manure was broadcast spread at normal rates over the surface of 4 plots totalling 40 acres. An equal area, divided into two plots, served as a control without manure. Fecal samples were directly taken from 60–80 beef cattle each year, and cattle were divided into groups and introduced on pasture plots. Animals were removed or additional *Salmonella* negative cattle were added to the fields in response to vegetation growth. There were 40–109 cattle on manured fields and 16–20 on control fields. The interval between manure application and cattle introduction ranged from 10–30 days. Fecal samples were taken monthly for 3 months. Soil and plant samples were taken before and 1–24 days after manure application. *Salmonella* from 25 g samples were enriched in Tetrathionate and Rappa-

port-Vassiliadis broths, plated on selective agars (HE and XLD), screened with somatic antibodies and serotyped by the National Microbiology Laboratory (Canada). Two thirds and 100% of hog manure samples were *Salmonella* positive each year. Twenty-nine *Salmonella* Krefeld and 17 *Salmonella* Derby isolates were isolated from the hog manure. Six *Salmonella* Typhimurium cultures were isolated from the vegetation up to 9 days after manure application. All isolates of each serovar were the identical serotype. *Salmonella* was not recovered from soil and was absent from the cattle fecal samples for the entire length of the study. During this study, *Salmonella* from hog manure did not appear to have been transferred to cattle grazing on the manure-treated pasture.

P1-68 Growth Inhibitory Effects of Kimchi (Korean Traditional Fermented Vegetable Products) against Foodborne Pathogens

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Kimchi is a unique Korean traditional vegetable fermented by lactic acid bacteria, which is consumed mainly as side dish with boiled rice. The main ingredients are brined Chinese cabbage, red pepper powder, fermented fish sauce with many spices such as garlic, green onion, ginger, and some seaweed. This study was performed to confirm the growth inhibitory effect of kimchi against six foodborne pathogens, *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Salmonella* Typhimurium, and *Vibrio parahaemolyticus* during fermentation. To evaluate the inhibitory effect of lactic acid bacteria (LAB) and pH in kimchi against 6 foodborne bacteria, the samples were heated (85°C, 15 min) and neutralized (pH 7.0) before fermentation (BF) and after fermentation (AF) (pH 4.2) for 2 days at 20°C, respectively. Each foodborne bacterium was spiked into kimchi and then was fermented at 20°C. All pathogens inoculated were counted by relevant selective medium. LAB counts in kimchi were maintained 7-9 log CFU/g during fermentation. During the incubation of kimchi, reductions of at least 1 log of *B. cereus* and 4 log of *L. monocytogenes* were achieved; *E. coli* and *S.* Typhimurium were undetectable after just 2 days of fermentation, *S. aureus* was undetectable after 4 days. *E. coli* and *S.* Typhimurium were not detected after 2 days of fermentation in neutralized BF and heated AF treatment; *S. aureus* was not detected after 4 days of fermentation in neutralized BF treatment. However, in heated BF treatment all kinds of foodborne bacteria tested showed higher growth than that of the control (untreated kimchi). The results show that the growth of LAB in kimchi, an important factor controlling pathogens, depends upon type of microorganisms.

P1-69 Isolation and Survival Characteristics of *Bacillus cereus* in Fermented Hot Pepper-soybean Paste (Kochujang)

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Kochujang, a fermented red pepper-soybean paste, is a popular spicy condiment in Korea and is characterized by a hot, sweet, and savory taste. Presumptive *Bacillus cereus* was detected in kochujang at the level of 5 log CFU/g. To isolate the proper level of *B. cereus* strains in kochujang, the primary dilution (10^{-1}) was heated for 5 min at 85°C. Voges-proskauer (VP) positive colony named as KBC and VP negative colony named as KBM strain were isolated. KBC and KBM strains were clearly determined to be *B. cereus* and *B. mycoides* with biochemical tests and 16S rDNA sequencing. D-values ranged from 45.05 (D_{85}) to 8.37 (D_{100}) min for strain KBC and from 39.82 (D_{85}) to 7.08 (D_{100}) min for strain KBM, and z-values were calculated to be 20.53°C (KBC) and 18.55°C (KBM), respectively. *B. cereus* (strain KBC) spores were artificially inoculated into kochujang at the level of 10^4–10^5spores/g. No significant difference ($P < 0.05$) was noticed in the number of *B. cereus* at each treatment from initial day to 60 days at 30°C during fermentation period of kochujang. From these results we can conclude that the *B. cereus* in kochujang does not grow during storage. In addition, when kochujang inoculated with 10^4 spores/g was heated for 15 min at 85°C, the number of *B. cereus* decreased slightly than that of unheated kochujang, but was not severely affected by the heat treatment. Therefore, we suggest that the above heat treatment (85°C, 15 min) was a useful method to isolate *B. cereus* present in low levels from kochujang.

P1-70 Moulds, Yeasts and Aerobic Plate Counts in Various Herbal Teas and Coffee Substitutes

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Various herbal teas including German chamomile, chrysanthemum Vascuflow herb tea, hop, jasmine and orange flowers, sweet marjoram, spearmint and thyme leaves, and papaya-mint tea as well as coffee substitutes (Bambu instant Swiss, Teeccino chocolate-mint, and Teeccino Mediterranean Espresso) were analyzed for fungal contamination and the presence of aerobic mesophilic bacteria (APC). The results of this investigation showed that mould and yeast (MY) counts reached levels as high as 5.8×10^5 colony forming units (CFU) per gram. The highest MY contamination was observed in German chamomile. A variety of microfungi including members of the toxigenic genera *Aspergillus, Alternaria, Eurotium, Fusarium* and *Penicillium* were isolated. *Aspergillus niger, Penicillium* spp., *eurotia* (*E. rubrum* and *E. chevalieri*), *A. flavus, Fusarium* spp. and *Alternaria alternata* were the most common moulds recovered from the analyzed samples. Less common were *A. carbonarius, A. ochraceus, A. versicolor, Ulocladium, Phoma* and *Rhizopus* spp. Yeasts were found in all herbal teas (except jasmine flowers and thyme leaves). Among the coffee substitutes, only the chocolate-mint coffee was contaminated with fungi. Low numbers ($<1.0 \times 10^3$ CFU/g) of *Eurotium rubrum* and *Ulocladium* spp. were isolated from 17% and *Phoma* spp. were recovered from 33% of the samples. Yeasts were found in 67% of the chocolate-mint coffee substitute samples in numbers reaching as high as 6.8×10^3. Aerobic mesophilic bacteria were recovered from 100% of the herbal tea, chocolate-mint and Mediterranean Espresso, and from 50% of the Bambu instant Swiss coffee samples. The highest APC counts of 1.2×10^7 CFU/g were observed in spearmint leaves and the lowest (<100 to 1.0×10^3 CFU/g) were found in Bambu instant Swiss coffee substitute.

P1-71 Isolation and Growth Pattern of Foodborne Pathogenic Bacteria from Seafoods and Korean Packaged Meals in South Korea

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The contamination frequency of major foodborne pathogenic bacteria was investigated in 96 seafood samples (sliced raw fish and shellfish) and 114 Korean packaged meals (kimbap, hamburger, dosirak, and sandwiches). Samples were analyzed for *Staphylococcus* aureus, *Salmonella* spp., *Vibrio parahaemolyticus, Escherichia coli* O157:H7, and *Listeria monocytogenes. S. aureus* and *V. para-*

haemolyticus were isolated from 11 (11.5%) and 8 (8.3%) of the seafood samples, respectively. *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 were not detected in the seafood samples. *S. aureus* was isolated from 12 (10.5%) of the packaged meal samples, no other pathogens were detected. The isolation rates by large and small establishments were 13.3% and 16.5%, respectively, not a significant difference. Total isolation rates by average temperature were 2.5% under 15°C, 18.8% at 16–20°C, and 23.5% at 21–27°C. As *S. aureus* and *V. parahaemolyticus* were closely related to temperature and time, temperature control under 10°C appeared to reduce the risk of food poisoning by these bacteria in seafood and Korean packaged meals.

P2-01 Evaluation of the Detection of Microbial Contamination in UHT Dairy Beverages Comparing the Standard 7-day Plating Method to a Rapid Method Utilizing the BacT/ALERT® Microbial Detection System

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Assessing the quality of aseptically processed foods involves sensory evaluations, measurement of specific parameters (e.g., pH), prolonged holding times (5–7 days) or combinations of these methods. A study was conducted comparing the standard 7 day plating method with shorter pre-incubation holding times (0 – 72 h) by monitoring contamination of a 10 mL sample using the BacT/ALERT® Microbial Detection system, a growth-based test method used to determine the presence or absence of microorganisms based on CO_2 production. Ultra High Temperature (UHT) milkshake beverages in 3 flavors were purchased from local grocery stores and inoculated with low levels (0.08 to 4.6 CFU/mL) of *Pseudomonas* fluorescens ATCC® 13525, *Staphylococcus epidermidis* ATCC 14990 and *Bacillus cereus* ATCC 10876. Samples were tested in triplicate at time zero or after pre-incubation at 32–35°C for 24, 48 and 72 h. For two of the flavors tested all 90 of the uninoculated samples were negative by both the 7 day standard testing method and the alternate rapid method. The third product contained a natural *Bacillus* contaminant, which resulted in 10 BacT/ALERT-positive samples in the uninoculated product controls (45 total), whereas only 1 was postive by the standard plating method. BacT/ALERT detection times for the naturally contaminated product ranged from 13.4 to 47.8 h. In the artificially contaminated samples, all samples were positive in the BacT/ALERT (13.0 ± 0.3, or 12 ± 0.6, 5.0 ± 0.9 h) following a 24-h pre-incubation. Samples tested at time zero generally took 12–26 h to flag positive by the BacT/ALERT and one replicate of the time zero test sets did not result in a positive in the BacT/ALERT. This sample was confirmed culture negative, suggesting that the organism was not present in the 10 mL tested. The pre-incubation beyond 48 h offered no significant time to detection advantage in the BacT/ALERT samples. The data demonstrated that by incorporating a larger 10 mL sample volume and the direct measurement of CO_2 using the BacT/ALERT Microbial Detection System it is possible to screen for bacterial contamination in UHT dairy beverages with a reduced pre-incubation of less than 48 h.

P2-02 Detection and Characterization of *Listeria monocytogenes* in São Jorge Cheesemaking Milk, Whey, Curd, and Cheese Via Phenotypic and Genotypic Methods

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Listeria monocytogenes is a Gram-positive opportunistic pathogen, often found in many natural and manmade environments. This pathogen causes foodborne infections, and may lead to miscarriage in pregnant woman. There is, therefore, great concern on the part of public health authorities on this issue as a wide range of food products, including dairy products, have been associated with both outbreaks and sporadic cases. In addition, European rules have become more strict toward enforcement of safety measures encompassing all food products and accordingly recommend application of GMP and HACCP. São Jorge, an Appélation d'Origine Protegé cheese in Azores, represents approximately 51% of the overall AOP cheese production in Portugal. It is manufactured on an small dairy farms, from raw cow's milk in the absence of any commercial starter. This study was designed to estimate occurrence of *L. monocytogenes* throughout the São Jorge cheese production chain. The ultimate goal was designing specific strategies, targeted at reducing the microbial viable load of this pathogen in the finished cheese. Three hundred thirty-five samples of raw milk, natural whey starter, curd and cheese along ripening, representative of the whole production chain of seven independent dairies, were collected over one year and assayed for *L. monocytogenes* and selected physicochemical parameters (pH, moisture and salt). While neither *L. monocytogenes* nor other *Listeria* spp. for that matter were detected in whey starter, curd or cheese samples, two of the 104 (1.9%) samples of raw milk tested positive for *L. monocytogenes*. These two raw milk isolates represented the same ribotype that has previously been reported to be the cause of multiple human listeriosis outbreaks and cases elsewhere. On average, physicochemical parameters of cheeses after 4 months of ripening presented values that likely minimize the risk of *L. monocytogenes* outgrowth during posterior storage and consumption. Our results indicate that raw milk used for São Jorge cheese manufacture, as well as the ripened product, are rarely contaminated with *L. monocytogenes*. However, there is room for improvement of the current manufacturing protocols so as to guarantee a safer final cheese.

P2-03 *Mycobacterium avium* subsp. *paratuberculosis* Prevalence Studies in Bulk Tank Raw Milk and Slaughtered Healthy Dairy Cows in Switzerland Using an F57 Sequence-based Real-time PCR Assay

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Mycobacterium avium subsp. *paratuberculosis* infections are prevalent and cause significant economic losses in dairy and beef cattle herds around the world. Moreover, an unresolved potential link of *M. avium* subsp. *paratuberculosis* to Crohn's disease has also raised some public health concerns in view of foodborne transmissions through dairy and beef products. We have applied a light cycler-based real-time PCR assay targeting the F57 sequence to monitor the prevalence of *M. avium* subsp. *paratuberculosis* in Swiss bulk tank raw milk samples and slaughtered dairy cattle. This assay had minimum detection limit of 10–100 cells per ml in milk spiked with *M. avium* subsp. *paratuberculosis* organisms. In feces the detection limit was approximately 10–100 cells in 200 mg of a spiked sample. Among the 100 bulk tank milk samples tested, we found 3 samples (3%) that were positive for *M. avium* subsp. *paratuberculosis*. In the slaughterhouse part of the study 8.9% (9/101) of the cows were positive for *M. avium* subsp. *paratuberculosis* DNA in fecal samples, 4.9% (5/101) in mesenteric lymph nodes, 0.9% (1/101) in ileum tissue and 3.6% (3/84) were positive in milk. Meanwhile in

2.9% (3/101) of the culled cows, *M. avium* subsp. *paratuberculosis* DNA was detected in samples of diaphragmatic muscles. This study reports the first set of prevalence data on *M. avium* subsp. *paratuberculosis* in Switzerland that are based on a F57 sequence targeting PCR system. This study confirms DNA-based evidence for *M. avium* subsp. *paratuberculosis* presence in both bulk tank raw milk and slaughtered healthy dairy cattle. These findings may be of relevance in view of milk and beef products as potential vehicles of *M. avium* subsp. *paratuberculosis* transmission to humans in the context of unresolved potential links of these microorganisms to some cases of human Crohn's disease.

P2-04 Combinations of Pasteurization Treatments and Hydrogen Peroxide to Inactivate Bacterial Spores in Milk and Dairy Products

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The objective of this study was to identify a method to inactivate *Bacillus cereus* spores in whole milk by treating with 0, 0.05, 0.1, 0.25, and 0.5% (v/v) hydrogen peroxide before high-temperature, short-time (HTST; 72 or 78°C, 15 s, lab-scale pasteurizer) or low-temperature, long-time (LTLT; 63 or 72°C, 30 min, vat) pasteurization. For LTLT treatments, survival was also evaluated during production of Cheddar cheese and whey, and in fluid milk stored at 4°C for 28 days or 13°C for 7 days. H_2O_2 was neutralized with catalase immediately after pasteurization and before enumeration of *B. cereus* on Mannitol-Egg Yolk Polymyxin agar. Data revealed that *B. cereus* populations remained unchanged in HTST pasteurized milk pretreated with </= 0.5% H_2O_2. In contrast, LTLT pasteurization at 63°C for 30 min inactivated 4.7, 3.7, and 0.6-log *B. cereus* in milk treated with 0.5, 0.25, and 0.1% H_2O_2, respectively. When heated at 72°C for 30 min, viable spores decreased > 4-log CFU/mL in 0.5 or 0.25% H_2O_2-treatments and 2.1-log in milk treated with 0.1% H_2O_2. No changes in *B. cereus* populations were noted for control milk or milk treated with 0.05% H_2O_2, regardless of the pasteurization temperature. Populations remained constant in all fluid milk stored 28 days at 4°C, but increased 1-log CFU/ml in milk treated with 0 or 0.05% H_2O_2 and stored at 13°C for 7 days. Differences in populations were also observed during cheese making, with an average of 1 to 2-log fewer spores recovered from cheese curd made with 0.1% H_2O_2-treated LTLT milk than for the control without H_2O_2. However, no significant difference in survival was observed in the whey derived from the different H_2O_2 treatments. This study demonstrated that a combination of LTLT pasteurization at 63°C and H_2O_2 decreases *B. cereus* viability in milk. This procedure may be used an intervention strategy if the presence of exceptionally high levels of spores are suspected in milk.

P2-05 Determination of Classical and Newly Defined Staphylococcal Enterotoxin Genes from Bovine Raw Milk in Korea

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Staphylococcal enterotoxin, a superantigen produced by *Staphylococcus aureus*, is one of the most prevalent etiological factors in human food poisoning. In addition to the classical staphylococcal enterotoxins (SEA to SEE), new types of staphylococcal enterotoxins (SEG to SElU) have been reported, but there are few studies investigating these newly described staphylococcal enterotoxins in Korea. The prevalence of enterotoxigenic strains in bovine milk samples was investigated by using multiplex PCR specific for 17 SE genes (*sea* to *selr*) and *tst-1* gene encoding staphylococcal toxic shock syndrome toxin-1. A total of 174 *S. aureus* were isolated from 5,640 bovine milk samples showing somatic cell counts > 50×10^4 somatic cells/ml from 63 dairy farms in 9 provinces in Korea during a three-year time period (2003–2005). Among isolates, 116 (61%) isolates harbored one or more toxin genes. The most common SE genes were *seg* (34%), *sei* (33%), *selm* (32%), and *selo* (34%) and the four genes were mostly found in the form of combination, a part of egc cluster (*seg, sei, selm,* and *selo* lacking *seln*). Among the classical staphylococcal enterotoxin genes, sea was most frequently detected (12%), followed by *sed* (9%), and *seb* (2%). The isolates having classical staphylococcal enterotoxin genes also possessed the newly described SE genes. Other staphylococcal enterotoxin genes were also found from the isolates (4–15%) but none of the isolates had *sec*, see, and *selp*. Although it is known that SEC producing *S. aureus* is associated with bovine mastitis by suppression of cellular immunity in the mammary gland, none of the isolates harbored *sec* gene in this study while the isolates with new staphylococcal enterotoxin genes, especially *egc* cluster, were widely distributed among the dairy farms in Korea. The association of the newly defined staphylococcal enterotoxin genes and bovine mastitis should be examined in further study.

P2-06 Effects of Cool Water Washing of Shell Eggs on Pathogen Detection

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A commercial study was conducted to determine the effect of cool water washing on shell-egg temperature and pathogen detection. Eggs were processed on a single day for three consecutive weeks at both an inline and offline facility. Inline facilities consist of laying houses which are directly connected to the processing facility through a series of belts. Offline facilities require the transport of eggs from off-site laying houses to the processing facility. Three temperature schemes were utilized in commercial dual washer systems: HH = 48.9°C, 48.9°C; HC = 48.9°C, 23.9°C; and CC = 23.9°C, 23.9°C. HH eggs maintained the highest surface temperature (26.25°C inline, 20.25°C offline, and 23.25°C combined, $P < 0.05$). The lowest temperatures were found in the CC eggs (21.25°C inline, 17.25°C offline, and 19.25°C combined). The frequency of *Enterobacteriaceae* detection in shell and membrane emulsions was greatest for CC eggs ($P < 0.05$ for inline and combined). There was no difference in *Enterobacteriaceae* detection for the offline facility. *Salmonella* was detected in 3 of 384 samples from the inline facility. The isolates were found in HC (2) and CC (1) shell emulsions. Two of 384 samples were positive for *Campylobacter* from the inline facility (CC). Three wash water samples were positive for *Listeria* in the offline facility (1 HC, 2 CC). No pathogens were detected in egg contents during this study. The results of this study indicate that warm followed by cool water washing has the potential of decreasing egg temperature while maintaining surface microbiology at an acceptable level.

P2-07 Effect of the Lactoperoxidase System on *Listeria monocytogenes* in Goat Milk and Goat Milk Cottage Cheese

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In developing countries, with high ambient temperatures, milk is transported unrefrigerated to processing plants. The delay between milking and processing can exceed five hours and this can contribute to poor quality and unsafe dairy products. An alternative process that can address this problem is the lactoperoxidase system in milk (LPS). In this study the effect of LPS alone and in combination with pasteurization on the growth of *Listeria monocytogenes* in raw goat milk and goat milk cottage cheese was determined. Goat milk samples were obtained from the University of Pretoria. Milk samples were taken for microbial and chemical analysis. The milk was divided into two, A and B, samples and then subjected to the activation of LPS by addition of sodium thiocyanate. After one minute of thorough mixing 30 mg/l sodium percarbonate was added as a source of H_2O_2. Samples A and B were then again divided into two, A1 and A2 and B1 and B2. A2 and B2 were pasteurized at 72°C for 15 s and A1 and B1 were not pasteurized. Cottage cheese was manufactured and stored at 4°C for 10 days. Samples were analyzed for *Listeria monocytogenes* and aerobic plate count (APC) on days 0, 1, 2, 5, and 10. After 6 h of LP activation there was no difference in *Listeria monocytogenes* counts between the LP milk and the control. However, after heat treatment the *Listeria monocytogenes* counts were lower in the LP activated milk than in the control. *Listeria monocytogenes* counts decreased most in the heat-treated and LP activated goat milk cottage cheese after 10 days at 4°C. Finally, a combination of LP and heat reduced *Listeria monocytogenes* in both goat milk and goat milk cottage cheese more than other treatments.

P2-08 Prevalence and Types of *Listeria monocytogenes* in Queso Fresco Cheese Processed in Sonora, Mexico

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Queso fresco (QF) is arguably the most popular Hispanic-style cheese consumed in Mexico. However, because it is often produced by small processors using raw milk, QF provides a favorable environment for the presence/growth of listeriae and serves as a common vehicle for listeriosis. The objective of this study was to determine the prevalence and types of *L. monocytogenes* in QF manufactured in Sonora, Mexico. We examined 101 QF samples that were obtained from 24 processors located in the northern, central, and southern regions of Sonora. For each cheese sample, a PCR-based and an enrichment/culture-based method were used to detect/recover *L. monocytogenes*. Isolates were characterized by serotyping, PFGE, and ribotyping. The pathogen was detected in 19 cheese samples by use of PCR and in eight samples by use of the enrichment/culture method. A total of 14 isolates were obtained from the eight cheese samples from which the pathogen was recovered by the enrichment/culture method. Ten of the isolates were recovered from six processors in northern Sonora and four of the isolates were recovered from two processors in southern Sonora. All of the isolates were serotype 4b, and ribotyping revealed that ten isolates were ribotype DUP1038 and four isolates were ribotype DUP1042. Additionally, PFGE analysis showed that the same 14 isolates associated into eight distinct, but similar, *XbaI* pulsotypes. This study established the prevalence of *L. monocytogenes* in QF processed in northern Mexico at between 8 to 19%. Additionally, each isolate was serotype 4b, the serotype that causes most foodborne outbreaks, and displayed two ribotypes that have been implicated in a number of foodborne outbreaks. These results emphasize the need to conduct additional studies to examine the impact that processing has on the prevalence of *L. monocytogenes* in this variety of cheese and to develop interventions that will reduce and/or eliminate the pathogen.

P2-09 Introduction of Lemon Juice into the Production of Wara, a West African Soft Cheese

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Wara, an important protein source for West African consumers, has a short shelf life of 2–3 days. Lemon juice was used in this study as a substitute coagulant during wara processing. The cheese was processed from pasteurized milk inoculated with 10^2 or 10^4 CFU/ml of *Listeria monocytogenes*, and sampled throughout processing and during a five-day storage period at 15 and 28°C for populations of *L. monocytogenes*, total aerobes, *Enterobacteriaceae*, psychrotrophs, as well as mold and yeast. On the fourth storage day, portions of the cheese were fried in vegetable oil, mimicking the practice of western Nigerian cheese processors. The results indicated that *L. monocytogenes* did not survive the processing of wara. In samples initially inoculated with 10^2 CFU/ml of *L. monocytogenes*, the *Enterobacteriaceae* counts increased from 1.47 log CFU/g on the first day to 3.20 log CFU/g at 28°C and 3.00 log CFU/g at 15°C by the 3rd day of storage. The counts dropped to undetectable levels from the 4th day of storage at both storage temperatures. Aerobic plate counts increased from 1.88 log CFU/g on the first day to 7.68 and 6.38 log CFU/g, respectively, on the 5th day of storage at 28 or 15°C. Psychrotrophic counts increased from 1.78 log CFU/g on the first day to 8.67 log CFU/g at 28°C and 8.56 log CFU/g at 15°C by the end of storage. The levels of mold and yeast in the 5-day old cheese increased to 10.06 and 8.73 log CFU/g, respectively, at 28° or 15°C from the initial undetectable levels. A similar trend was observed in samples with an initial *Listeria* inoculation level of 10^4 CFU/ml. While storage temperatures had a significant effect on the four types of microorganisms sampled, frying considerably reduced the populations of total aerobes as well as mold and yeast. The study suggests that lemon juice could be used as an effective coagulant during wara processing.

P2-10 Prevalence and Antibiotic Resistance of *Salmonella* Isolates Recovered from Finishing Swine Herds and Slaughter Facilities in Southern Brazil

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The objective of this investigation was to determine the distribution and types of *Salmonella* in 12 swine finishing herds and a slaughter facility in Santa Catarina, Brazil, during 2004. A total of 1,258 samples were colleted and pre-enriched in buffered peptone water, after which each sample was divided into two portions and enriched in both Tetrathionate and Rappaport-Vassiliadis broth. Following enrichment, a portion from each broth was plated onto Brilliant-Green and XLT4 selective agars. As appropriate, one to four isolates were selected, biochemically confirmed, and serotyped. From 487 positive samples, a total of 1,255 isolates were recovered and

confirmed as *Salmonella*. The distribution of positive samples were as follows: finishing pen floors 26% (16/61); feed 29% (42/143); feces 44% (52/119); pooled feces 59% (35/59); slaughter holding pens 90% (36/40); lymph nodes 46% (221/478); pre-chilled carcass surfaces 24% (24/98); and post-chilled carcass surfaces 24% (62/260). The most prevalent serotypes were Typhimurium, Panama, Senftenberg, Derby, and Mbandaka. The antibiotic resistance profiles against 15 antibiotics were determined for 582 of the isolates. Results demonstrated that there were 62 different profiles among 582 isolates, 17% (98/582) of isolates were susceptible to all 15 antibiotics tested, 83% (484/582) were resistant to at least one antibiotic, and 43% (248/582) of the isolates were resistant to four or more (multi-resistant). The antibiotics to which resistance was observed most often were: tetracycline 79% (459/582); ampicillin 46% (266/582); kanamycin 40% (234/582); gentamicin 38% (221/582); streptomycin 35% (206/582); and sulfamethoxazole/sulfizoxazole 24% (138/582). Results of this study demonstrate that *Salmonella*, including multi-antibiotic-resistant strains, are distributed widely over the swine production and slaughter system in Brazil. Furthermore, these findings are important for the identification of risk factors for *Salmonella* carriage in swine and for the identification of *Salmonella* control points in the pork production chain.

P2-11 DSC Use of Carbon Monoxide Combined with Carbon Dioxide for Modified Atmosphere Packaging of Fresh Pre-rigor Pork Sausage to Improve Shelf Life

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Fresh pork sausage typically has a short shelf life due to color loss, microbial growth, rapid rancidity, and related quality changes. Modified atmosphere packaging (MAP) has potential to reduce oxidative and microbial developments that result in quality loss. The hypothesis for this study was that MAP with 0.4% CO/ 99.6% CO_2 gas combination will provide improved color stability, increased microbial inhibition and decreased rancidity to improve quality and shelf life of fresh pre-rigor pork sausage. Pork sausage patties were manufactured in the Iowa State Meat Laboratory, placed individually in either MAP bags with 0.4% CO and 99.6% CO_2 or overwrapped with permeable film on foam trays (control), and stored at 2–4°C under fluorescent lights for 1, 3, 6, 8, 10, 13, 15, 17, 20, 22, 24, 27, 29, and 31 days post-packaging. The experiment was replicated three times and analyzed with standard statistical methods (SAS 9.1). Total plate counts, anaerobic microorganisms, and psychrotrophic microorganisms were enumerated using standard microbiology methods (Vanderzant and Splittstoesser, 2001). Quality measurements of the pork sausage included raw and cooked color (Hunter L*a*b* Scan), purge and lipid oxidation using the TBA (2-thiobarbituric acid) procedure (Tarladgis, 1960). Results indicated that MAP packaging significantly reduced the total microbial load from days 6–22 ($P < 0.05$) and reduced the psychrotrophic and anaerobic bacteria from days 13–22 ($P < 0.05$) in comparison to the controls. Color retention was increased by 7 days ($P < 0.05$) and lipid oxidation was reduced ($P < 0.05$) for MAP packaging relative to controls. A potential disadvantage of MAP was increased purge observed during the storage period. In conclusion, MAP, with a combination of CO and CO_2, extended pre-rigor pork sausage shelf life by reducing microbial growth and improving quality attributes, particularly color, compared to conventional over-wrap packaging.

P2-12 DSC Validation of Heat Acid Coagulated Fresh Hispanic Cheese Manufacture Process to Achieve a 5-log Reduction of *Listeria monocytogenes* and *Escherichia coli* O157:H7

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Currently the Food and Drug Administration (FDA) requires the use of pasteurized milk for cheeses aged less than 60 days. However, some cheeses undergo a heat treatment equivalent to or higher than pasteurization during their manufacture. This study measured the thermal inactivation of *Listeria monocytogenes* and *E. coli* O157:H7 during the manufacture of a heat acid coagulated fresh Hispanic cheese (Queso Blanco). Cheese was made from Ultra High Temperature (UHT) milk inoculated with *L. monocytogenes* (DUP 1053 serotype 1/2a, 1044 serotype 4b) and incubated at 35°C for 24 h to achieve approximately 8 log CFU/ml. In other trials milk was inoculated with an acid tolerant clinical strain of *Escherichia coli* O157:H7 to achieve approximately 8 log CFU/ml. *L. monocytogenes* strains (DUP 1044 and 1053) had a $_{D60°C}$ = 0.47 min and 0.59 min with a Z-value of 3.71°C and 4.72°C respectively. *E. coli* O157:H7 had a $_{D60°C}$ = 0.75 min with a Z-value of 4.06°C. Laboratory scale cheese was manufactured following a typical time/temperature heat profile used during pilot plant manufacture. Samples were removed at various time/temperatures and enumerated on 3M™ Aerobic Count petrifilm. Milk temperature was raised from cold storage to 85°C over approximately 30 min and held for 5 min. This process resulted in a 5-log reduction of *L. monocytogenes* and *E. coli* O157:H7 by 65°C (approximately 13 min). Cells were undetectable long before the additional hurdles of acidification and salt addition. Use of a heat acid coagulated manufacturing process (Queso Blanco) provides safety equivalent with use of pasteurized milk. Standards of identity in the Code of Federal Regulation (21 CFR 133) should be revised in order to include a category for heat acid coagulated cheeses taking into consideration validated process lethality from the manufacture process.

P2-13 DSC The 60-day Aging Requirement Does Not Ensure Safety of Bloomy Rind Cheese Manufactured from Raw or Pasteurized Milk when *Listeria monocytogenes* are Introduced as Post-processing Contaminants

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The Code of Federal Regulations (21CFR133.182) permits manufacture of soft ripened cheeses from raw milk provided that these cheeses are aged for 60 days at ≥ 1.67°C (35°F). Due to renewed interest in specialty cheeses, artisan and farmstead producers are manufacturing soft ripened cheeses from raw milk, using the 60-day holding standard to achieve safety. This study compared the survival of *L. monocytogenes* on cheeses manufactured from raw or pasteurized milk and held for ≥ 60 days at 4°C. Final cheeses were within Federal Standards of Identity for soft ripened cheese, with low moisture targets to facilitate the holding period. After brining and drying, wheels were surface inoculated with a 5 strain cocktail of *L. monocytogenes* to contain approximately 0.2 CFU/g (low level) or 2 CFU/g (high level) and ripened for 12 days at 14°C +/- 1°C at 90 +/- 2% RH to allow proper surface flora growth. Wheels were then wrapped in microperforated paper and held at 4°C +/- 1°C for 58 days. Weekly, duplicate 1-cm deep surface samples (100 g) were removed, diluted, surface plated on CHROMagar *Listeria* selective agar, incubated and enumerated. After an initial decline to undetectable levels, growth initiated at day 28 for all treatments, reaching on average 2.58 log CFU/g and 4.51 log CFU/g by 70 days for low

and high level inoculations respectively. No significant differences ($P < 0.05$) were observed in pH development, growth rate, or population levels between milk types. Lower moisture soft ripened cheeses held for 60 days supported extensive growth from very low levels of *L. monocytogenes* introduced as post-process contaminants independent of the milk type used for manufacture. The safety of cheeses within this category must be achieved through control strategies other than a 60-day holding period and revision of current federal regulations are warranted.

P2-14 Effect of Cooling Rate on Pathogen Survival in Yogurt

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The effect of cooling rate on the survival of pathogens was compared in full-fat and nonfat yogurt with strawberry fruit preparation added (final pH 4.4). Products were inoculated with 4.5-log CFU/g of acid-adapted *Listeria monocytogenes*, *Salmonella* sp., or *Escherichia coli* O157:H7 and dispensed into yogurt cups. One set of packages was immediately chilled to 7°C (US Pasteurized Milk Ordinance cooling requirement), whereas a parallel set of packages was cooled from 27°C to 7°C within 96 h (typical industry cooling procedures) and then stored at 7°C for the duration of the study. Triplicate samples of each treatment were enumerated by plating on appropriate selective agar at 0, 1, 2, 3, 4, 7, 10, and 14 days storage. In yogurt cooled slowly according to typical industry procedures, populations decreased 1.3 to 1.7-logs for *Listeria*, 2.2 to 3.0-logs for *Salmonella*, and 0.8-log for *E. coli*, with the greater decrease seen in the nonfat yogurt compared with full-fat varieties. In contrast, log reductions in yogurt chilled immediately to 7°C were 0.5 for *Listeria*, 1.7 to 1.9 for *Salmonella*, and 0.2 to 0.3-log for *E. coli*, with the greater decrease observed in the full-fat yogurt. The pH decreased in all yogurt treatments during the 2-week storage, but the decrease was more rapid in the yogurt cooled slowly (final pH 4.1) than in samples chilled to 7°C immediately after inoculation (final pH 4.2). Results from this study agree with other studies that demonstrate greater inactivation of pathogens in yogurt when cells are stressed at elevated temperatures under acid conditions (pH 4.4). These data support the safety implications of filling yogurt with active cultures at 27°C followed by cooling to < 7°C within 96 h. To ensure a safe product to the consumer, manufacturers should also comply with the described processing parameters, good manufacturing practices, and environmental controls.

P2-15 An Examination of the Relationships between Foodborne Pathogens and Implicated Food Vehicles

DSC

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An outbreak database, created to archive foodborne and waterborne outbreaks, was used to examine the relationships between foodborne pathogens and the food categories they contaminate. Information gathered between the years of 1998 and 2004 was used for this report, which included 1,409 outbreaks and 65,574 confirmed cases of illness. This report examines the pathogens associated with each food category as well as why that pathogen has an affinity for that food item. *Salmonella*, *Escherichia coli*, *Clostridium perfringens*, norovirus and *Listeria monocytogenes* were the main pathogens examined. *Salmonella* was associated with 99% of all outbreaks in the "eggs" category, while 67% of all "beef" outbreaks were attributed to *E. coli*. Norovirus was implicated in 37% of the outbreaks in the "beverages" category. *L. monocytogenes* had the fewest reported outbreaks (28) and 43% of these involved dairy products. The "produce" category had the largest number of outbreaks in the database, namely *Salmonella* contaminating melons, sprouts and lettuce. Although the "chicken" category is often recognized by consumers to be one of the most common sources of foodborne illness, examination of data suggests otherwise. Categories such as "beef", "eggs" and "multi-ingredient foods" have a higher number of reported outbreaks. In this report, associations between foods and pathogens are highlighted in an attempt to identify foods that are of higher risk for contamination by certain pathogens. These relationships suggest that pathogens have a tendency to contaminate certain foods often and others rarely. Establishing the relationships between pathogens and foods, where contamination occurred, and factors that allowed survival and proliferation of the pathogens, are vital steps in prevention of future outbreaks of foodborne illness.

P2-16 Ecology and Transmission of *Bacillus* and Related Sporeformers Present in Dairy Production Systems

DSC

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Bacillus species and related sporeformers (e.g., *Paenibacillus* spp.) are important spoilage organisms in various sectors of the food industry, including dairy processing. The ability of these thermoduric microorganisms to survive high-temperature-short-time pasteurization makes their presence in raw milk a major potential cause of milk spoilage. Applying a recently developed *rpoB* subtyping method to track *Bacillus* and related sporeformers through two New York State (NYS) fluid-milk processing plants, from raw receiving tanks to packaged products, we have gained insight into the ecology and transmission of these microbial contaminants. Eighty-two raw and pasteurized milk samples were collected over three consecutive weeks from 2% milk production runs at 2 NYS fluid-milk plants currently achieving shelf lives >14 days, yet unable to attain code dates past 18 or 21 days, respectively. Fourteen raw and 32 pasteurized in-line sampling sites were included in this sampling, to assess potential in-process contamination. Heat-treated (80°C for 12 min) raw milks and pasteurized samples were held at 6°C and plated systematically throughout storage. End of shelf life (Day 18 or 21) plate counts (PC) ranged from 2300 to > 70,000,000 CFU/mL. Fifteen hundred representative colony-types were isolated from PC's over shelf life and 379 isolates from strategic sites were subsequently subtyped. Our results indicate the presence of 93 *rpoB* allelic types, at least 4 of which can be tracked from raw receiving tanks, through pasteurization to the packaged product. The persistence and transmission of these thermoduric spoilage organisms suggests the need to improve our understanding of the ecology of psychrotolerant *Bacillus* species and related sporeformers throughout the dairy production chain, especially in raw milk. This new information will further identification and elimination of bacterial niches that harbor these spoilage organisms, hence reducing the prevalence of milk contamination and improving product quality and shelf lives.

P2-17 Diversity of Bacterial Communities Associated with Cold-water Dispenser Systems

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Bacteria exist in water distribution systems as polymicrobial communities, the formation of which are known to affect the microbiological composition of the water. Little is currently known about the dynamics and diversity of these populations and the quantitative effect they have on the bacteriological quality of water. In this study, water samples were taken from a variety of water coolers: mains-fed, bottle-fed and traditional vending-machine (from a university campus and office block) and a laboratory model system. Samples were analyzed using molecular (RAPD-PCR, ARDRA-PCR, ITS-PCR) and traditional microbiological techniques. Outlet plate counts varied from 3.8 log (vending machine) to 5.6 log (mains-fed dispenser) CFU/ml. Inlet plate counts varied from 1.9 (model system) to 3.8 (office mains) CFU/ml. The inlet was found to be significantly different from the outlet ($T < 0.005$) in three out of four systems, with the greatest difference being within the model system (3.2 1og CFU/ml). RAPD analysis on individual isolates revealed each dispenser possessed its own distinctive bacterial population at the strain level, with evidence of a dominant strain in some cases. Marked differences were observed between the inlet and outlet in each case. Community profiles showed species, though not strains, being present across dispensers. This research shows that the biofilm associated with the tubing within a water dispenser is a significant contributory factor in the bacteriological quality in dispensed water. The difference between machines on the same site suggests that the environment may play an important part in determining the bacteriological constituent of water rather than the inlet population or operator contamination. The presence of a dominant member may imply a selection pressure or structuring principle determining community composition.

P2-18 Antimicrobial Resistance of *Staphylococcus aureus*, *Streptococcus* spp. and *Enterococcus* spp. Isolated from Bovine Milk in Korea

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Bovine mastitis is one of the most costly diseases in the dairy industry. *Staphylococcus aureus* and *Streptococcus* spp. are major pathogens of bovine mastitis and *Enterococcus* spp. are frequently isolated from raw milk, though they are not the major microorganism causing bovine mastitis. The antimicrobial susceptibility was determined by disc diffusion method for *S. aureus* and broth dilution method for *Streptococcus* spp. and *Enterococcus* spp. in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. A total of 33,595 raw milk samples were collected from dairy cows of 385 dairy farms in Korea from 2003 to 2005. Among them, 178 *S. aureus*, 5 *S. uberis*, 2 *S. dysgalactiae*, and 77 *Enterococcus* spp. were isolated from 5,640 milk samples ($\geq 5 \times 10^5$ somatic cells/ml) from 63 dairy farms and the isolates were also determined by PCR specific to each microorganism. The result showed that 21%, 52%, and 56% of *S. aureus* were resistant to gentamycin, ampicillin, and penicillin, respectively. However, all isolates were susceptible to imipenem and more than 90% to the other antimicrobials. Two *S. dysgalactiae* isolates were susceptible to all antimicrobials used in this study. Unlike *S. dysgalactiae*, 5 *S. uberis* isolates showed resistance to penicillin, erythromycin, and tetracycline. *Enterococcus* spp. showed resistance to gentamycin, chloramphenicol, streptomycin, and tetracycline, with resistance of 17%, 25%, 33%, and 92%, respectively, and no resistance to vancomycin.

P2-19 Metabolic Activity of Probiotic Bacteria in Whey Cheese Matrices: Extension of Shelf Life

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Whey cheese matrices have been proposed in the recent past as probiotic carrier. In this research effort, *Lactobacillus* and *Bifidobacterium* strains were able to maintain their viable cell numbers (approximately 10^7 CFU/g) in said food matrices. As a result of bacterial metabolism, sugars are consumed and organic acids are concomitantly produced; such an acidification contributes to extending shelf life and brings about specific organoleptic features. Experimental whey cheese matrices were prepared, using a combination of ovine whey added to 10% ovine or bovine milk, via protein denaturation by heat supplied at 90°C. The resulting matrices were inoculated with *L. acidophilus* strain LAC-1, *L. paracasei* ssp. *paracasei* strain LCS-1 and *B. animalis* strain Bo (at 10%), and stored at 7°C for 20 days. The qualitative and quantitative profiles of sugars and organic acids were determined by HPLC. The viable numbers of contaminating microflora and the inoculated probiotic microflora were monitored accordingly. Lactose was depleted by 20 days of storage in all matrices. Citric, formic and acetic acids developed at low concentrations; lactic acid was present at concentrations of approximately 2 mg/g. Correlation between growth and glycolytic activity, between the feedstock compositions as well as contaminant microflora, was putatively established. Statistically significant differences were also detected.

P2-20 Evolution of Qualitative and Quantitative Profile of Yeasts in (Organic) Ewe's Raw Milk Cheese, According to Feeding Regime and throughout Ripening

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Dairy products offer a unique ecological niche, which constrain ocurrence and activity of specific yeasts. On the other hand, high numbers of yeasts are frequently observed in cheeses produced from raw milk – the origin may be related not only to the quality of milk, but also to the environmental contamination throughout the production and distribution chain. The presence of yeasts in cheeses may contribute positively to their flavor development during the stage of maturation or, conversely, may lead to product spoilage. The main contribution of yeasts during cheese ripening is utilization of lactic acid and reduction of growth of other contaminants, which in turn increase pH and thus favor bacterial growth at the onset of the second stage of cheese ripening. In this study, three batches of cheese were manufactured with organic raw milk produced by ewes fed with various supplement concentrations: No Supplement (NS), High Supplement (HS) and Low Supplement (LS). The LS and HS groups received 200 and 400 g, respectively, of supplement twice a day in the milking parlor; the NS sheep received no supplementation whatsoever, depending solely on dry feed to fulfill their nutritional requirements. Two batches of cheese were produced in parallel by two different techniques, in separate vats,

using milk obtained from a group of animals at Mid Lactation (ML – 8 wk). Each batch of cheese was sampled and analyzed at 4 ripening times (0, 7, 14 and 28 d), so as to monitor microbiological changes throughout maturation via enumeration of yeasts on Potato Dextrose Agar. From each sample, 20 colonies were isolated at random; after purification, they were phenotypically identified. In general, yeasts decreased in viable number by 1–1.5 log CFU in the latest stage of ripening. Differences between batches, associated with animal feeding, were visible mainly in cheese by 7 and 28 days. The results obtained are relevant in attempts to improve the quality of the final product from an upstream perspective, and allowed rationalization of the role of yeasts throughout the chain.

P2-21 Monitoring of Different Microbiological Parameters in Semi-hard Raw Milk Cheese Produced by Bio-farms in Switzerland

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To establish the bases of a microbiological monitoring system for a semi-hard raw milk cheese produced by bio-farms, 46 cheese batches were examined at various stages of production (after 0, 7, 14, 21, 28, 35, 63 and 91 days of ripening). Additionally, 403 bulk-tank milk samples from 11 producers and 37 cheese-dairy milk samples used for the production of these cheese batches were examined. Samples were analyzed (1) quantitatively for total viable count (TVC), *Enterobacteriaceae* and coagulase positive *Staphylococcus* (CPS), and (2) qualitatively for Shiga toxin-producing *E. coli* (STEC) and *Listeria*. TVC median values ranged from 2.7 to 3.7 log CFU/ml in bulk-tank milk samples. During cheese production, TVC median value rose from 3.6 log CFU/ml in cheese-dairy milk samples to 8.0 log CFU/g and remained constant during ripening. The proportion of *Enterobacteriaceae* positive bulk-tank milk samples from the different producers ranged from 22% to 80% and the proportion of CPS positive bulk-tank milk samples from 9% to 100%. In cheese batches, highest *Enterobacteriaceae* and CPS counts were obtained at the 7th day of ripening (median: 3.6 and 4.0 log CFU/g). 78% of *S. aureus* isolated at the 7th day of ripening tested positive for the sea gene. By day 63 of ripening, mean counts of *Enterobacteriaceae* were 2.5 log CFU/g and CPS counts below detection limit in most samples. Moreover, STEC were detected in seven bulk-tank milk samples and in five cheese batches. *Listeria* spp. were not detected in bulk-tank milk samples and cheese batches. In a microbiological monitoring system, examinations for *S. aureus* should be performed on the 7th day of ripening and for *Enterobacteriaceae* after 60 days. In view of product safety, a minimal ripening period of 60 days is necessary for this semi-hard raw milk cheese.

P2-22 Microbiological Contamination of Pig Carcasses at Different Stages of Slaughter in Two EU-approved Abattoirs

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At sequential steps of slaughter (scalding, dehairing, singeing, polishing, trimming, washing, chilling), pig carcasses were examined for aerobic plate counts (APC), *Enterobacteriaceae* and coagulase positive *Staphylococcus* (CPS). After each stage, 100 carcasses from each abattoir were sampled at four sites (neck, belly, back, ham) by swabbing. Before scalding, mean log APCs were > 5.0 log CFU/cm^2 and *Enterobacteriaceae* and CPS were detected on all carcasses. At abattoir A, average APCs and the proportion of *Enterobacteriaceae* positive carcasses were reduced ($P < 0.05$) after scalding (1.8 log CFU/cm^2, 12%), singeing (1.9 log CFU/cm^2, 66%) and chilling (2.3 log CFU/cm^2, 17%), and increased ($P < 0.05$) after dehairing (3.4 log CFU/cm^2, 100%) and polishing (only APCs: 2.9 log CFU/cm^2). Otherwise, the proportion of CPS positive carcasses decreased constantly to < 5% after singeing and remained at this level. At abattoir B, mean APCs and the proportion of *Enterobacteriaceae* and CPS positive carcasses were reduced ($P < 0.05$) after scalding (2.4 log CFU/cm^2, 29%, 20%), polishing (only APC: 3.7 log CFU/cm^2) and chilling (2.6 log CFU/cm^2, 55%, 77%), and increased ($P < 0.05$) after the combined dehairing/singeing process (4.7 log CFU/cm^2, 97%, 100%). At both abattoirs, the neck tended to yield higher results ($P < 0.05$) than the other sites from trimming to chilling. In consequence, at abattoir B, on average higher APCs as well as higher *Enterobacteriaceae* and CPS detection rates and counts were obtained during slaughter. For the implementation of a HACCP based monitoring system in accordance with new EU hygiene legislation, abattoir-specific data on the microbiological contamination of pig carcasses at different stages are required in order to identify process stages contributing to contamination and to establish more effective HACCP based systems.

P2-23 Implementation of Quality Management Systems among Mexican Sausage-making Industries

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Sausage-making is a major segment of the food industry in Mexico. Since domestic and international markets are increasing their concern for food safety, the future of suasage plants may depend on the extent to which these plants are willing to adopt food safety protocols. The objective of the study was to determine the extent to which Mexican sausage-making industries have adopted quality management systems in relation to whether they were locally, regionally, nationally or internationally distributed. A survey instrument was distributed to all 38 registered sausage-making companies, and only 22 (58%) answered it. Over 80% of the companies were from small to medium size, and only 14% indicated having HACCP system fully implemented. In contrast, 9% of them declared no interest in establishing any food safety systems in the near future. ISO 9000 was operating in 27% of the companies. Of their production, 38 and 57% went to nationwide supermarket chains and distributors, respectively. Exports represented over 4% of total production and went mainly to Central America and Puerto Rico, in addition to some fast-food chains that operate in Mexico with similar requirements to those enforced by international trade. Decision of the industries to implement HACCP was mainly based on the internal factors, improvements in product quality and efficiency on processes and of the external factors, customer satisfaction and regulatory requirements. Staff training and motivation were pointed out as the two major restraints faced in HACCP implementation. It was concluded that adoption and awareness of quality management systems such as HACCP are low among Mexican sausage-making companies, this being a weak point in the process of facing increasing demands of domestic and international markets.

P2-24 Migration of *Salmonella* spp. into Whole-muscle Pork Roasts during Marination

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The interior of any intact, whole-muscle food is typically presumed to be sterile. However, marination — a common value-added process to improve meat quality—can introduce bacterial pathogens into roasts, with such cells likely to exhibit enhanced thermal resistance. While current USDA-FSIS performance standards dictate a 6.5-log reduction for *Salmonella* during cooking of pork and other meats, these standards do not account for bacterial migration into roasts during marination with potential survival after cooking. Consequently, the objective of this study was to determine the numbers of *Salmonella* that migrate into whole-muscle pork roasts during marination. A typical marinade containing 80.7% water, 12.6% NaCl, and 6.7% phosphates was inoculated with an 8-strain *Salmonella* cocktail to contain 10^8 CFU/ml. Cylindrically-shaped irradiated whole-muscle boneless pork loin (longissimus dorsi) roasts measuring 12 cm × 8.7 cm × 4.5 cm (0.75 kg each) were submerged in the marinade for 15, 30, or 60 s. After marination, a 1-cm-thick center slice was cut across the muscle fibers, using an electrosurgical unit with a heated cauterizing blade (Surgistat™ II-20, Valleylab™) and sectioned into 1-cm cubes to assess multidirectional migration of *Salmonella*. Samples were diluted 1:10 in 0.1% pep-tone and homogenized for 90 s in a masticator. Surviving salmonellae were enumerated by plating serial dilutions in duplicate on PetrifilmTM aerobic count plates and then incubating at 37°C for 48 h. *Salmonella* populations decreased significantly ($P < 0.0001$) from 5.4 log CFU/g at the surface to 2.6 log CFU/g at the geometric center of the roast, but marination time (15, 30, or 60 s) did not significantly affect the numbers of salmonellae recovered. Given these findings, a presumption of interior sterility may not be valid for marinated products, which might affect the microbial safety of those products.

P2-25 Effect of Beef Physical Structure on *Salmonella* Thermal Inactivation

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Numerous studies have assessed thermal inactivation of *Salmonella* in beef. However, the impact of muscle structure has only recently been considered, with several studies reporting enhanced thermal resistance in whole muscle compared to ground meat. The functional relationship between meat product structure and *Salmonella* thermal resistance has not been reported, and it is not known whether thermal resistance decreases with the degree of grinding. Therefore, the objective of this study was to determine the relationship between thermal resistance of *Salmonella* and degree of grinding (whole muscle, coarse ground, fine ground, and beef puree). Each of the four product types was irradiated to sterility and inoculated with a marinade containing an eight-serovar *Salmonella* cocktail (10^8 CFU/mL). Samples (5.00 ± 0.05 g) were packed into sterile brass tubes (12.7 mm diameter), sealed, and cooked in a water bath at 60°C for 8–11 durations (at 30 s intervals). The internal temperatures of the samples were monitored with a thermocouple. Samples were then plated on Petrifilm™ aerobic count plates to enumerate surviving salmonellae. All samples had the same composition, thermal history, and initial *Salmonella* counts; therefore, differences in thermal resistance were due entirely to the degree of grinding (i.e., product structure). The thermal resistance of *Salmonella* was higher ($P < 0.0001$) in whole muscle (D = 2.66 min) than in the coarse ground (D = 1.16 min), fine ground (D = 1.12 min), and pureed samples (D = 1.23 min). However, no significant difference in thermal resistance was seen between coarse-ground, fine-ground, and pureed samples. Therefore, *Salmonella* thermal inactivation models need to account for whether a product is whole muscle or ground, but not for the degree of grinding.

P2-26 Efficacy of Potassium Sorbate Formulated Antimicrobial Product against *Salmonella* spp. and the Extending Shelf Life on Poultry Carcasses

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This study evaluated the efficacy of a commercial potassium sorbate formulated product at 20% concentration for its ability to reduce the bacterial counts of mesophiles (MAB) and psychrotrophic (PAB) bacteria, in an attempt to extend the shelf life of poultry carcasses and to reduce *Salmonella* from inoculated poultry carcasses. 102 poultry carcasses were obtained from a municipal poultry processing plant. The groups of carcasses were treated with: sprayed water (A), with sprayed product before the chiller (Ba), before and after the chiller (Bd) and no treatment (C). All poultry carcasses were maintained at 1°C and were analyzed at 0, 1, 3, 5 and 7 days post treatment, rinsing with 400 ml of peptonated water (PW) for counts of MAB and PAB as well as *Salmonella*. In the evaluation for *Salmonella*, the poultry carcasses were inoculated immersed in a mix of 5 strains of *Salmonella* rifampicin resistant (10^{6-7} CFU/carcass) and were treated through spraying-inoculation and inoculation-spraying with the commercial product. Other groups of treatments were included, spraying potable water and the control group without any treatment. After treatment, the carcasses were rinsing with PW for counting of rifampicin-resistant *Salmonella* per carcass (log CFU/C). The treatment of the poultry carcasses with the commercial product had a reduction effect on MAB counts of 1 log CFU/C on day 5 and 0.91 log CFU/C on 7 day post treatment in the group Bd ($P < 0.05$). For carcasses sprayed twice (Bd), reductions 5 days post treatment of PAB counts were 2 log CFU/C compared to group C and 1.5 log CFU/C compared to group A. In the *Salmonella* reduction evaluation, the treatment inoculation-spraying (0.91 log CFU/C) was better than spraying-inoculation treatment (0.62 log CFU/C). The commercial potassium sorbate product analyzed could be a good alternative for bacterial reduction on poultry carcasses.

P2-27 Dissemination of *Salmonella* Enteritidis in a Commercial Chicken Production Chain: Phenotypic and Genotypic Characterization

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Salmonella is one of the most important foodborne disease agents all over the world, and chicken is recognized as an important vehicle of the infection. Chicken production in Brazil has increased in the last couple of years and the country is now ranked 2nd as producer/exporter of this commodity. For this reason, there is an increased concern over the safety of these goods. This study deals with

the dissemination, antimicrobial resistance and genetic characterization of *S.* Enteritidis strains isolated from an industrial chicken production chain. 108 isolates, phagetypes PT4, PT7a and PT1, were obtained at different steps of the commercial production from farm to frozen cuts, and the birds were from different producers supplying the same processing plant. Tests for susceptibility to 12 human and veterinary antimicrobial agents were performed. The strains were also typed by PFGE, RAPD, ribotyping, and PCR-ribotyping. 6.5% of the strains were susceptible to all drugs tested and 33.3% were resistant to 1 or 2 of them. Intermediate resistance to up to 4 agents was observed in 83.3% of the isolates. Combining all the typing methods allowed the division of the strains into 13 genotypes with elevated degree of similarity. However, 75% of the strains belonged to 3 main genotypes spread along the production chain. There was no correlation between phagetypes and genotypes, or phagetypes and resistance profiles. However, most strains from one sub-region were from 2 genotypes and showed intermediate resistance to, or were resistant to furazolidone. The high degree of similarity amongst the genotypes indicates the clonal origin of the strains. The relatively high resistance to antimicrobial agents is a cause of concern, and trying to diminish the selective pressure has to be a goal for bird producers. Acknowledgement: FAPESP (03/02077-9).

P2-28 DSC Development of a Mathematical Model to Describe the Growth of *Salmonella* spp. in Raw Poultry Stored under Aerobic Conditions

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Salmonella spp. contamination continues to constitute a major concern for poultry processors. Since live birds may naturally carry *Salmonella* in their gut, any carcass may potentially be contaminated with *Salmonella*. US regulations for poultry slaughter and processing (9 CFR 381.66) mandate certain cooling times for freshly slaughtered birds, as well as maximum temperatures allowed during further processing of raw meat. The objective of our study was to develop a mathematical model that predicts the growth of *Salmonella* spp. in raw poultry stored under aerobic conditions over a variety of temperatures to better assist in determining the severity of cooling or other temperature deviations. One hundred twenty-four *Salmonella* spp. growth rates were extracted from 13 published studies and used to develop a model relating growth rate of *Salmonella* spp. on poultry to incubation temperature. A square-root or Ratkowsky equation was used to model the data with good results (R2 = 0.89). Model predictions were used to analyze the effect of different poultry carcasses chilling rates allowed in current regulations. Results show that the worst case scenario allowed by the regulations (8 h chilling allowed for carcasses over 8 lbs) results in a 0.8 log CFU/g increase in *Salmonella* spp. concentration. If the carcass chilling takes 9 h the model predicts a 0.9 log CFU/g increase. The model also estimates that chilling times of 10 h result in a 1.0 log CFU/g increase in *Salmonella* spp. concentration. A properly constructed and applied model for *Salmonella* spp. growth can provide a faster and cost-effective alternative to laboratory studies to estimate the effects of storage temperature on product safety. The developed model allows the end user to estimate the effect of storage temperature on *Salmonella* spp. concentration in poultry meat held under aerobic storage conditions over temperatures from 5.9 to 40°C.

P2-29 Reduction of *Listeria monocytogenes* and *Salmonella* on Chicken Skin by *Pseudomonas* Biosurfactants

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The presence of bacterial pathogens in retail poultry meat is, in part, dependent on their ability to attach to carcasses during slaughter. Biosurfactants produced by other bacteria, which may both act as antimicrobials and reduce attachment, represent a potential mechanism to control pathogens. Four biosurfactants (A-D) produced by different *Pseudomonas* strains were purified. The antimicrobial activity of the biosurfactants against two *Listeria monocytogenes* (1/2a and 4b) and two *Salmonella* (Enteritidis and Typhimurium) strains was investigated. Each of the pathogens was exposed to the biosurfactants in water and numbers monitored over time by plate counts. The ability of the same biosurfactants to reduce numbers of the pathogens on chicken skin was also determined. An in vitro assay was used in which fresh chicken skin was exposed to the pathogens in water, with biosurfactants added either during or after attachment, and bacteria on the skin enumerated by plate counts. Biosurfactants A and B in water displayed significant ($P < 0.05$) antimicrobial activity against *L. monocytogenes* 4b (reduction of 1.7 log CFU ml-1) and *S.* Enteritidis (reduction of 2.2 log CFU ml-1), respectively. In all other cases no antimicrobial activity of biosurfactants in water was apparent. When added after attachment to chicken skin, a significant reduction ($P < 0.05$) in numbers of *S.* Enteritidis (between 0.9–1.3 log CFU ml-1) and *S.* Typhimurium (between 0.8–0.9 log CFU ml-1) was achieved by biosurfactants A, B and C, and biosurfacants B and C, respectively. When added after attachment to chicken skin, a significant reduction ($P < 0.05$) in numbers of *L. monocytogenes* 1/2a and 4b (between 1.0-2.2 log CFU g-1) was achieved by all biosurfactants. In all other cases biosurfactants resulted in no reduction in bacterial numbers on chicken skin. The biosurfacants studied have potential to reduce pathogens on chicken skin but this effect is both strain and biosurfactant specific.

P2-30 DSC Microbiological Quality of Beef and Pork Carcasses Processed by Four Small and Very Small Meat Processing Plants in Georgia

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The purpose of this study was to investigate the microbiological quality of beef and pork carcasses slaughtered by small and very small meat processing facilities in Georgia. A sterile sponge was used to sample a 100 cm^2 area of rump, midline, and neck area of 72 beef carcasses along with the ham, belly, and jowl area of 72 pork carcasses, over a period of two months, from 4 different slaughtering facilities. All samples were collected at the end of the slaughter line but prior to the application of any antimicrobial interventions. Each collected sample was analyzed for the population of total aerobes, total coliforms, and *Enterobacteriaceae*, and for incidence of *Salmonella*. No significant differences ($P > 0.05$) were found in the populations of aerobic bacteria and *Enterobacteriaceae* recovered from the different locations on the beef carcasses. The total coliforms were higher ($P \leq 0.05$) on the neck area (2.11 $\log_{10}$ CFU/cm^2) than on the rump area (1.53 $\log_{10}$ CFU/cm^2) and intermediate at the midline area (2.02 $\log_{10}$ CFU/cm^2). In pork carcasses, total aerobes, total coliforms, and *Enterobacteriaceae* recovered from the belly area (2.91, 2.94, and 2.19 $\log_{10}$ CFU/cm^2, respectively) were

higher ($P \leq 0.05$) than respective populations recovered from the ham and jowl areas. The total aerobes on the jowl area (2.52 $\log_{10}$ CFU/cm^2) were higher ($P \leq 0.05$) than on the ham area (2.06 $\log_{10}$ CFU/cm^2). Out of 72 beef carcasses evaluated, only 1 was positive for *Salmonella* (1.38% incidence rate), whereas of 72 pork carcasses evaluated, 4 were positive for *Salmonella* (5.5% incidence rate). The findings of this study may help to identify critical control points as well as effective intervention in small and very small meat processing plants.

P2-31 Microbial Populations and Pathogen Incidence of Poultry Carcasses, Carcass Parts, Necks, and Giblets following Processing

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A surveillance study was conducted to determine levels of total aerobic (APC), total coliform (TCC), and *Escherichia coli* (ECC), as well as incidence of *Salmonella* and *Campylobacter* on poultry carcasses, carcass parts, necks, and giblets (including gizzards, hearts, and livers) following commercial processing (and immediately prior to packaging). Samples were collected from four commercial poultry processors in the Western United States during the period of January to December 2005. Poultry carcasses yielded: (1) APC, TCC, ECC of 2.6, 1.1, 0.7 log CFU/ml, respectively; and, (2) *Salmonella* and *Campylobacter* incidence of 6.7 and 14.7%, respectively. Carcass parts yielded: (1) APC, TCC, ECC populations of 2.7, 0.9, and 0.5 log CFU/ml, respectively; and, (2) *Salmonella* incidence of 12%, respectively. Necks yielded: (1) APC, TCC, ECC of 2.8, 1.3, 1.1 log CFU/ml, respectively; and, (2) *Salmonella* and *Campylobacter* incidence of 4 and 22.7%, respectively. Gizzards yielded: (1) APC, TCC, ECC of 0.8, 0, 0 log CFU/ml, respectively; and, (2) *Salmonella* and *Campylobacter* incidence of 2 and 0%, respectively. Hearts yielded: (1) APC, TCC, ECC of 0.2, 0, 0 log CFU/ml, respectively; and, (2) *Salmonella* and *Campylobacter* incidence of 0 and 0%, respectively. Liver yielded: (1) APC, TCC, ECC of 1.6, 0.3, 0 log CFU/ml, respectively; and, (2) *Salmonella* and *Campylobacter* incidence of 1.3 and 22.7%, respectively. Results of this study provide evidence that the commercial poultry processing industry is effectively controlling microbial load and incidence of pathogens such as *Salmonella* and *Campylobacter* entering the food supply.

P2-32 Detection of *Campylobacter* spp. from Broiler Chicken Related Samples Using BAX® and Conventional ISO Culture

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Campylobacter spp. continues to be the leading cause of bacterial foodborne illness in the UK, and can often be isolated throughout chicken production. Samples are currently tested using the standard enrichment protocol (ISO 10272:1995), which can be a time consuming process with a presumptive positive result taking four days and a confirmed result taking six days to be confirmed as *Campylobacter* spp. More rapid detection methods are needed for this important human pathogen. The DuPont Qualicon BAX® system is a rapid molecular assay that can be used to detect *C. jejuni* and *C. coli*. The BAX® system entails automated PCR amplification and detection, providing a result in less than 4 h. Broiler chicken caeca (n = 100), fresh chicken carcass rinses (n = 60) and bootsocks (gauze sock walked through a broiler chicken house; n = 50) were enriched according to ISO 10272:1995 using Bolton broth with and without lysed blood. Samples enriched without blood were used in subsequent BAX® detection (blood has been reported to inhibit PCR). BAX® detected *C. jejuni/C. coli* in significantly more ($P < 0.001$; McNemar's test) samples than the ISO enrichment method (all samples included). There was no significant difference ($P > 0.05$) in the number of *Campylobacter*-positive samples detected by use of direct plating onto mCCDA and direct BAX® (BAX detection on un-enriched samples) for caeca but there was a significant difference ($P < 0.0009$; McNemar's test) when using fresh carcass rinses. There was no significant difference ($P > 0.05$) in the number of *Campylobacter*-positive samples detected using direct BAX® and conventional ISO enrichment culture in combined bootsocks and caeca. The BAX® system is a rapid method that can be effectively applied to detection of campylobacters in diverse sample types.

P2-33 Thermal Inactivation of Newcastle Disease Virus (Ulster Strain) in Chicken Meat: Determination of Dt and Z Values

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Newcastle disease (ND) viruses and avian influenza (AI) viruses cause respiratory or intestinal infections in chickens and other gallinaceous birds. Some virulent ND viruses and some highly pathogenic AI viruses can cause systemic infections and replicate to high titers in skeletal muscle fibers. To prevent transmission of these viruses through contaminated meat, the World Organization for Animal Health (OIE) recommends trading poultry products from countries, zones, or compartments infected with ND or certain AI virus subtypes only when the products are effectively processed to destroy these pathogens. ND and AI viruses are heat-labile and will be inactivated in sufficiently cooked poultry meat, but more studies are needed to determine thermal processing guidelines. For this study, thermal death calculations were performed for a model low-virulence ND virus (Ulster strain) in chicken meat. Small (0.05 g) pieces of breast meat were artificially infected with virus (107.5 to 108.5 EID50 per g of meat) and heated in a thermocycler block. Meat samples were ground with small pestles to release virus from the tissue, and the supernatants were tested for virus inactivation by titration in embryonating chicken eggs. The time required for a 90% reduction in virus titer (Dt value) was calculated for temperatures of 57, 58, 59, and 61°C. Dt values were inversely related to temperature, and ranged from 38.1 s at 61°C to 472.1 s at 57°C. The temperature increase required for a 90% reduction in thermal death time was 3.7°C. These results indicate that current USDA performance standards for a 7.0 log reduction of *Salmonella* in Ready-to-Eat poultry meat products are sufficient to inactivate high titers of a low-virulence ND virus in chicken breast. Future studies will determine whether this is also the case for virulent ND viruses and highly pathogenic AI viruses.

P2-34 Inactivation of Avian Influenza Virus in Disinfectants and in Egg Products (Mayonnaise)

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Since chicken and egg products are widely exported and imported, there is a distinct possibility that Avian Influenza Virus (AIV) may be transmitted and cause epidemics amongst poultry. There is the additional fear that AIV may become infective to humans. From the viewpoint of food safety and risk, we attempted to determine AIV survivability in disinfectants and egg products. We have investigated the inactivation of AIV by hypochlorite and ethanol, which are used as disinfectants in egg-processing factories. We have also investigated the inactivation of AIV in mayonnaise, which is a typical and widely-consumed egg product. The following isolates of AIV virus were used in this experiment : A/whistling swan/Shimane/499/83 (H5N3)(H5 AIV), A/whistling swan/Shimane/42/80 (H7N7)(H7 AIV) and A/duck/Hokkaido/26/99 (H9N2)(H9 AIV). In 30 ppm of hypochlorite, AIV was not inactivated after 300 s. At concentrations over 100 ppm of hypochlorite, AIV was inactivated within 10 s. In 50% (v/v) of ethanol/water, AIV was also inactivated within 10 s. These concentrations of disinfectants were enough to inactivate AIV on the surface of eggs, utensils, process lines and employees' hands at egg handling processes in egg-processing factories. A model mayonnaise was made of salad oil, vinegar, egg yolk and salt, similar to commercial products. After the model mayonnaise and AIV were mixed, H7 AIV and H9 AIV were inactivated immediately, and their infectivity titers decreased to under the detection limit. In the case of H5 AIV, the infectivity titer decreased from 105.0EID50/0.1mL to under the detection limit after 30 min. These results demonstrated that different isolates of AIV were inactivated in mayonnaise. The inactivation of AIV may be caused by the chemical properties of mayonnaise, such as the salt concentration, acid concentration and/or pH.

P2-35 Food Safety Practices and Technologies Used by United States Poultry Slaughter Plants: Results of a National Mail Survey

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In response to the PR/HACCP final rule, some poultry slaughter plants have implemented practices and technologies to control foodborne pathogens, chemical hazards, and physical hazards. Plants may implement these procedures to meet FSIS microbiological performance criteria or as part of their SSOPs or HACCP plan. The use of food safety practices and technologies may subsequently help reduce the risk of foodborne illness in the United States. To characterize the use of food safety practices and technologies in the poultry slaughter industry, we conducted a census of poultry slaughter plants, using a mail survey approach. We received 219 completed surveys (78% response rate). The survey collected information on current and planned use of specific food safety practices and technologies, microbiological testing practices, and employee food safety training. Some of the most frequently used practices and technologies are the requirement that poultry producers use specific practices to control pathogens (61% of plants) and to control chemical residues (77% of plants). Also, 86% of plants use some type of carcass recontamination strategy and 50% of plants with processing activities use antimicrobial chemicals or some other type of pasteurization method. Most plants conduct voluntary microbiological testing (85% of plants) and environmental sampling (75% of plants). The vast majority of plants have one or more production employees that have completed formal HACCP training (93% of plants). Nearly all plants provide food safety training for new hires and on a continuing basis. In general, large and small plants are more likely to use most types of food safety practices and technologies than are very small plants ($P < 0.05$). Some plants expect to increase use of food safety technologies in the next few years. The survey findings can be used to establish a baseline of current practices and conduct analysis of food safety risk management practices.

P2-36 Distribution of *Salmonella* Enteritidis within Shell Eggs, Inoculated from Different Sides and Incubated with or without Rotation

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The influence of site inoculation and egg rotation on the distribution of *Salmonella* Enteritidis (SE) in shell eggs were studied. The objective of the study was to attain a uniform distribution of SE for use in validation studies of various shell egg pasteurization technologies. Shell eggs were inoculated with an overnight culture of SE (10^7 CFU/ml) either from the narrow end of the eggs or along their sides. Inoculated eggs were then incubated at 37°C with rotation either vertically or horizontally with respect to the long axis of the egg at 120 rpm for 24 h. Controls were also incubated in similar positions with no rotation. Albumin was sampled from different places in the intact eggs. However, the yolk was sampled by breaking the egg and carefully seperating it from the albumin. In eggs inoculated through their narrow end and incubated with rotation, average counts of SE were one log higher in the albumin (10^8 CFU/ml; vertical rotation, 10^7 CFU/ml; horizontal rotation) than in yolk (10^7 CFU/ml; vertical rotation, 10^6 CFU/ml; horizontal rotation). In the case of no rotation, counts of SE were the same in both albumin and yolk (10^7 CFU/ml). In eggs inoculated along their sides with vertical rotation, average counts of SE were 1 log higher in albumin (10^7 CFU/ml) than in yolk (10^6 CFU/ml). In the case of horizontal rotation versus no rotation and vertically-placed eggs inoculated along their side, counts of SE were the same in both albumin and yolk (10^7 CFU/ml). Using any of these combinations, one can simulate all possibilities of natural contamination. However, narrow end inoculation and vertical placement with no rotation would be the best combination for validating various pasteurization technologies for shell eggs where uniformity of treatment or process and integrity of albumin and yolk may be a concern. There was no significant difference ($P < 0.05$) between the counts of SE sampled from different places in the intact egg.

P2-37 A Retail Survey of Brazilian Milk and Minas Frescal Cheese and the Corresponding Dairy Plant Producing These Products to Determine the Prevalence and Sources of *Listeria monocytogenes* and to Implement Corrective Measures

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A study was designed to recover *Listeria monocytogenes* from pasteurized milk and Minas frescal cheese (MF) and to identify the sources of contamination of these products in the corresponding dairy processing plant. Fifty milk samples (9 brands, 5–7 samples per brand) and 50 MF samples (10 brands, 5 samples per brand) were analyzed for LM by a microbiological enrichment method and by PCR assay. Samples were obtained in June–September of 2005 (the dry/cold season) from 37 retail sites (chosen by stratified random sampling) located in seven areas of Juiz de Fora, State of Minas Gerais, Brazil. All 50 milk samples tested negative (< 1 CFU/ml) for LM. However, 4 of 5 samples of MF from 1 brand, brand "F", tested positive for LM. In October of 2005 we visited the farm/dairy that produced brand F and took samples from the milking parlor (bulk tank milk, teat cups, filters, milk buckets, and rinse water obtained from buckets; all 5 tested negative), samples from food contact and non-food contact areas of the processing plant (e.g., floors, walls, sinks, refrigeration units, molds, and excess liquid from MF on trays, in sinks, and in refrigerators; 9 of 22 sites/samples tested positive), and samples of MF (5 of 5 samples tested positive). Based on our findings, in collaboration with the producer and local health department officials, failures in the hygienic process and plant design were identified and corrected. The plant remained closed until December of 2005 when microbiological testing confirmed that the processing environment (29 samples) tested negative for *Listeria monocytogenes*. Moreover, analysis of MF produced in January of 2006 confirmed that all 5 samples tested negative for *Listeria monocytogenes*. Studies are ongoing to quantify the prevalence, levels, and types of *Listeria monocytogenes* in dairy products and in the processing plant to better manage the threat of listeriosis.

P2-38 Characterization of *Enterobacter* spp. Isolated from Shell Eggs Using Pulsed-field Gel Electrophoresis

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The prevalence of *Enterobacter* associated with shell eggs was determined previously to assess the sanitation practices of three commercial facilities in the Southeastern United States. *Enterobacter* were recovered from 117 of 837 (13.97%) samples tested. In the present study we established the relatedness of the 176 isolates (1–5 isolates per positive sample) recovered from 96 of these 117 positive samples. Pulsed-field fingerprinting generated 121 XbaI pulsotypes (34–96% related) distributed as follows: *Enterobacter cloacae* (104 isolates; 73 pulsotypes, 39–100% related); *E. amnigenus* (32 isolates; 25 pulsotypes, 33-100% related); *E. sakazakii* (27 isolates; 12 pulsotypes, 33-100% related); and *E. taylorae* (9 isolates; 7 pulsotypes, 38–100% related). There was no predominate or persistent pulsotype among isolates from a given plant for any of the 3 visits; the 101 plant X isolates displayed 73 pulsotypes, the 14 plant Y isolates displayed 8 pulsotypes, and the 61 plant Z isolates displayed 41 pulsotypes. Multiple isolates were recovered from 47 of the 96 (49%) positive samples and multiple pulsotypes were displayed by isolates from 34 of these 47 samples (72%). For the majority of visits to each plant, more *Enterobacter* isolates/pulsotypes were recovered before washing/processing than during or after washing. Pulsed-field fingerprinting of isolates from all three stages indicated that processing was effective at reducing the prevalence and types of *Enterobacter* and also demonstrated the possibility of multiple sources of contamination. These data establish that new/unique isolates of *Enterobacter* are introduced into the shell egg environment with each batch of eggs and that there is considerable heterogeneity among isolates and species associated with shell eggs. Also, examination of multiple *Enterobacter* isolates from positive samples is necessary to determine the variety of potential contaminants of egg shells before, during, and after processing.

P2-39 Identification of Yeasts Isolated from Commercial Shell Eggs Stored at Refrigerated Temperatures

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Yeasts and molds, which are able to withstand harsh environmental stresses, can grow on or in eggs and cause spoilage. Egg meats readily absorb off odors, including those caused by yeast or mold growth, so their presence on eggs may constitute a quality concern. Washed and unwashed eggs (treatments) were collected aseptically on three separate days (replications) from a commercial processing facility and stored for ten weeks at 4°C. Enough eggs were collected so that ten eggs from each treatment could be sampled weekly (120 eggs/treatment/replication). Yeast and mold populations were enumerated from external rinses of the shells by plating onto acidified potato dextrose agar. Prevalence and level data were described in a previous report. Yeast colonies were picked randomly and stored for subsequent identification by gas chromatographic analysis of fatty acid content, using the MIDI Microbial Identification System. Of the 688 isolates analyzed, 457 were identified to genus or species. Genera identified by this method included *Candida*, *Cryptococcus*, *Hanensula*, *Hyphopichia*, *Metschnikowia*, *Rhodotorula*, *Sporobolomyces*, and *Torulaspora*. Almost 80% of the isolates were identified as *Candida* spp. (390/457). *Candida famata* was the most commonly identified species (n = 165), followed by *Candida zeylanoides* (n = 20). Subsequently, a group of 20 isolates were subjected to molecular and biochemical analyses for comparison with the MIDI results. While biochemical tests and sequencing of rRNA were in agreement for 10 of the isolates, only 2 of the 20 MIDI identified isolates were in agreement with the sequenced samples when Genebank data were used. *Candida famata*, a synonym of *Debaromyces hansenii* var. *hansenii*, was the most commonly identified isolate by biochemical and molecular methods. These data indicate that there is little correlation between MIDI system and the corresponding library is of limited use in properly identifying yeasts isolated from commercial shell eggs.

P2-40 Thermal Inactivation and Injury of Freeze-stressed *Campylobacter jejuni* in Ground Chicken

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Although foods of animal origin, mainly poultry and poultry products, are often associated with *Campylobacter jejuni* infection, relatively little is known about the effect of environmental conditions, such as cold or freeze stress on subsequent heat tolerance. Therefore, inactivation and injury of *C. jejuni* by heating ground boneless chicken breasts with and without prior freeze-stress were investigated. Ten-gram portions of ground chicken were irradiated and artificially contaminated with approximately 10^8 CFU/g of a

three strain *C. jejuni* cocktail. A set of samples stored at -20°C for 14 days and an otherwise similar set of samples that was not frozen were both heated to 50, 60, and 74°C. Frozen storage at -20°C for 14 days produced a reduction of viable cell counts to 10^5 CFU/g. At various time intervals, samples were serially diluted and were surface plated onto Mueller Hinton agar (MHA) to enumerate surviving cells, as well as plated onto MHA with 3% NaCl (MHAS) to detect thermally-injured cells. The MHA and MHAS plates were incubated for 48 h at 42°C in sealed jars under microaerophilic conditions. Based on the average of triplicate experiments, the corresponding D-values were 59.3 min, 1.89 min, and 4.28 s in samples without prior freeze-stress and 36.25 min, 1.80 min, and 3.94 s in samples subjected to freeze-stress and then heated at 50, 60, and 74°C, respectively. In general, the recovery of cells on MHAS was lower than on MHA for samples with and without prior freeze-stress as compared to those subsequently heated at 50, 60, and 74°C, indicating cell injury. This study showed that freeze-stress of *C. jejuni* in ground chicken at -20°C for 14 days did not appreciably affect the thermal inactivation of this pathogen ($P > 0.05$) and that heating at 74°C for 30 s inactivated *C. jejuni* in ground chicken.

P2-41 Development and Validation of an Isothermal-based Pathogen Growth Prediction Tool for Evaluating Non-isothermal Processing of Raw Pork

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We developed an easy-to-use, isothermal-based pathogen growth prediction tool (IBPT) for use in evaluating non-isothermal processes. This tool will assist the meat industry in critical limit validation and process deviation decision-making. The IBPT was developed using small-scale (25g) meat inoculation studies, using inocula of either five strains each of *Salmonella* serovars and *Escherichia coli* O157:H7 or five strains of *Staphylococcus aureus* dispersed throughout 25 g samples of ground pork and incubated [isothermal experiments at 2.8°C (5°F) intervals from 10-49°C (50-120°F)]. Log CFU/g vs. time data from three concurrent trials were analyzed using DMFit software to determine Estimated Critical Times [ECT; time until growth reached a pre-defined increase of concern: 0.3 log CFU (*Salmonella* serovars and *Escherichia coli* O157:H7) or 1.3 log CFU (*Staphylococcus aureus*)] for each pathogen. The ECT values ranged from 60 min at 43.3°C (*Escherichia coli* O157:H7) to 3694 min at 10°C (*Salmonella* serovars) with R2 values for DMFit-derived growth curves ≥ 0.90 for 71% of all pathogen/temperature combinations. By determining the percent ECT for each temperature interval in a product's time/temperature history and then summing all percent ECT values, the IBPT predicts concern (cumulative percent ECT < 100%) or no concern (cumulative percent ECT < 100) for pathogens in raw pork products. To validate the IBPT, non-isothermal challenge studies were conducted with ground and intact cured raw pork products held for up to 18 h at ≤ 49°C during early stages of slow or partial-cooking. The IBPT predicted growth to our level of concern in 36 of 45 pathogen/product/trial combinations (80%); for these 36 combinations growth was actually observed in nine. Overall, the IBPT was either accurate (40% of combinations) or fail-safe (60%), and was never fail-dangerous. The IBPT will be a useful conservative tool in critical limit validation, and process deviation decision-making for pork processors.

P2-42 Survival of *Campylobacter jejuni* on Vacuum-packed Beef and Pork at Refrigerated Temperatures

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Campylobacter jejuni is the most common cause of food related gastroenteritis. The most common vehicle of human *Campylobacter* infection is poultry. However, this organism is frequently present in the gut flora of meat animals and has been found on beef carcasses, beef cuts and in frozen bulk packed beef. *C. jejuni* does not grow at temperatures below 28°C and is intolerant of aerobic conditions. Therefore, its survival rather than growth on meat must be a major factor affecting the incidence of campylobacterosis. Despite that, studies of *C. jejuni* survival on meat are few. The present study examined the survival of *C. jejuni* on beef and pork stored at refrigerated temperature and under vacuum packed conditions. *C. jejuni* NCTC 11168 was inoculated at 10^5 CFU/25 cm^2 sterile cores of beef and pork, packed under vacuum and stored, with uninoculated controls for 42 days at 4 and 10°C. At bi-weekly intervals, the numbers of surviving bacteria were determined by direct plating on Tryptic Soy Agar and culturable bacteria were also recovered by enrichment in *Brucella* broth containing *Campylobacter* growth supplement and plating on *Campylobacter* Blood-Free Selective Agar and incubation at 42°C for 48 h under microaerophilic conditions. Numbers of *C. jejuni* dropped to 1 log unit within 7 days of storage, and were undetectable at day 9 by direct plating. However, *C. jejuni* was detected up to day 28 following enrichment. Studies on pork adipose tissue show no significant reduction in survival even after 2 weeks of storage. These results demonstrate that *C. jejuni* survives longer on meats under standard preservative packaging conditions than has been reported in the literature and this may be part of the explanation of its importance with regard to foodborne illness.

P2-43 Enzyme-linked Immunosorbent Assay for Detection of Poultry Content in Heat-processed Meat

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An indirect enzyme-linked immunosorbent assay (ELISA) was developed for the detection of undeclared poultry content in heat-processed meat products, based on the recognition of a thermal-stable myofibril protein, troponin I, by the detecting monoclonal antibody (Mab) 5G8. Meat species adulteration has implications of economic fraud, loss of traceability, and food safety issues. Lean meat from each species, including 4 poultry (chicken, turkey, duck and goose) and 8 mammalian meats (pork, beef, lamb, rabbit, horse, deer, buffalo, and elk) was ground separately and divided into 3 portions to prepare raw, cooked (100°C for 30 min) and autoclaved (121°C for 30 min) protein extracts in 0.01 M phosphate buffered saline containing 0.5 M NaCl for examining the species-specificity of the assay. The microtiter plates were coated with meat extracts containing 2g of protein. Mab 5G8 (IgG1) supernatant was then added to the coated well to capture the antigen in the extract. The ELISA showed strong signals with cooked and autoclaved chicken and turkey meat extracts, with stronger reactivity with thigh than breast protein extract. The assay showed no reactivity with mammalian meats or any of the proteins commonly used as food additives in meat products. The assay could achieve a detection limit of at least 1% cooked chicken in pork or turkey in beef mixture. The reaction signal increased proportionally to the increase of poultry content, suggesting that the Mab 5G8 based ELISA could quantitatively measure chicken or turkey content in a cooked meat mixture. The reactivity in chicken breast increased with the increasing severity of the heating conditions. Therefore, the assay could also be used to probe improper heating condition of a whole poultry product, which differentiates pasteurized poultry from sterilized product. The method will provide valuable information concerning meat quality and safety.

P2-44 Food Safety Practices and Technologies Used by United States' Meat Slaughter Plants: Results of a National Mail Survey

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Practices and technologies implemented by meat slaughter plants for controlling foodborne pathogens and other hazards may subsequently help reduce the risk of foodborne illness in the United States. Plants may implement these procedures to meet FSIS microbiological performance criteria or as part of their SSOPs or HACCP plan. To characterize the use of food safety practices and technologies in the meat slaughter industry, we conducted a nationally representative mail survey of meat slaughter plants. We took a census of HACCP large and small plants and selected a systematic sample of HACCP very small plants. We received 598 completed surveys (65% response rate). The survey collected information on current and planned use of specific food safety practices and technologies, microbiological testing practices, and employee food safety training. Some of the most frequently used practices and technologies are use of some type of carcass decontamination intervention (75% of plants) and use of antimicrobial chemicals or some other type of pasteurization method during processing (41% of plants). Many plants conduct voluntary microbiological testing (70% of plants) for generic *E. coli*, *E. coli* O157:H7, and other pathogens. Also, 52% of plants conduct environmental sampling. Most plants have one or more production employees that have completed formal HACCP training (87% of plants) and provide informal or formal food safety training for new hires (92% of plants) and on a continuing basis (86% of plants). In general, large and small plants are more likely to use most types of food safety practices and technologies than very small plants are ($P < 0.05$). Some plants expect to increase use of food safety practices and technologies in the next few years. The survey findings can be used to establish a baseline of current practices and analysis of food safety risk management practices.

P2-45 Further Characterization of *Escherichia coli* O157:H7 Strains from Ground Beef Isolated by the Food Safety and Inspection Service

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Current regulations by the United State Department of Agriculture's Food Safety and Inspection Service (FSIS) require that *E. coli* O157:H7 isolates meet two criteria: (1) isolates must be serologically O157, and (2) isolates must express shiga toxin or contain the genes for either shiga-toxin 1 or 2. Characterization of the isolates is limited to biochemical confirmation and pulse-field gel electrophoresis. In this study, 52 *E. coli* O157:H7 isolates confirmed by FSIS over a three year period were further characterized as to which shiga-toxin genes were present, antibiotic resistance and plasmid profile. Real-time PCR was used to detect the individual shiga-toxin genes *stx*1 and *stx*2. PCR rsults show that 52% of the isolates contain both *stx*1 and *stx*2, 42.3% contain *stx*2 only, and 5.7% contain *stx*1 only. Comparison of the strains based on *Xbal* PFGE pattern and shiga-toxin type also yielded no discernible association. However, grouping isolates by the BlnI PFGE pattern showed that nearly all (75%) of isolates containing both toxins had similar patterns. Antibiotic resistance profiles for the *E. coli* O157:H7 isolates were determined by the microdilution method, using the Sensititre detection system. Resistance to sulphamethoxazole, tetracycline, streptomycin, and chloramphenicol was identified. No correlation between resistance pattern and shiga-toxin geneotype or pulsotype was identified. Eleven of the isolates contained plasmids ranging from 2 kb to greater than 12 kb in size. Characterization of FSIS *E. coli* O157:H7 isolates from ground beef identified no unique or unusual features in the strains.

P2-46 Effect of Individual and Multiple-sequential Interventions on Microbial Populations during Processing of Poultry Carcasses and Parts

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The study evaluated changes in aerobic plate counts (APC), total coliform counts (TCC), *Escherichia coli* counts (ECC), and *Salmonella* incidence on poultry carcasses, carcass parts, and in processing water before (Pre) and after (Post) individual interventions as well as on poultry carcasses exposed to multiple-sequential interventions at various stages during the slaughter process. The study was conducted in three commercial processing plants in the Western United States during the period of January to December 2005. Individual interventions evaluated included: New York (NY) Wash; Post-Evisceration Wash; Inside/Outside Bird Wash 1 (IOBW1); IOBW2; Chlorine Dioxide (ClO_2) Wash; Trisodium Phosphate (TSP) Wash; Chlorine (Cl_2) Chiller; ClO_2/Cl_2 Chiller; Chiller-Exit Spray, Post-Chiller Wash; Dropped Carcass Wash; Dropped Parts Wash; Red Water; Carcass Parts Dip; Neck Tube-Chiller; Neck Ice-Chilling; Liver Tube-Chiller; Heart Tube-Chiller; and Gizzard Tube-Chiller. Multiple-sequential interventions were evaluated at two processing plants namely: (1) Plant A (NY Wash, Post-Evisceration Wash, IOBW1, IOBW2, ClO_2 Wash, ClO_2/Cl_2 Chiller, Chiller-Exit Spray, Post-Chiller Wash); and, (2) Plant B (NY Wash, IOBW1, IOBW2, TSP Wash, Cl_2 Chiller). Results indicate that the majority of individual interventions effectively, if not significantly ($P < 0.05$), reduced microbial populations on/in carcasses, carcass parts, and processing water. Reduction in APC, TCC, and ECC due to individual interventions ranged from 0 to 1.2, 0 to 1.2, and 0 to 0.8 log CFU/ml, respectively. Most individual interventions resulted in substantial reduction of *Salmonella* incidence ranging from 0 to 28%. Multiple-sequential interventions at plant A resulted in significant ($P < 0.05$) reduction in APC, TCC, ECC, and *Salmonella* incidence of 2.4, 2.8, 2.9 log CFU/ml, and 27%, respectively. Multiple-sequential interventions at plant B resulted in significant ($P < 0.05$) reduction in APC, TCC, ECC, and *Salmonella* incidence of 1.8, 1.7, 1.6 log CFU/ml, and 40%, respectively. These results enable processing plants verify that interventions employed are effective in controlling microbial populations and *Salmonella* incidence.

P2-47 Baseline Incidence of *Escherichia coli* O157:H7, Enterohemorrhagic *E. coli* (EHEC), and *Salmonella* in/on Beef Carcasses, Trim, Ground Beef, and Variety Meats

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A surveillance study was conducted to establish baseline incidence of *Escherichia coli* O157:H7, enterohemorrhagic *E. coli* (EHEC), and *Salmonella* in/on beef carcasses, trim, ground beef, and variety meats. Samples for this study were collected at six beef processing facilities throughout the United States during the period of January to December 2005. Samples were enriched in trypticase

soy broth for 12 h at 37°C and tested for pathogen incidence, using a commercial multiplex polymerase chain reaction (PCR) assay. Incidence of *E. coli* O157:H7 was 0 (n = 6,285), 0.03 (n = 292,386), 0 (n = 3,527), and 0.01% (n=35,014) in/on beef carcasses, trim, ground beef, and variety meats, respectively. Incidence of EHEC was 0.11 (n = 6,285), 0.28 (n = 292,386), 0 (n = 3,527), and 0.17% (n = 35,014) in/on beef carcasses, trim, ground beef, and variety meats, respectively. Incidence of *Salmonella* was 1.51 (n = 10,602), 2.30 (n = 107,079), 6.28 (n = 3,527), and 5.20% (n = 16,816) in/on beef carcasses, trim, ground beef, and variety meats, respectively. Incidence of *E. coli* O157:H7 was highest on trim during April and June and on variety meats during March and April. Incidence of EHEC was highest on carcasses during March and May, on trim during April/May and August/September, and relatively consistent on variety meats during January to September. Incidence of *Salmonella* was highest on carcasses during February to April, on trim during March/April and August/September, in ground beef during March and August, and relatively consistent on variety meats during January to September. Results of this study provide a baseline incidence of *E. coli* O157:H7, EHEC, and *Salmonella* on beef carcasses and products and may be used by the industry for risk assessment and for optimizing food safety programs to control these hazards.

P2-48 Effect of Individual Interventions on Beef Carcasses, Hearts, and Heads during Beef Processing

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The objective of this study was to evaluate the efficacy of individual interventions for decontaminating carcasses, hearts, and heads in two Midwestern commercial beef processing facilities during the period of January 2003 to November 2005. The study evaluated changes in aerobic plate counts (APC), total coliform counts (TCC), and *Escherichia coli* counts (ECC) on carcasses, hearts, and heads before (Pre-) and after (Post-) individual interventions. Interventions evaluated included: (1) steam vacuuming (at five anatomical locations: inside round, midline-rear pattern, brisket, neck, and gooseneck); (2) hock washing; (3) pre-evisceration carcass washing, applying hot water (82 C) followed by lactic acid (2.5%) rinsing; (4) post-evisceration lactic acid (2.5%) rinsing; (5) hot water (82 C) pasteurization; (6) pre-chiller lactic acid (2.5%) rinsing; (7) heart lactic acid (2.5%) rinsing; and (8) head lactic acid (2.5%) rinsing. Results indicated that the majority of individual interventions significantly ($P < 0.05$) reduced APC, TCC, and ECC on carcasses, hearts, and heads. Reduction in APC, TCC, and ECC due to steam-vacuuming ranged from 0.4 to 1.0, 0.3 to 1.0, and 0.3 to 1.0 log CFU/100 cm^2, respectively. Pre-evisceration carcass washing resulted in reduction of 1.4, 0.6, and 0.5 log CFU/100 cm^2 in APC, TCC, and ECC, respectively. Hot water pasteurization resulted in a reduction of 0.6, 0.2, and 0.1 log CFU/100 cm^2 in APC, TCC, and ECC, respectively. Post-evisceration and pre-chiller lactic acid rinsing resulted in reduction of APC by 0.1 and 0.2 log CFU/100 cm^2, respectively. Heart lactic acid rinsing resulted in a reduction of 0.5, 0.2, and 0.1 log CFU/100 cm^2 in APC, TCC, and ECC, respectively. Head lactic acid rinsing resulted in a reduction of 0.5, 0.4, and 0.2 log CFU/100 cm^2 in APC, TCC, and ECC, respectively. These results indicate that individual interventions applied in these facilities are able to control microbiological populations on beef carcasses, hearts, and heads.

P2-49 Identification of Microflora Associated with "Blown-Pack" Spoilage of Ground Beef Chubs during Refrigerated Storage

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The objective of this study was to identify microflora associated with spoiled ("blown-pack" appearance) ground beef chubs obtained from a commercial beef processing plant. Ground beef samples were analyzed for microbial populations (total plate counts [TPC], total coliform counts [TCC], *Enterobacteriaceae* counts [EBC], lactic acid bacteria counts [LAB], psychrotrophic total plate counts [Psy-TPC], anaerobic total plate counts [An-TPC], and psychrotrophic anaerobic total plate counts [Psy-An-TPC]). Furthermore, 10-g portions of ground beef chub samples were enriched using peptone yeast glucose starch (PYGS) broth containing Durham tubes and incubated at 7°C until visual signs of gas production were observed. Gas-positive samples were streaked onto selective agars to isolate gas-producing organisms. The isolated organisms were identified using phenotypic characterization and 16s rRNA sequencing. In addition, volatiles produced from gassy ground beef chubs were analyzed using gas chromatography-mass spectrometry utilizing the solid phase micro extraction (SPME) technique. Mean TPC, Psy-TPC, An-TPC, and Psy-An-TPC ranged between 5.8 and 6.2 log CFU/g. Mean LAB and EBC were 5.5 log CFU/g and 2.5 log CFU/g, respectively. Gas production in meat-inoculated PYGS broth was observed between 7-11 days of storage. Predominant microorganisms isolated from the PYGS enrichment were *Enterobacteriaceae* and LAB. *Hafnia alvei* and *Serratia* spp. dominated the *Enterobacteriaceae* community while *Carnobacterium* spp. and *Lactococcus piscicum* dominated the LAB community. GC-MS analysis indicated that 3-methylbutanal, 2-methylbutanal, 3-methy-lbutanol, methyl-isobutyl-ketone, hexanal, and ethanol were the predominant volatiles associated with "blown-pack" ground beef chubs. Subsequent analysis of volatile profiles from pure cultures of *Hafnia alvei, Serratia* spp., *Carnobacterium* spp., and *L. piscicum* matched profiles of "blown-pack" ground beef samples. Based on these results we hypothesize that the effect of individual and/or interactions of psychrotrophic *Enterobacteriaceae* such as *Hafnia alvei* or *Serratia* spp. with LAB may stimulate gas production in commercially processed ground beef chubs.

P2-50 Pathogen Reduction in Smokehouse Versus Dehydrator-prepared Beef Jerky

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Beef jerky is a heat-treated, shelf-stable, Ready-to-Eat meat product. Commercial jerky processors need to show their processes can reduce *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* populations by > 5 logs. Many small processors use dehydrators rather than smokehouses to process their product, and while humidity control during drying and its effect on pathogen survival has been questioned, controlling humidity in a dehydrator is more difficult compared to a smokehouse. Beef jerky strips were made using a horizontal-flow dehydrator at 62°C (143.6°F) and a commercial-type smokehouse with a dry-bulb/wet-bulb setting of 63°C/43°C (145°F/110°F) (33% relative humidity) with or without pretreatments of acidic calcium sulfate (1:2 and 1:3 water:calcium sulfate ratios), and chlorine dioxide (500 and 1200 ppm) to determine effectiveness of treatments in inactivation of *Salmonella, E. coli*

O157:H7, and *L. monocytogenes*. *E. coli* O157:H7 populations were reduced by > 5 logs CFU/strip ($P < 0.05$) for all treatments except for jerky pretreated with 500 ppm chlorine dioxide and dried in the dehydrator. For *L. monocytogenes*, 5 log reductions ($P < 0.05$) were noted for all treatments regardless of the drying method. *Salmonella* populations were reduced by > 6.5 logs ($P < 0.05$) on jerky strips that were pretreated with the higher concentration of calcium sulfate and dried in the dehydrator and jerky pretreated with 1200 ppm chlorine dioxide and dried in the smokehouse. Populations were reduced almost as well on calcium sulfate jerky when dried in the smokehouse. While effective treatments may be attained using a dehydrator coupled with an antimicrobial pretreatment, processing jerky in the smokehouse with similar temperature conditions was more effective. Generally, acidic calcium sulfate was a more effective pretreatment than chlorine dioxide. Small processors using dehydrators with limited humidity control within the drying chamber may find using these antimicrobial treatments beneficial in achieving the desired level of pathogen reduction.

P2-51 Biogenic Amine Content Related to Physico-chemical Parameters and Microbial Counts in Spanish Traditional Sausages

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"Androlla" and "Botillo" are two traditional raw fermented sausages made in the north-west of Spain from pork meat. In 20 commercial samples of Androlla and 15 commercial samples of Botillo, the content of nine biogenic amines (agmatine, tryptamine, 2-phenylethylenediamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) were quantified using HPLC methods, following the technique described by Eerola et al. (1993). In the sausage samples, some physico-chemical parameters (moisture and NaCl contents, and pH and a_w values) and the counts of some microbial groups (total aerobic mesophilic flora, salt-tolerant flora, lactic acid bacteria and *Enterobacteriaceae*) were also determined. Statistical correlations between the contents of each biogenic amine and the physico-chemical parameter and microbial count values were carried out by Pearson's correlation coefficient, using the computer program Statistics 5.1 for Windows (Statsoft Inc, 1996, Tulsa, OK, USA). Both in Androlla and Botillo sausages, the main biogenic amines were cadaverine (mean values of 232 ppm in Androlla and 296 ppm in Botillo), followed by putrescine (215 ppm in Androlla and 228 ppm in Botillo) and tyramine (170 ppm in Androlla and 124 ppm in Botillo). The minor biogenic amines were spermidine (12 ppm in Androlla and 11 ppm in Botillo) and agmatine (13 ppm in Androlla and 5 ppm in Botillo). There was a significant ($P < 0.05$) negative correlation between the moisture content and the agmatine, 2-phenylethylenediamine, tyramine, spermidine and spermine values. There was also a significant ($P < 0.05$) negative correlation between the a_w values and the agmatine, 2-phenylethylenediamine, tyramine, spermidine and spermine contents. A significant ($P < 0.05$) positive correlation between the tryptamine levels and the counts of the different microbial groups investigated was observed; this same correlation was negative in the case of spermine and spermidine. The putrescine content was negatively ($P < 0.05$) correlated with the salt tolerant flora counts.

P2-52 Validation of *Escherichia coli* O157:H7 in Direct Acidified Venison Summer Sausage

DSC

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The objective of this study was to determine effects of typical thermal processing temperatures and times on reducing *E. coli* O157:H7 in directly acidified venison summer sausage. In addition, the effect of different fat contents (approximately 10.5 and 18%) and acid types (citric versus lactic) on lethality was examined. To achieve targeted fat contents, beef was added to ground venison along with seasonings, cure (6.25% sodium nitrite), sodium eyrothorbate, water and encapsulated citric or lactic acid. Four treatments for both control and inoculated summer sausage batter were: (1) 10.5% fat and citric acid, (2) 10.5% fat and lactic acid, (3) 18% fat and citric acid and (4) 18% fat and lactic acid. To inoculated treatments, a five-strain cocktail of *E. coli* O157:H7 (ATCC 43889, ATCC 43890, ATCC 43894, ATCC 43895, and USDA-380-90) was mixed into the batter. Meat batter was stuffed into 64-mm casings and processed in a commercial smokehouse. The thermal process included a come-up period of 2 h with dry bulb starting 46.1°C and reaching 79.4°C until internal temperature (I.T.) of the product reached 70°C with relative humidity at 50% throughout the process. A cold shower then was applied until product I.T. reached 37.8°C. Initial inoculum levels in raw batter ranged from 6.8 to 8.1 log CFU/g. For all treatments in venison summer sausage, log reductions ranged from 4.8 to 6.7 CFU/g on MaConkey sorbitol agar (MSA) and phenol red sorbitol agar (PRSA) when product reached 70°C I.T. However, approximately 0.6 log CFU/g outgrowth was observed on MSA and PRSA when product reached 37.8°C I.T. after the thermal process and cold shower. Therefore, it is important to increase the final internal temperature of 64.4°C or holding time before cold showering to achieve and maintain a consistent >5.0 log CFU/g reduction as required by USDA/FSIS.

P2-53 Effectiveness of Bacteriophage in Reducing *Escherichia coli* O157:H7 on Beef Steaks and in Ground Beef Slurries

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A study was performed to evaluate the effectiveness of bacteriophage specific for *Escherichia coli* O157:H7 in killing the pathogen on beef steaks and in beef slurries stored at 4°C, 10°C and 37°C. Beef steaks (50 cm^2) and ground beef slurries (300g) were either surface inoculated or inoculated and mixed with 3.3 log CFU/cm^2 or 3.0 log CFU/g of *E. coli* O157:H7 B6914, respectively. A bacteriophage (EcoPhage, Intralytix, Inc.) was applied to steaks (7.3 PFU/cm^2) or in slurries (6.3 log PFU/g), and steaks and slurries were stored at 4, 10 and 37°C for up to 7 days. Populations recovered from bacteriophage-treated steaks were 0.2 log CFU/cm^2 lower than from control (no bacteriophage) steaks after 5 days at 4°C. Control slurries contained populations (2.5 log CFU/g) greater than treated slurries (1.5 log CFU/g) after 7 days of storage at 4°C. Populations of *E. coli* O157:H7 on steaks (2.2 log CFU/cm^2) and in slurries (1.9 log CFU/g) treated with bacteriophage were lower than on control steaks (3.2 log CFU/cm^2) and in slurries (3.9 log CFU/g) when stored at 10°C for 5 days. After 7 days at 10°C, no cells were recovered by surface plating from treated slurries, while control slurries contained populations of 3.0 log CFU/g. Populations of *E. coli* O157:H7 on treated steaks were 0.5 log CFU/cm^2 greater than on

control steaks after 5 days at 37°C. Treated slurries incubated at 37°C for 3 days had populations 2 log CFU/g greater than those in control slurries. Storage of steaks and slurries at 10°C may promote limited growth of *E. coli* O157:H7, assisting in bacteriophage propogation and in increased killing of bacterial cells. More interaction between bacteriophage and bacterial cells may occur in ground beef slurries than on steaks, leading to greater reductions in populations of *E. coli* O157:H7 in slurries.

P2-54 Evaluation of the Incubation Temperature, Time and Compositing on the Detection of *Escherichia coli* O157:H7 in Raw Ground Beef Using the VIP Immunoprecipitate Assay as a Screening Method

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The VIP immunoprecipitate assay for *E. coli* O157:H7 has been used by the Canadian Food Inspection Agency for several years. To harmonize with the United States Department of Agriculture, the sample size for raw ground beef was increased from 25 g to 65 g per subsample and each subsample was enriched individually. This modification in the method resulted in a significant increase in materials and cost. Historically, raw ground beef has had a low incidence of *E. coli* O157:H7, so the decision was made to composite the 5 incubated enrichment broths before performing one VIP analysis. The Compendium of Analytical Methods MFLP-87 states both 35°C and 42°C as incubation temperatures, with an incubation time of at least 18 h. The focus of this study was to determine if a positive VIP result would be obtained if only one of the five 65 g subsamples was contaminated with low levels (1–10 cells) of *E. coli* O157:H7. This was investigated at both 35°C and 42°C incubation temperatures, as well as at various incubation times. It was concluded that a positive VIP result would be obtained when compositing the 5 enrichment broths if 42°C was used as the incubation temperature. An incubation temperature of 35°C produced a 20% false negative rate, and therefore should not be used.

P2-55 Detection of Bovine Central Nervous System Tissue in Retail Meat Products by Real-time RT-PCR

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Detection of prohibited bovine central nervous system (CNS) tissue in meat products as a means of prevention of bovine spongiform encephalopathy and its human new variant, Creutzfeldt-Jacob disease, is an important task for food control. A sensitive and rapid method for the detection of bovine CNS tissue based on the amplification of mRNA for glial fibrillary acidic protein (GFAP) sequence by real-time RT-PCR has been developed. Total RNA was isolated from samples of meat products using Macherey–Nagel NucleoSpin RNA II kit (Macherey–Nagel GmbH & Co. KG, Duren, Germany). The primers and probe for the detection of GFAP mRNA were designed. The amplifications of the meat products samples were carried out in a final volume of 20 ml in a reaction mixture containing 10 ml of QuantiTect Probe RT-PCR Master Mix, 0.2 ml of QuantiTect RT Mix (Qiagen GmbH, Hilden, Germany), 2 ml of solution of primers and probe, 5.8 ml of water and 2 ml of sample. The final concentration of the solution of each primer was 1.0 mM and of probe was 0.2 mM. The probe was labeled with the fluorescent reporter dye 6-carboxyfluorescein (FAM) on the 5' end and with the Black Hole 1 (BH 1) fluorescent quencher dye on the 3' end. The amplifications were run in the LightCycler (Roche Diagnostics GmbH, Mannheim, Germany) using the following program: reverse transcription at 50°C for 20 min and initial activation step at 95°C for 15 min followed by 45 cycles at 95°C for 0 s and 60°C for 60 s. Dilution series. A model series of pasties containing 5, 1, 0.5, and 0.1% amounts of bovine CNS were prepared. All mixtures were homogenized in a blender and heated to 70°C for 10 min in a water bath. The samples containing bovine brain tissue were kept at 48°C for 8 days before RNA isolation. The set of 20 samples of pasties and 15 samples of cooked sausages from the market of the Czech Republic have been tested. Positive (pasty containing 0.5% of bovine brain) and negative (pasty without brain) control samples were run with each RNA isolation and real time RT-PCR amplification of the measured samples. It can be concluded that the method described in this paper is suitable for testing meat products for the presence of bovine CNS. The specificity of the primers and dual-labeled probe (TaqMan) has been tested using swine and chicken brains and bovine peripheral nerve RNA samples. The method allows the detection of 0.1% of the bovine CNS tissue in heat processed meat products. Twenty samples of pasties and 15 samples of cooked sausages have been tested for the presence of bovine CNS with negative results.

P2-56 Evaluation of Acid and Thermal Resistance Properties of Fluorescent-marked Nonpathogenic *Escherichia coli* Strains for Use as Surrogates for Enteric Pathogens

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The purpose of this study was to compare the acid and thermal resistance of non-pathogenic *Escherichia coli* Biotype I strains isolated from cattle hides with that of *E. coli* O157:H7 and *Salmonella*. Non-pathogenic *E. coli* strains with similar resistance properties could be used as surrogates for *E. coli* O157:H7 and *Salmonella* in carcass intervention validation studies when pathogens can not be utilized in commercial food processing environments. Thirteen strains, including fluorescent-marked and ampicillin-resistant *E. coli* Biotype I (4 strains), *E. coli* O157:H7 (5 strains) and *Salmonella* (4 strains, serotypes Agona, Anatum, Montevideo and Typhimurium) were tested for acid and heat resistance. The acid resistance of stationary-phase and acid-adapted cultures was evaluated in phosphate buffered saline (PBS) at pH values of 2.5, 3.0 and 3.5 (adjusted with 88% L-lactic acid) for 2 h at 37°C. The thermal resistance of stationary-phase and acid-adapted cultures was evaluated in PBS (pH 7.4) by heating bacterial suspensions in capillary tubes submerged in a water bath at 55, 60 and 65°C. *E. coli* Biotype I strains showed greater acid resistance than *Salmonella* and similar resistance to *E. coli* O157:H7 ($P < 0.05$). Reductions (log CFU/ml) at pH values of 2.5, 3.0 and 3.5 were: 4.2, 2.0 and 0.6 for *E. coli* Biotype I; 4.4, 2.2 and 0.5 for *E. coli* O157:H7 and 5.4, 2.7 and 1.7 for *Salmonella*, respectively. *E. coli* Biotype I strains were more tolerant to heat than E. coli O157:H7 and *Salmonella* ($P < 0.05$). D55°C values ranged from 29.7 to 40.2 min for *E. coli* Biotype I, from 18.8 to 22.8 min for *E. coli* O157:H7 and from 4.8 to 11.1 min for *Salmonella* strains. Similarly, heat resistance was greater for *E. coli* Biotype I than for *E. coli* O157:H7 and *Salmonella* at 60 and 65°C.

P2-57 DSC Use of Fluorescent Surrogate Organisms for Enteric Pathogens in Validation of Carcass Decontamination Treatments

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During the slaughter process, meat products can become contaminated with enteric pathogens such as *Escherichia coli* O157:H7 and *Salmonella* Typhimurium. Surrogates for these pathogens would be beneficial for validating carcass decontamination treatments. The surrogates proposed are non-pathogenic *E. coli* strains that were previously isolated from beef cattle hides. They were later transformed to express a fluorescent protein (red: RFP; green: GFP; yellow: YFP) that is detectable under an ultraviolet light source. Hot boned beef carcass surface areas (clod, brisket, outside round) were treated in a model spray cabinet, using an initial water wash (28°C). Then decontamination treatments such as 2% L-lactic acid (55°C), hot water (95°C at source) and a combination of the two were applied. Treatments were compared for their effectiveness at reducing populations of inoculated (4.7 to 6.7 log CFU/cm^2) *E. coli*, *S.* Typhimurium, RFP, GFP and YFP. Log reductions for each inoculated organism were calculated individually and then log reductions of the surrogates were averaged together and labeled "surrogate cocktail". All decontamination treatments reduced the inoculated numbers of pathogens and surrogates to near or below the detection limit of 0.5 log CFU/cm^2. The combined treatment resulted in the greatest log reductions. The three individual surrogate organisms varied in log reductions according to the different decontamination treatments applied; however, the log reduction for the surrogate cocktail did not differ significantly from that of *E. coli* O157:H7. With the exception of YFP, the surrogates and surrogate cocktail were significantly more resistant to microbial interventions, including lactic acid, than *S.* Typhimurium. Since abattoirs utilize different carcass decontamination treatments, it is difficult for one single fluorescent protein-producing isolate to accurately represent the behavior of *E. coli* O157:H7 or *S.* Typhimurium. Instead, surrogates should be used as a cocktail to accurately represent the effectiveness of different treatments for reduction of enteric pathogens.

P2-58 *Listeria innocua* as a Surrogate for *Listeria monocytogenes* for Aerosol Studies

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Aerosol studies for *L. monocytogenes* in food processing plants have been limited by lack of a suitable surrogate microorganism. The objective of the study was to investigate the potential of using *Listeria innocua* as a surrogate for *L. monocytogenes* for aerosol studies. These studies were carried out at ambient temperature in a laboratory bioaerosol chamber and a pilot food processing facility. Four strains of *L. innocua* and 5 strains of *L. monocytogenes*, all labeled with jellyfish green florescent protein genes, were used. In the first study, *Listeria* cells were released into the chamber by a nebulizer at 10^5 and 10^3 CFU/l air. Two conditions were used for airflow: (1) no fan use after plates were opened, and (2) continuous fan blowing throughout the entire 3-h study. Trypticase™ soy agar (TSA) plates, oven roasted breast of chicken, and oven roasted breast of turkey were placed on the floor of the chamber to monitor *Listeria* cell numbers deposited from aerosols. Every 30 min, new plates and meat were exposed and previously exposed samples were removed. A similar experimental design was used in the pilot plant study; however, only *L. innocua* was used. Results revealed that *L. monocytogenes* and *L. innocua* survived equally well on chicken and turkey breast meats and TSA plates. No fan and continuous fan applications had no significant effect on settling rates of aerosolized *L. monocytogenes* and *L. innocua* in the bioaerosol chamber. Aerosolized *L. innocua* behaved similarly in the pilot plant as in the bioaerosol chamber. Results indicate that *L. innocua* could be used as a surrogate for *L. monocytogenes* in an aerosol study.

P2-59 Host Range of *Listeria*-specific Bacteriophage from the Environment of Turkey Processing Plants in the United States

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Even though at least 400 *Listeria* phages have been isolated from various sources, limited information is available on phage from the environment of food processing plants. Phages in the processing plant environment may play critical roles in determining the *Listeria* population that successfully become established in the plant. In this study, we pursued the isolation of *Listeria*-specific phage from environmental samples from three turkey processing plants in the United States. These environmental samples were also employed to isolated *Listeria* spp. Nineteen isolated phages were classified in terms of their host range. Of these, twelve showed wide host range including multiple serotypes of *L. monocytogenes*, as well as one or more of other *Listeria* spp. (*L. innocua, L. welshimeri, L. seeligeri, L. ivanovii*). The remaining phages infected only certain strains of serotype 4b as well as *L. innocua, L. welshimeri,* and / or *L. ivanovii*. Serotype 4b isolates from the processing plant environment could be readily infected by the wide-range phages that were isolated from the environment of the same processing plants. However, many isolates of other serotypes (1/2a/3a and 1/2b/3b), which represented the majority of *L. monocytogenes* isolated from the environmental samples, were resistant to infection by these phages. Experiments with several of these isolates suggested that the resistance involved reduced phage adsorption onto the host cells. These findings suggest that phages in the processing plant environment may play important roles in determining the types of strains of *L. monocytogenes* that predominate in these environments.

P2-60 Molecular Epidemiology of *Listeria monocytogenes* Isolated from Brazilian Poultry Abattoirs

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Listeria monocytogenes (Lm) can be found throughout the food processing industry, including food contact surfaces and food handlers. The presence of Lm in two Brazilian poultry slaughterhouses differing only in automated (Plant A) versus manual (Plant M) evisceration was undertaken. Phenotypic [carbohydrate utilization, hemolysis, chromogenic media, antimicrobial susceptibility testing (MIC)] and genotypic [pulsed-field gel electrophoresis (PFGE), molecular serology] methods were used to characterize 221 strains isolated from 869 samples, comprised of workers' hands, factory environments, and raw food products, as well as water used for rinsing and chilling carcasses. Biochemical characterization revealed that 72.9% (161/221) and 71.9% (159/221) of strains were

positive for *Listeria monocytogenes* by use of blood hemolysis and chromogenic media, respectively. Molecular serotyping of Plant A isolates revealed that 61.6% of strains belonged to serotype 4b/4d/4e; 19.2% to 1/2a/3a; 4.0% to 1/2b/3b/7 and 15.2% to 1/2c/3c, whereas Plant M showed 72.9% strains belonging to serotype 1/2a/3a and 27.1% to 4b/4d/4e. Immunological serology on randomly chosen strains revealed that only a single 4b strain did not match the 1/2b/3b/7b molecular serology result. MIC testing using a disc diffusion assay showed 100% susceptibility to 11 antibiotics. Five strains were resistant to erythromycin, 5 to chloramphenicol and 127 to clindamycin. Intermediate resistance was observed for 1 strain by use of ciprofloxacin and 62 clindamycin. However, these strains had a small MIC break point with a spiral gradient endpoint assay. PFGE using ApaI showed identical pulsotypes for 74 and 92 strains of Plant M and A, respectively, whereas 67 and 106 strains from Plant M and Plant A had identical AscI pulsotypes. Serovars 1/2a/3a and 1/2c/3c clustered together, but separately from serovars 4b/4d/4e and 1/2b/3b/7, using AscI, with 1/2a/3a serovars clustering separately from the 1/2c/3c and 1/2b/3b/7 with ApaI. We are continuing to investigate the molecular epidemiology of *Listeria monocytogenes* to identify possible niches in Brazilian poultry slaughterhouses.

P2-61 Comparative Growth of *Listeria monocytogenes* on Ham Slices and in Ham Juice

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Accurate estimations of microbial behavior require the collection of numerous data which can involve time-consuming viable count determinations. Compared to enumeration in liquids, enumeration of microorganisms in solid food is complicated by particulates, multiple handling steps, higher potential for contamination, and fewer options for automation. In contrast, transparent matrices permit microbial kinetics to be studied more easily via optical measurements using automated systems. The objective of this study was to compare the behavior of *Listeria monocytogenes* growth on cured ham slices to that in filtered ham juice to determine if the latter would be an appropriate surrogate medium. Test matrices were ham juice (0.45µm filtered) and ham slices (5 cm – 5 cm – 0.5 cm), all prepared from the same irradiation-sterilized product. Samples were inoculated with ~10^4 CFU of a 6-strain *L. monocytogenes* cocktail in 0.1% peptone water. Samples were incubated at 10, 25 and 35°C, and at selected time intervals the levels of *L. monocytogenes* were determined by surface-plating samples on tryptic soy agar. Growth parameters were calculated for each storage condition. There was no difference ($P < 0.05$) in *L. monocytogenes* growth rates between the two matrices, with values of 0.039, 0.207 and 0.360 log CFU h-1at 10, 25 and 35°C, respectively. Similarly, lag phase duration was not different at 10 and 35°C (mean = 15.5 and 1.2 h, respectively), but was significantly lower at 25°C in ham juice than in slices (mean = 1.5 versus 3.9 h). In general, for each temperature, maximum population density was slightly lower on ham slices (range = 8.2-8.7 log CFU/cm^3) than in ham juice (range = 8.6-8.9 log CFU/cm^3). These findings demonstrate that filtered ham juice is a suitable medium to study the growth rate of *L. monocytogenes* on cured ham, and thus will facilitate experimentation using automated optical instrumentation such as Bioscreen®.

P2-62 Control by Competitive Bacteria of *Listeria monocytogenes* in Biofilms and *Listeria* sp. in Floor Drains in a Ready-to-Eat Poultry Processing Plant

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The ability of *L. monocytogenes* and two competitive bacteria, *Lactococcus lactis* subsp. *lactis* (#C-1-92) and *Enterococcus durans* (#152), to form biofilms on coupons comprised of different materials (plastic, rubber, glass and silicone) was determined at 4 and 8°C. Characteristics of biofilms formed on stainless steel were observed by scanning electron microscope (SEM). Well-formed biofilms of *L. monocytogenes* produced at 37°C for 24 h on the surfaces of coupons were treated with the two competitive bacteria individually at either 4 or 8°C for 3 weeks and assayed for reduction of *L. monocytogenes*. These two competitive isolates were further evaluated as a treatment of floor drains in a Ready-to-Eat poultry processing plant for control of *Listeria* sp. Results revealed that *L. monocytogenes* and the two competitive isolates could form biofilms on coupons as determined by SEM. Treatment of biofilms of *L. monocytogenes* on coupons with #C-1-92 or #152 at 4°C for 3 weeks reduced listeriae from 6.3–7.3 to 4.0-5.5 and 3.7-5.5 log CFU/cm^2, respectively; and at 8°C for 3 weeks reduced listeriae from 7.2-8.3 to 2.4-4.7 and 3.8-5.4 log CFU/cm^2, respectively, depending on the coupon composition. For in-plant studies, six floor drains were consistently positive for *Listeria* spp. before competitive bacteria treatment, with a maximum *Listeria* spp. count in drains of 100 CFU/100 cm^2. Following four competitive bacteria treatments within one week, 5 of 6 drains were *Listeria* spp.-negative, and all drains were *Listeria* spp.-negative at 8 weeks following a series of 4-week treatments. Control floor drains that were not treated with competitive bacteria were *Listeria* spp.-positive throughout the study.

P2-63 Multiple Antibiotic Resistances of *Escherichia coli* Isolated from Commercial Broiler Chicken Farms

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Farm animals can carry in their gastrointestinal tract some pathogenic *Escherichia coli* that can cause serious infections in humans. The broiler industry uses non-therapeutic antibiotics in feed to improve gain and feed conversion and to control sub-clinical diseases; however, there is a concern that this practice leads to the development of antibiotic-resistant bacteria. In this study, a total of 158 *E. coli* strains were isolated from ceca, fecal, and litter samples from nine broiler chicken farms using antibiotic feed supplementation in the Fraser Valley (British Columbia). All the *E. coli* isolates were identified by API 20E and their susceptibility to 18 antimicro bials were determined by Sensititre, using the Clinical and Laboratory Standard Institute's breakpoints. Detection of virulence and tetracycline resistance genes was performed by PCR. Overall, more than 50% of the tested strains were multi resistant. All the *E. coli* strains were resistant to penicillin, erythromycin, tylosin, clindamycin, and novobiocin and displayed different resistance levels to the other antibiotics. Among the 43 isolates screened by PCR, 14% and 34.8% were positive for eaeA and e-hlyA, respectively. Of the 49 tetracycline resistant strains tested, 28.6% and 75.5% were positive for tetA and tetB, respectively. These findings show that atypical and multi-antimicrobial-resistant *E. coli* strains are present in chicken production and support current recommendations for prudent use of antibiotics in broiler chicken production.

P2-64 Antibiotic Resistance of *Enterococcus* spp. Isolated from Broiler Chicken Farms Using Antimicrobial Agents as Growth Promoters

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Enterococci are ubiquitous, commensal inhabitants of the gastrointestinal tract of humans and animals. However, *enterococci*, particularly *E. faecium* and *E. faecalis*, are important in food safety and public health. The antibiotic resistance of these bacterial isolates from nine broiler chicken farms in British Columbia was determined. A total of 71 *enterococci* were isolated from chicken fecal and litter samples and the strains were identified by API 20 Strep. Susceptibility to antibiotics was performed using the Sensititre test, with the National Antimicrobial Resistance Monitoring System plate for enteric bacteria and by the agar dilution method using the Clinical Laboratory Standard Institute's breakpoints. Four species of *enterococci* (49 *E. faecium*, 8 *E. gallinarum* 7 *E. faecalis* and 7 *E. durans*) were identified from this study. While 70% of the *enterococci* strains were resistant to bacitracin, ampicillin, lincomycin, tetracycline, kanamycin, virginiamycin, tylosin, and quinupristin/dalfopristin, all of them were found to be susceptible to chloramphenicol, flavomycin, and linezolid. 100% and 4% of *enterococci* were resistant to salinomycin and vancomycin, respectively. Overall, more than 50% of the tested strains were multi resistant. Results from this study showed that pathogenic (*E. faecium* and *E. faecalis*) and vancomycin resistant *enterococci* can be isolated from broiler chicken production and can be multiresistant to antimicrobials used in human medicine. The resistance spectrum of our isolates from poultry farms suggests that *enterococci* might be useful as indicators of the development of antibiotic resistance resulting from the usage of antimicrobial agents as growth promoters in poultry production.

P2-65 Comparison of Retail Raw Chicken Carcasses Bought from Two Different Grocery Stores for Total *Campylobacter* and Total Ciprofloxacin-resistant *Campylobacter* Loads in 2005

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Over a period of 27 weeks a total of one hundred-eight retail raw chicken carcasses (RTCC) from two different grocery stores in Fayetteville, Arkansas, were sampled at the rate of four carcasses per week. The RTCC sampled from two different stores were processed by one commercial processor but at different processing plants. Thermophilic-group of total *Campylobacter* CFU and total ciprofloxacin-resistant *Campylobacter* CFU (irrespective of species) were concurrently quantified in rinses form RTCC by direct plating of centrifuged pellets from 50/400 ml rinse sub-samples concurrently on *Campylobacter* agar (CA) and ciprofloxacin-containing *Campylobacter* agar (CCA) and incubated at 42°C. The minimum detection limit for the *Campylobacter* count was 0.90 log CFU/carcass. Countable *Campylobacter* CFU were recovered from 94% and 51% of RTCC sampled from grocery-store A and B, respectively. Countable ciprofloxacin-resistant *Campylobacter* CFU were recovered from 41% and 12% of RTCC sampled from grocery-store A and B, respectively. Total *Campylobacter* and total ciprofloxacin-resistant *Campylobacter* loads in RTCC rinses ranged from 0.90–4.60 and 0.90–3.86 log CFU/carcass, respectively, for grocery-store A, and 0.90–4.38 and 0.90–2.64 log CFU/carcass, respectively, for grocery store B. These results indicate continuing persistence and high variability in ciprofloxacin-resistant *Campylobacter* loads in RTCC sampled from different grocery stores in 2005, and lower counts of store B samples correlated with lower product temperature at purchase.

P2-66 Contamination of the Surface of Beef Carcasses with *Mycobacterium avium* subsp. *paratuberculosis*

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Johne's disease is an infection of the gut tissue of cattle and other animals caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The organism may be involved in Crohn's disease, a gut condition in humans similar to Johne's disease. Although a connection between the two diseases has not been established, reduction of the exposure to MAP is being sought in some countries, as a preventative measure. Animals infected with MAP can shed large numbers of the organisms in feces. Since infection of cattle herds with MAP is common, contamination of beef carcasses with MAP, along with other fecal organisms, must be expected. However, there is no published data on MAP contamination of beef carcasses. To obtain initial information about the presence of MAP on beef carcasses, swab samples were collected from 100 cm^2 crotch regions of beef carcasses after skinning but before evisceration and after completion of dressing and pasteurizing. Samples were processed for the detection of MAP DNA, using a quantitative real time polymerase chain reaction (PCR). The PCR tests found that 4% of skinned and 6% of pasteurized culled beef carcasses samples was positive for MAP. Using a highly sensitive nested PCR test, 45% of skinned and 69% of pasteurized carcasses were positive. The findings indicate that while MAP may be frequent on beef carcasses, they may be present at numbers of the order of only one organism per 100 cm^2.

P2-67 Culture of *Mycobacterium avium* subsp. *paratuberculosis* from Edible Tissues of Johne's Infected Cattle

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Johne's disease, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a chronic granulomatous infection of the intestinal tract of wild and domestic ruminants. Survival within macrophages is a hallmark of MAP, which could allow them to be transported throughout the body of an infected animal by way of the circulatory and lymph systems. Although not much work has been done attempting to culture viable MAP from blood, a landmark paper was published by Nasser et al. describing the culturing of viable MAP from the blood of Crohn's patients. Therefore it is not improbable that MAP may be found in muscle tissue via the circulatory system. Our laboratory tested various tissues from five cattle diagnosed with advanced Johne's Disease, using a MAP PCR method that was validated using a USDA fecal checktest samples and cultured on Herrolds egg yolk agar. We tested 17–18 tissues samples from each of the five cattle. Twenty-three of the 83 tissues tested were found to be PCR positive for MAP. The ileum, ileocecal lymph node, and mesenteric lymph node were positive for viable MAP in all five animals. Fecal samples were available for only 2 of

the cattle, but in both cases they were positive for viable MAP. We also isolated viable MAP from the following tissues: tenderloin (2/5), shinshank (1/5), prepectoral lymph node (1/5), kidney (1/5), liver (1/5), short ribs (1/5), and ribs-prepared muscle (1/5). Although no preliminary decontamination on the outside of the meat samples was done, we believe these results may indicate that MAP is a systemic infection and it is possible that low levels of viable MAP exists within edible muscle tissue. Further research must be done to verify these findings and investigate possible kill steps such as freezing and cooking.

P2-68 The Use of a Novel Sample Preparation and PCR/ECD-based Assay for the Detection of *Mycobacterium avium* Subspecies *paratuberculosis*

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Mycobacterium avium ssp. *paratuberculosis* (MAP) is recognized as the causative agent of Johne's disease in cattle and may also be linked to Crohn's disease in the general population. The threat of MAP transmission arises from environmental sources and dairy products. MAP's menacing presence in the cattle industry and potential for human transmission pointedly aims at the necessity for rapid detection. Here, we have combined the use of a new method for cell lysis with PCR and an electrochemical biosensor system for both rapid and sensitive detection of MAP derived from bovine samples. A one milliliter bacterial suspension was centrifuged for 5 min to form a pellet and the supernatant discarded. The pellet was resuspended in 1 mL of PBS and centrifuged again for 5 min. The supernatant was removed and the remaining pellet was resuspended in 100 uL of AnzenBio UltraLyse and then placed in a heating block for 30 min at 95°C. Samples were then centrifuged for 1 min at high speed. Five microliters of the supernatant was added to the PCR primer/mastermix solution (25 ul reaction volume) and amplified on a MJ Research thermocycler. The target DNA was amplified using biotin and 6-FAM labeled primers designed to hybridize to MAP IS900. The PCR product was analyzed by electrochemical detection (ECD) biosensors and gel electrophoresis. The efficacy of the bisensors will be compared to the gel analysis method.

P2-69 Effects of pH Enhancement on Consumer Ratings of Various Meat Products

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The objective of this research was to determine the effect of pH enhancement with ammonium hydroxide on consumer acceptability of deli-style roast beef (RB), grilled chicken breast (CK), grilled pork loin (PL), beef prime rib (PR), beef pot roast (PT), and barbeque beef brisket (BR). Approximately one-half of the products served as controls (CON), while the others were pH enhanced (PHE) to approximately 120% by weight with a solution containing water, ammonium hydroxide, carbon oxide, and salt using a patent process of Freezing Machines, Inc. To produce RB, USDA Select, *semimembranosus* and *adductor*, muscles were seasoned, cooked in a smokehouse to 59°C (held 1.5 h), cooled, sliced (.3 cm), and served cold. Chicken breast was grilled on gas grills to 77°C and then cut into 2.5 × 1.3 cm cubes. Chops (2.5 cm) were cut from PL and cooked on gas grills to 71°C. On each panel day, PR, USDA Select, *longissimus* only, was seasoned, cooked in a smokehouse to 54°C (held 1.9 h), cut into steaks (2.5 cm), and then cut into wedge-shaped pieces for serving. To produce PT, USDA Select, *triceps brachii*, was seasoned, cooked "sous vide" in a smokehouse to 82°C (held 3 h), cooled, shredded, and reheated to 74°C for serving. To produce BR, USDA Select, *pectoralis profundi*, was seasoned, smoked and cooked in a smokehouse to 82°C (held 1 h), cooled, sliced (.4 cm), barbecue sauce added, and reheated to 74°C for serving. A CON and PHE sample of each product was served to a diverse population of consumers (n = 196) in the order as listed above, and rated on 10-pt scales for palatability characteristics. Products were evaluated for raw purge loss, cooking loss, and calculated moisture retained in cooked product. Control had less purge loss than PHE for RB, CK, PR, PT, and BR, while control had less cooking loss than PHE for RB and BR ($P < 0.05$). Although purge and cooking losses were higher for PHE, all PHE products had higher calculated moisture retained in the cooked product than respective CON product ($P < 0.05$). Consumer mean ratings for 'Overall Like' were higher for PHE than CON for RB (6.90 vs. 6.49), CK (6.83 vs. 6.19), PL (7.13 vs. 5.33), PR (8.36 vs. 7.90), PT (8.15 vs. 7.76), and BR (7.50 vs. 6.92) ($P < 0.05$). Ratings for 'Like of Flavor', 'Like of Texture/Tenderness', and 'Like of Juiciness' were higher for PHE than CON for all six products with the exception of 'Like of Juiciness' for RB and 'Like of Flavor' for PT ($P > 0.05$). When asked "Overall, which sample do you prefer?" a majority of consumers responded in favor of PHE: 60.5% for RB, 64.3% for CK, 89.1% for PL, 65.1% for PR, 60.2 for PT, and 64.1% for BR ($P < 0.05$). In conclusion, pH enhancement was effective at improving percent moisture retained after cooking, thus yielding a juicier end product. Moreover, pH enhancement was successful at improving consumer palatability ratings of various meat products, especially grilled pork loin.

P3-01 Pellicle Formation in Hot-smoking of Salmon: Smoke Decreases Survivability of *Listeria* and *Staphylococcus* Species

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Other researchers have indicated that bacteria may resist the inhibitory action of wood smoking by protection under a protein film that develops after brining of fish. When dried during hot-smoking of salmon, a pellicle forms and may prevent phenolic and acid compounds in smoke from interacting with bacteria to cause cell death. The objective was to determine survivor rates for *Listeria* and *Staphylococcus* species during drying and hot-smoking processes of inoculated salmon. Salmon fillet strips (~100 g) were brined in 5% NaCl for 24 h then drained. Overnight cultures of *L. innocua* ATCC 33090 and *S. epidermidis* ATCC 14990 were grown in brain heart infusion and 100 µl spread on each drained strip (approximately 10^8 cells/g of pre-pellicle). Strips were air dried by fanning for 0.5 hr then hot-smoked (final temperature of 72°C) or processed without smoke in a programmable smokehouse. Alternatively, 60% liquid smoke was substituted for wood smoke. During the 6-h, five-step smokehouse program, pellicle samples from several strips were excised and diluted in 0.1% peptone water. Serial dilutions were surface-plated on Baird-Parker agar and incubated for enumerating

S. epidermidis and on modified Oxford agar and incubated for enumerating *L. innocua* and survival curves were plotted. Pellicle thicknesses of 0.1–0.3 mm formed on hot-smoked salmon. Strips in which smoke was added initially, the *L. innocua* and *S. epidermidis* counts declined 4–6 logs in the product. When no smoke was added, a 2–3 log reduction occurred. Liquid-smoked strips resulted in a 3-log reduction early in the smokehouse program followed by a 2-log reduction. Liquid smoke added at a middle step showed a 4-log reduction while controls showed 3-log reductions. We conclude wood chip smoke or liquid smoke addition was more effective than its absence at reducing *L. innocua* and *S. epidermidis* in smokehouse-processed salmon strips.

P3-02 Influence of Processing Steps in Cold-smoked Fish Production on Survival and Growth of *Listeria monocytogenes*

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Cold-smoked fish products have been defined as high risk products with respect to the foodborne pathogen *Listeria monocytogenes*. Generally, when producing cold-smoked fish, the raw fillets are brined to 3–6% NaCl in the water phase of the final product, matured, smoked and dried at 22–28°C, frozen, sliced, and kept at refrigeration temperature. These processing steps are not believed to eliminate the bacterium. The purpose of the present study was to examine the possible reducing effect of various processing steps in the cold-smoking process on different strains of *L. monocytogenes*. In particular, we assessed if persistent strains were more or less sensitive than, e.g., clinical strains. The effect of salting and smoking on the level of lactic acid bacteria and total aerobic counts were studied in an industrial setting where the combined steps reduced the aerobic count (on Long and Hammer agar) by 1–2 log CFU/cm^2. Dry-salted and brine injected salmon fillets were surface inoculated with approx. 10^3 CFU *L. monocytogenes*/ cm^2 and dried and smoked at 24°C. This caused an immediate reduction of 1–2 log CFU/cm^2. No systematic variation between strains was detected ($P > 0.05$). *L. monocytogenes* was inoculated between two slices of cold-smoked salmon to at a level of 10^3 per g and vacuum packed. This level did not change during storage at -20°C or -40°C for 30 days; however, *L. monocytogenes* grew to about 10^7 CFU/g when the fish were thawed and stored at 5°C. Growth rates of the strains exposed to a freezing period were similar to strains inoculated directly on cold-smoked salmon stored at refrigeration temperature. However, the former had an extended lag phase when refrigerated. In conclusion, cold-smoking appeared to reduce numbers of *L. monocytogenes* significantly ($P < 0.001$), and short term frozen storage under controlled laboratory conditions had no effect on the *L. monocytogenes* level.

P3-03 Survival of *Listeria monocytogenes* on the Surface of Domestic Raw Shrimp Stored at Frozen Temperatures with a Cetylpyridinium Chloride Wash

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This study determined the concentration of CPC that would effectively reduce *Listeria monocytogenes* on the surfaces of white shrimp stored at -20°C. An 18 h Lm culture was decimally diluted and 1 ml was inoculated onto the surface of shelled raw, shelled cooked or peeled raw shrimps (5 g). The samples were allowed to air dry under a laminar flow hood for 1 hour and then treated with different concentrations of CPC (0, 0.05, 0.4, or 1.0%) solutions for 1 min, with or without a water rinse for 1 min. The samples were frozen at -20°C and bacterial counts determined on day 0, 7, 14, 21, 30, 60, and 90. Samples were plated onto Oxford agar, incubated at 37°C for 48 h and the log CFU/g determined. Peeled raw shrimps that were not treated with CPC on day 0 had significantly lower *Listeria monocytogenes* counts then the shell-on raw or cooked shrimp. Lm counts on all shrimps treated with 1.0% CPC with or without a water rinse were significantly reduced from the control non-treated shrimp sample on day 0 with the largest reduction of 3.0 log CFU/g occurring for the shell-on cook shrimp. When *Listeria monocytogenes* -inoculated CPC-treated shrimp with or without a water rinse were stored at -20°C for 90 days, all concentrations of CPC were significantly lower than the controls, regardless of shrimp type. The largest reduction of *Listeria monocytogenes* counts after 90 days at -20°C occurred on the surface of shell-on cooked shrimp when treated with 1.0% CPC. *Listeria monocytogenes* counts were significantly reduced (2.25 log) with a water rinse and 1.69 log without a water rinse from control counts. Our study has shown the potential of CPC as a washing solution to reduce *L. monocytogenes* on the surface of raw and cooked shrimp stored at freezer temperatures.

P3-04 Withdrawn

P3-05 Study of the Efficacy of Peroxyacetic Acid, Chlorine Dioxide and Ozone for Inactivating *Vibrio parahaemolyticus* and *Escherichia coli* on Black Tiger Shrimp (*Penacus monodon*)

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The objectives of this study were to determine the efficacy of peroxyacetic acid (PAA) (60, 80, 100 ppm), chlorine dioxide (ClO_2) (3, 5, 10 ppm), and ozone (O_3) (0.2, 0.5, 0.8 ppm) to reduce *Vibrio parahaemolyticus* and *Escherichia coli* inoculated on black tiger shrimp under the effect of low temperature (10°C) and ambient temperature (30°C) washing and the influence to the survivors after treatment during chilled (4°C) and frozen (-18°C) storage. The study showed that the proper conditions of washing treatment at 30°C to inactivate *V. parahaemolyticus* (3.2–3.9 log CFU/ml) on shrimps were PAA 60 ppm 10 min, ClO_2 5 ppm 10 min, and O_3 0.5 ppm 15 min. These conditions achieved the *V. parahaemolyticus* reduction of 99.02%, 94.35%, and 92.35%, respectively, while tap water (15 min) only reduce 92.92%. Meanwhile, *E. coli* (3.5–4.0 log CFU/ml) were inactivated by PAA 60 ppm 10 min, ClO_2 5 ppm 15 min, and O_3 0.2 ppm 10 min, resulted to the reduction of 99.14%, 94.72%, and 93.28%, respectively, whereas, 80.61% reached by tap water (15 min). PAA exhibited the highest efficacy for inactivating *V. parahaemolyticus* and *E. coli* on shrimps (~2 log reduction). The further study of low temperature washing (10°C) showed that the efficacy of PAA was not greatly affected by the temperature. Storage studies at 4°C showed *E. coli* was not detected in washed shrimp since the first day of storage, whereas *V. parahaemolyticus*, except for the samples treated with PAA, can be detected until day 4. Both microorganisms were able to survive until the end of the 4°C storage experiment (day-10), but they were able to survive until day-30 of frozen storage at -18°C.

P3-06 The Response of Human Viruses and Viral Surrogates in Oyster Slurry to Hydrostatic Pressure

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Viral foodborne infections account for approximately 80% of the cases of foodborne illness caused by known etiological agents. For illnesses attributed to consumption of contaminated shellfish, oysters are the most commonly implicated type, as they are often consumed raw or partially cooked and can concentrate viruses like hepatitis A virus (HAV), Aichi virus (AiV) and noroviruses. High hydrostatic pressure (HHP) processing is a non-thermal treatment that can minimize microbial contamination while maintaining the fresh quality of a food product and tested in this study on a model food system, an oyster slurry. HHP was used to inactivate human pathogens HAV and AiV as well as viral surrogates feline calicivirus (FCV) and F-specific RNA bacteriophage Q. TCID50 and plaque assays in mammalian and bacterial cells were used to determine virus inactivation. The oyster slurry was inoculated with virus, mixed, and separated into equal size samples, which were pressure treated at 250, 400, and 500 MPa for 1 or 5 min. at 9°C. The virus was recovered, serially diluted, and analyzed for infectivity. After a 500 MPa treatment, HAV was reduced by >5-log PFU/ml and at 250 MPa there was a 1-log PFU/ml reduction. FCV was reduced by >2-log PFU/ml at 500 MPa and no reduction was seen at 250 MPa. There was no reduction of AiV after 500 MPa. Q was reduced by 0.13-log PFU/ml at 400 MPa for 5 min, and by 0.6-log PFU/ml at 500 MPa for 1 min. Increasing the temperature to 60°C and 80°C increased reduction of Q by 1.5 and 7 log ($P < 0.05$); however, increasing hold time to 10 min did not enhance inactivation. While the need for surrogates is great, they differ in resistance to HHP compared to pathogens in oyster slurry and the role of temperature must be considered.

P3-07 Prevalence and Numbers of *Vibrio parahaemolyticus* in Korea Retail Oysters as a Function of Environmental Factors

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Vibrio parahaemolyticus is a halophilic estuarine organism and is frequently isolated from coastal waters and seafood. It is one of the leading causes of foodborne disease in Korea. Outbreaks caused by *V. parahaemolyticus* occur mainly in August and September with a few cases in May. Recently *V. parahaemolyticus* outbreaks in September have been increasing. In this study, total viable bacteria and *V. parahaemolyticus* in oysters at retail were monitored monthly using a Bacteriological Analytical Method. In addition, we investigated the relationship between the prevalence/level of *V. parahaemoyticus* in oysters and environmental factors such as seawater temperature, seawater salinity and air temperature. Counts of 3.81~5.19 log MPN/g total viable bacteria and 2.12~4.39 log MPN/g for *V. parahaemolyticus* were detected in oysters purchased from May through October at retail and *V. parahaemolyticus* was not isolated in December. The level of *V. parahaemolyticus* in oysters in September was slightly higher than that in August, which might be attributed to the higher seawater temperature in September (24°C) than in August (21°C). The numbers of *V. parahaemolyticus* were greatly influenced by the seawater temperature (r=0.77) among the environmental factors tested in this study. Lower numbers of *V. parahaemolyticus* were isolated from retail oysters stored in the icebox than from retail oysters packed in salt water at ambient temperatures, or oysters on ice and exposed to air. This quantitative data can be used for risk management plan and establishment of a Food Safety Objective (FSO) for *V. parahaemolyticus* in oysters at retail as well as to establish intervention strategies to reduce the health risks associated with raw oyster consumption in Korea.

P3-08 Change of Hygienic Quality and Freshness in Tuna Treated with Electrolyzed Water and Carbon Monoxide Gas during Refrigerated and Frozen Storage

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Among different fish slices used for sashimi preparation, tuna is the most popular and preferable fish type for Taiwanese people. To improve the hygienic quality of fish slices, electrolyzed (EO) water containing 10, 50 and 100 mg/L chlorine, was used in combination with CO gas treatment. Effect of different treatment on aerobic plate count (APC), volatile basic nitrogen (VBN), K value and Hunter L*, a*, b* values of yellow-fin tuna steak during storage (4°and -20°C) were evaluated. It was found that APC, VBN and K values increased with storage time for all treatment. Except for K value, APC and VBN of tuna steak treated with the combination of more than 50 mg/l chlorine EO water and CO gas had the lowest value after 8 days of refrigerated storage. Hunter a* value of tuna steak treated with only CO gas was the highest, followed by those treated with EO water and CO gas. These demonstrated that EO water containing 50 mg/L chlorine combined with CO gas treatment in tuna fish steak would be an effective method for enhancing the hygienic quality and freshness for tuna meat and extending refrigerated storage time. Tuna treated with EO water containing 100 mg/l chlorine and CO gas combination had the lowest APC immediately after treatment and reduced further to below detection limit after 1 month frozen storage at -20°C.

P3-09 *Vibrio vulnificus*-related Deaths and Illnesses, 1996 – 2005

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Vibrio vulnificus is a bacterium from the same family as those that cause cholera and it normally lives in warm seawater. *V. vulnificus* can cause disease in people who eat contaminated seafood or have an open wound that is exposed to seawater. Among immunocompromised persons, particularly those with chronic liver disease, *V. vulnificus* can infect the bloodstream, causing a severe and life-threatening illness. *V. vulnificus* bloodstream infections have a 50% fatality rate. A recent study showed that people with these pre-existing medical conditions were 80 times more likely to develop *V. vulnificus* bloodstream infections than were healthy people (CDC). The bacterium is frequently isolated from oysters and other shellfish in warm coastal waters during the summer months. *V. vulnificus* is a rare disease, but it is also underreported. While there is no national surveillance system for *V. vulnificus*, Alabama, Florida, Louisiana, California, Texas, and Mississippi have reported over 300 *V. vulnificus* illnesses and deaths between 1996 and 2005, an average of 33 cases of *V. vulnificus* each year. In April 2003, California public health officials banned the sale of raw oysters harvested in warm months from the Gulf of Mexico because of concerns about *V. vulnificus*. The ban prevents California retailers, distributors, and wholesalers from accepting raw Gulf Coast oysters harvested during April through October unless the oysters have been processed

to eliminate *Vibrio*. Between 1996 and 2002, there were 38 *V. vulnificus* illnesses and deaths in the state of California. The April 2003 ban of Gulf Coast oysters in California reduced the number of *V. vulnificus* illnesses from an average of five each year to zero in each year following the implementation of the ban.

P3-10 DSC Multi-locus Sequencing Used for Identification of a New Species of *Morganella* Associated with Outbreaks of Histamine Poisoning

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All over the world cases and incidents of histamine fish poisoning (HFP) are important problems. Histamine that causes HFP when ingested in high concentrations is formed by bacterial decarboxylation of histidine. *Morganella morganii* is the best known histamine producing bacterium from seafood. Below 7–10°C this organism produces very little histamine. Nevertheless histamine in toxic concentrations is observed in seafood stored at 0–5°C. This study describes the identification of a novel psychrotolerant species of *Morganella* with the ability to grow and produce toxic concentrations of histamine in seafood at 2°C. Nineteen *Morganella* isolates from seafood, one clinical strain as well as the type strains of *Morganella morganii* subsp. *morganii* and *Morganella morganii* subsp. *sibonii* were subjected to polyphasic taxonomic analysis including multi-locus sequencing. Phylogenetic analysis of fragments from seven housekeeping genes (atpD, dnaN, gyrB, hdc, infB, rpoB and tuf) and 16S rDNA sequences divided the strains into two distinct groups that perfectly comprised the mesophilic and psychrotolerant isolates, respectively. The 16S rDNA sequence similarity between the two groups of strains was 98.6%, but sequences of all the housekeeping gene fragments showed dissimilarities higher than 9.1%. This clear separation was supported by DNA-DNA hybridization studies. The psychrotolerant isolates grew at 0–2°C and also differed from the mesophilic *M. morganii* isolates with respect to growth at 37°C, growth with 8% NaCl and fermentation of D-galactose. For this new species the name *Morganella psychrotolerans* sp. *nov.* is proposed. *M. psychrotolerans* has been isolated from both fresh and cold-smoked tuna products involved in incidences of HFP in Denmark. Furthermore, *M. psychrotolerans* produced toxic concentrations of histamine in a challenge test at 2°C. Thus *M. psychrotolerans* is important for histamine formation in chilled seafood and this novel species deserves further attention in an attempt to reduce the frequency of HFP.

P3-11 Summary and Recommendations of the AOAC Presidential Task Force on Best Practices for Validation of Microbiological Methods

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In 2005, supported by the U.S. Food and Drug Administration, AOAC established a Task Force to identify the best practices for validation of microbiological methods for food analysis (BPVMM). One of the key objectives was to determine appropriate levels of validation and performance criteria depending on the intended purpose of the method; e.g., screening, acceptance sampling, regulatory testing, etc. The task force recognized that the precision, or "Uncertainty of Measurement" of data generated by a microbiological method might be different, but still acceptable, depending on the application. Therefore, validation procedures and statistical analysis must consider the application of the method and be designed to provide the level of confidence needed (fit-for-purpose). The task force recommended the use of international approaches to define the performance characteristics of methods, including uncertainty. The task force also made recommendations on levels of validation necessary to extend the application of already validated methods to other matrices and target organisms. Another key development was the recommendation of alternative methods for determination of levels and limits of detection, and validation of methods in the absence of reference methods. It was also recommended that methods studies be designed to allow an LOD_{50} to be determined for qualitative methods, rather than the traditional approach of comparing the number of positives and negatives to a reference method. In 2006, the Task Force is focused on developing specific guidelines and procedures for validation of microbiological methods; including those for detection of toxins and viruses that can be considered for adoption by AOAC and other organizations. Special attention is being given to developing procedures to be employed when a reference method does not exist or is less sensitive than the new method. In addition, the task force will recommend procedures for verification of validated method performance by the user laboratories.

P3-12 Isolation and Characterization of Lactic Acid Bacteria Isolated from Raw Fish and Nham-Pla, a Thai Fermented Fish Sausage with Respect to Their Antibacterial Activity and Probiotic Properties

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A total of 135 lactic acid bacterial strains were isolated from raw fish and nham-pla, a Thai fermented fish sausage in order to select the most suitable strains for probiotic formulation of fermented fish starter cultures, according to their antibacterial activity and probiotic properties. Among those bacterial strains, 30 strains were found positive to inhibit at least one species of four bacterial species, including *Staphylococcus aureus, Listeria monocytogenes, Pediococcus acidilactici*, and *Lactobacillus bulgaricus*, using the agar spot test method, and were further screened for their acid, salt and bile tolerance. Two of these thirty strains exhibited the highest resistance to acid, sodium chloride, and bile salts by tolerating the lowest pH of lactic acid (pH 2.9) and hydrochloric acid (pH 2.2), and the highest concentration of sodium chloride (6%) and bile salts (5.25%) in MRS broth, and were found to inhibit additional bacterial species, such as *Bacillus cereus, Escherichia coli*, and *Vibrio parahaemolyticus*. The isolates were identified using sequence analysis of 16S rRNA, and showed to be *Lactococcus lactis* and *Lactobacillus curvatus*. These cultures were further characterized for their fermentation ability. Effects of temperature (25°C, 30°C, and 35°C), sodium chloride (0%, 1.0%, 1.5%, 2.0%, and 2.5%) and garlic (0%, 1.0%, 3.0%, and 5.0%) on growth of these lactic acid bacteria in Nham-Pla model broth during 5-day fermentation were investigated. The concentrations of 1% sodium chloride and 1% garlic were suitable for fermentation with good growth of these lactic acid bacteria at 30°C. The pH level of the fermentation medium was decreased to < 4.5 within 1 day of fermentation. These results indicated that these two strains of lactic acid bacteria could potentially be used as probiotic starter cultures for fermented fish products.

P3-13 Rapid Detection of the *Vibrio cholerae* ctx Gene in Food Enrichments Using Real Time PCR

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Standard methods for the detection of *Vibrio cholerae* in food are based on enrichment in alkaline peptone water (APW) followed by isolation on selective agar and identification using biochemical utilization patterns, toxigenicity assays and serological confirmation. The current FDA BAM method employs conventional PCR to detect the cholera toxin (ctx) gene from APW enrichments. However, recent developments in real time PCR (qPCR) methods allow more rapid detection of the target genes. This study was conducted to validate a qPCR assay for ctx gene detection in food. Five food matrices (milk, bottled water, shrimp, potato salad and oysters) were artificially contaminated with a low inoculum (1–2 CFU/g) of three toxigenic strains of *V. cholerae* O1. Six lots of each food type were inoculated with each of the strains and diluted (1/10 and 1/100) samples were enriched in APW at 42°C for 6 and 18 h. *V. cholerae* in the enriched samples were detected by streaking onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar and by the real-time PCR method that utilizes a TaqMan® –style probe to target the B subunit of the ctx operon. The frequency of detection of the ctx B subunit by PCR was 98% (88/90) and 100% (90/90) after 6 and 18 h enrichment, respectively. Streaking on TCBS agar detected 87% (78/90) and 83% (75/90) after 6 and 18 h, respectively. Low detection rates were observed with shrimp and oysters due to the presence of organisms that were difficult to distinguish from *V. cholerae* on the plates. It was necessary to dilute oysters and potato salad samples 1:100 for enrichment in APW as 1:10 dilutions were often inhibitory to PCR. The data indicate that this real-time PCR assay for ctx gene B subunit is a reliable, sensitive and rapid alternative to the culture method and is applicable to diverse food products.

P3-14 Enumerating Chromogenic Agar Plates Using the Color QCount Automated Colony Counter

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Over the past decade, chromogenic substrates have been used in culture media for the detection, isolation, identification, and enumeration of microorganisms. Agar plates with chromogenic substrates will produce colonies with various colors and the hand-counting method used to differentiate and enumerate these colonies is time-consuming and laborious. The Color QCount was developed to improve efficiency in the microbiology laboratory by automatically counting colonies by color. In this study, color colony counts prepared using mixed microbe cultures were compared using both automated and manual count methods. Inoculum levels were prepared by serial dilution in a phosphate buffer to span the total counting range of 30–300 CFU/ml, 26 chromogenic agar types were used, and a total of 581 plates were analyzed. Plates were prepared, inoculated, and incubated according to manufacturers instructions, manually counted once by 2 scientists, and counted in duplicate automatically. The Pearson correlation coefficient comparing the automated and manual counts for the entire pooled population of data was 0.987. The slope and intercept for the linear regression line were 1.0067 and 0.031, respectively. The mean log value difference between the automated and manual count methods for pooled data was -0.042. The mean log value differences between the manual and automated counts demonstrated that 85.6% of the plates analyzed were within 0.1 log and 98.2% were within 0.2 log. These results demonstrate that the Color QCount automatic colony counter is a suitable alternative to the standard method of manually counting colonies by color on chromogenic agar plates.

P3-15 Induction of Guaiacol Production in *Alicyclobacillus acidoterrestris* ATCC 49025 by Different Carbon Sources

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Production of guaiacol by *Alicyclobacillus* spp. is of great concern in the fruit juice industry, as its characteristic "medicinal" odor is considered undesirable. Currently, methods are available for the detection of guaiacol in *Alicyclobacillus* spp. but usually requires 24–48 h as guaiacol formation is slow. This research investigates the effect of different carbohydrates on inducing the production of guaiacol by *A. acidoterrestris* ATCC 49025. *A. acidoterrestris* was grown in KV broth with or without glucose, adjusted to pH 4.0 with 10% tartaric acid, and supplemented with sucrose, arbutin, cellobiose, or succinic acid (0.1% or 1%). KV broth without any carbohydrate supplement was used as the control. Each treatment was sampled every 3 h over a period of 18 h and vanillic acid utilization/guaiacol production quantified by HPLC. No guaiacol was produced in KV broth (with or without glucose) containing succinic acid. For KV broth containing glucose, production of guaiacol was detected in treatments containing 0.1% sucrose and 1% arbutin by 9 h, and in all treatments including the control by 12 h. For KV broth without glucose, production of guaiacol was detected in the control by 12 h. Guaiacol formation was detected in treatments containing 0.1% sucrose, 1% sucrose, and 1% cellobiose by 8 h, 8 h, and 9 h, respectively. These results indicate that different carbohydrates affect the production of guaiacol. The findings can be applied to further research involving the enzymatic mechanisms of guaiacol formation in *Alicyclobacillus* spp.

P3-16 Comparison of KV Method with Conventional HPLC Method for Detecting Guaiacol from *Alicyclobacillus* spp.

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Alicyclobacillus spp. are sporeformers implicated in the spoilage of pasteurized apple juice. Spoilage by this microorganism is characterized by the formation of guaiacol, often described as medicinal or phenolic. Currently, use of high pressure liquid chromatography (HPLC) is the standard method for detecting the presence of guaiacol. In our previous study, a simple colorimetric KV method which utilizes the chemical reaction of phenolics and peroxidase to form reddish-brown pigments was developed to indicate the ability of *Alicyclobacillus* spp. to form guaiacol. This research focuses on comparing the two methods and evaluating KV method as a simple alternative to HPLC. A total of 127 *Alicyclobacillus* spp. isolates were grown in K broth containing 100 ppm vanillic acid and incubated at 43°C for 48 h. After incubation, presence of guaiacol was evaluated using HPLC and KV method. With the HPLC method, 19 out of 127 isolates did not form guaiacol, whereas 16 isolates were identified as non-guaiacol forming with the KV. All of the isolates that were identified as guaiacol forming isolates by HPLC correlated with those identified with the KV method. However, 3 isolates (2.36%) were identified as false positive using the KV method. The false positive results may be due to the presence of phenolics other than guaiacol contributing to the color formation. Our results indicate that while KV method may yield a certain percentage of false-positive results, the percentage is relatively low, and therefore can be considered as a simple and cost-efficient alternative to the current standard HPLC method.

P3-17 Development of ELISA and Immunochromatographic Assay for the Detection of Chloramphenicol Residues in Animal Plasma, Tissues, and Milk

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Monoclonal antibody against chloramphenicol was prepared, and the competitive direct enzyme-linked immunosorbent assay (ELISA) and the immunochromatographic assay were developed using the antibody for the detection of illegal residue of chloramphenicol in livestock and dairy products. No cross-reactivity of the antibody was observed with other derivatives, indicating that this antibody is highly specific for chloramphenicol. Based on a competitive indirect ELISA, the detection limits of chloramphenicol were 10 ng/ml in both rabbit and chicken plasma, 15 ng/ml in rabbit muscle, and 5 ng/ml in chicken muscle, respectively. Depletion profiles of chloramphenicol were successfully determined in rabbit and chicken plasma after intramuscular administration. The chloramphenicol concentration rapidly increased to 2,000 ng/ml up to 1 h in both rabbit and chicken plasma, and sharply decreased to less than 100 ng/ml after 8 h of withdrawal. The half-lives of chloramphenicol have been estimated to be 1.12 h in rabbit plasma and 2.23 h in chicken plasma after intramuscular injection. A rapid test based on an immunochromatographic assay was also developed using the monoclonal antibody conjugated with colloidal gold. The detection limits were less than 10 ng/ml of chloramphenicol in plasma, muscle, and cattle milk. Using the immunochromatographic assay, depletion profiles of chloramphenicol were also confirmed in rabbit and chicken after intramuscular administration, which were coincident and consistent with those of ELISA. In conclusion, the colloidal gold-based immunochromatographic assay could be applied to the detection of chloramphenicol in veterinary fields due to its rapid and simple procedure. For greater accuracy, however, the detection should be supported by a more sensitive laboratory method such as competitive direct ELISA method. The assays developed in this study could complement each other as well as the veterinary field and laboratory findings.

P3-18 Immunomagnetic Capture and Detection of *Yersinia pestis* from Milk

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Yersinia pestis is the causative agent of human plague and a potential threat with respect to food security. While there are 11 species in the genus *Yersinia*, only *Y. pestis* causes plague; *Y. enterocolitica Y. pseudotuberculosis* are known foodborne pathogens, and none of the remaining 8 species are known to be pathogenic to humans. Currently, rapid detection methods do not exist to distinguish foodborne *Y. pestis* from other *Yersinia* spp. As a first step toward developing a rapid method to isolate and identify *Y. pestis* from food, we have developed and characterized immunomagnetic beads for the specific capture of the organism. Anti-*Y. pestis* immunomagetnic beads (IMBs) were generated by biotinylating commercially-available monoclonal antibodies directed against the *Y. pestis* F1 capsular antigen followed by coupling of the biotinylated antibodies to streptavidin-coated magnetic beads. The anti-*Y. pestis* IMBs demonstrated efficient and specific capture of *Y. pestis* cells as demonstrated by determining the capture efficiency for five strains of *Y. pestis* (at least one strain from each of the three serovars), three strains of *Y. pseudotuberculosis*, eight strains of *Y. enterocolitica*, and strains of *Listeria monocytogenes*, non-pathogenic *Escherichia coli*, *E. coli* O157:H7, *Lactococcus lactis*, *Enterococcus faecalis*, and *Pseudomonas fluorescens*. The specific capture of *Y. pestis* cells by the IMBs was not diminished in the presence of excess *Y. enterocolitica* cells. The temperature at which the cultures were grown significantly affected the capture of *Y. pestis* by the IMBs. The IMB capture of *Y. pestis* from artificially-contaminated milk was also examined. While *Y. pestis* survived for several days in milk at both 22°C and 4°C, the efficiency of IMB capture of *Y. pestis* cells from milk was dependent upon incubation temperature of the cultures used to inoculate the milk and the temperature at which the milk was stored after contamination.

P3-19 Rapid Cell Lysis for DNA Isolation and Amplification from Common Food Pathogens

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A persistent technical bottleneck for the rapid sample preparation and amplification of nucleic acids has been the inability to overcome the obstacles associated with cell lysis and subsequent amplification of the nucleic acid target. Several methods of sample preparation have been described involving differential solubility, precipitation, or adsorption of nucleic acids from contaminants (primarily onto silica or derivatives using chaotropic salts). Using a new buffer formulation, we have demonstrated cell lysis and direct amplification from the lysis buffer without the need for nucleic acid purification. Samples of the common food pathogens *Listeria monocytogenes*, *Salmonella enterica*, *Clostridium perfringens*, *E. coli* O157:H7, and *Campylobacter jejuni* (10^4 cells) were centrifuged to form a pellet and the supernatant was removed. The pellet was resuspended in 1 ml of PBS and centrifuged again for 5 min. The supernatant was discarded and the pellet was resuspended in 100 µL of AnzenBio UltraLyse buffer, and incubated for 30 min at 95°C. Samples were then centrifuged for 1 minute at high speed. Aliquots of 1 µL, 2 µL, 5 µL, and 10 µL of the supernatant were added to the PCR primer/mastermix solution (final reaction volume of 25 µL). Amplification was performed on a BioRad IQ Real-time thermocycler and the products run on agarose gels. The AnzenBio UltraLyse buffer system provided readily amplifiable DNA from all of the organisms tested and at even 40% of the reaction volume, did not affect amplification efficiency. This new buffer formulation provides a simple, robust, and effective method for the preparation of food pathogens for identification via PCR.

P3-20 Optimization and Validation of Improved Culture and Molecular Methods for the Detection of *Shigella* spp. in Fresh Vegetables and Fruits, and Softshell Clams

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Methods for the detection of *Shigella* spp. in foods were not available either internationally or in Health Canada's Compendium of Analytical Methods, that met CFIA requirements for sensitivity. Teams were identified at CFIA and Health Canada to collaborate on the optimization and validation of a classical cultural method and a polymerase chain reaction method for the detection of *Shigella* spp. in foods. Fresh fruits and vegetables as well as softshell clams have been targeted because these commodities are historically related to shigellosis. In the first part of the project, the following parameters were studied: detection limit of the method, repeatability of the

results, influence of the food matrices, ability of the method to detect different species of *Shigella*, and recuperation of *Shigella* after long term storage at refrigeration temperatures. In the second part, a collaborative study was initiated through the CFIA, Health Canada and MAPAQ (Ministére de l'agriculture, des pêcheries et de l'alimentation du Québec) laboratories with the goal of evaluating method reproducibility. During this study, seven shipments each containing seven unknown samples spiked in duplicate at 10, 1, 0.1 and 0 CFU/g were sent to nine participating laboratories. Following this initiative, the detection limit of both methods was evaluated at 1 CFU/g. At that detection limit, reproducibility was observed by all participating laboratories. MFLP-25 and MFLP-26 methods have both been accepted in Health Canada's Compendium of Analytical Methods and they are now used in CFIA laboratories for routine analyses of fresh fruit and vegetables samples obtained through the CFIA's inspection service.

P3-21 Evaluation of Multiplex PCR of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in Various Food Samples

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We described the development of a multiplex PCR method, which is capable of identifying *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 directly from enrichment culture of meat samples. In our previous study, we evaluated the optimization of pre-enrichment medium, DNA extraction method and the multiplex PCR setting; this multiplex PCR assay was capable of detecting as few as 1 CFU/25 g of any test organism in raw meat after enrichment cultivation for 24 h. In this study, we evaluated the sensitivity of the multiplex PCR for simultaneous detection of each pathogen in other food materials (meat, cabbage, smoked salmon, egg yolk, milk, cheese samples, etc.). When this protocol was used for the detection of each of the above-mentioned pathogenic bacterium in more than 30 kinds of spiked food samples, 100 cells/25 g of inoculated sample was detectable after enrichment cultivation for 20 h in all foods tested. Moreover, the inoculated samples which were stored at -20°C for 2 weeks were also detectable with this protocol. On the other hand, the conventional culture method failed frequently to detect the inoculated pathogens from frozen samples. Therefore, the multiplex PCR assay method described in this study is capable of detecting as few as 1 CFU/25 g of any test organism in more than 30 kinds of food samples and the detection rate from frozen samples was higher than that of the conventional culture method.

P3-22 The Detection of *Enterobacter sakazakii* and Other *Enterobacteriaceae* from Milk Powders Using Paramagnetic Cationic Particles

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A method for the detection of *Enterobacter sakazakii* and other *Enterobacteriaceae* in infant feed formulas and milk powders is described. PATHATRIX is a novel patented technology that makes use of cationically–(positively-) charged paramagnetic particles to selectively bind and capture *E. sakazakii* from a variety of food matrices. The approach is unique in that it is the only commercially-available system that can analyze a 250 ml sample volume by re-circulating it through a "capture phase". 100 gram samples of infant feed formula were pre-enriched in 900 mL Buffered Peptone Water for 6 h at 42°C. A 250 ml sub-sample was further analyzed on the PATHATRIX system in which the cationically-charged paramagnetic beads were immobilized. The samples were recirculated for 30 min over the particles, after which they were washed, eluted from the system and plated onto Violet Red Bile Glucose Agar (VRBGA) and DFI chromogenic selective agar. Individual infant feed formula samples (n = 51) were spiked with 1 – 10 CFU per 100 gram. In all cases (51/51 = 100%) *E. sakazakii* was recovered. The method was further extended to adopt the PATAHTRIX pooling strategy in which 50 mL sub-samples from each of 5 pre-enrichments were wet composited (n = 28). With spike levels of 1 – 10 CFU per 100 gram, all samples (28/28 = 100%) were found to be positive. It was also possible to demonstrate that this approach captured other *Enterobacteriaceae* including *Citrobacter diversus, C. freundii, E. cloacae, Klebsiella oxytoca, K. pneumoniae, K. aerogenes, Serratia marcescens, Salmonella* Enteritidis, *S.* Typhimurium and *S. poona*. The method is simple to perform and has considerable promise for the early detection of *E. sakazakii* and other *Enterobacteriaceae* in infant feed formulas.

P3-23 Sanitizer Efficacy When Tested in Suspension and on Surfaces against Food-associated Bacteria and the Potential for Development of Resistance

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Sanitizers are used to kill bacteria associated with food contact surfaces, wash water, and commodities (fresh fruits and vegetables). The efficacy of sanitizers depends on a number of factors including pH, temperature, concentration, and presence of organic and inorganic material. Bacteria that survive exposure to a sanitizer may develop resistance to that sanitizer and cross resistance to other antimicrobial agents. In the present study, the efficacy of sodium hypochlorite, chlorhexidine digluconate, and benzalkonium chloride against food-associated bacteria was determined using a suspension and surface test. Bacteria surviving exposure under conditions of surface testing were evaluated for cross-resistance to each of the other sanitizers and to tetracycline and ampicillin. Development of resistance to sanitizers through repeated exposure was also tested. Pathogens *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Enteritidis, and *Staphylococcus aureus*, and the food spoilage bacteria *Pseudomonas aeroginosa* were used. The surface test was conducted using bacteria suspended in water and inoculated onto stainless steel coupons. For the suspension test bacteria were suspended in sanitizer diluted to test concentrations in water. Results of the suspension test showed that sanitizers at 128 ppm resulted in an 8-log reduction of each microbe tested. For the surface test no reduction in bacterial populations occurred for sodium hypochloride and chlorhexidine digluconate at 128 ppm. However, a 2–3 log decrease in bacterial populations occurred following exposure to 128–ppm benzalkonium chloride. Survivors isolated following exposure to selected disinfectants exhibited a 4-fold decrease in susceptibility to chlorhexidine digluconate, but exhibited no change in susceptibility to sodium hypochlorite or benzalkonium chloride. Cross-resistance experiments for the antibiotics suggest that no intrinsic mechanism was activated thereby providing resistance. Repeated exposure to a given sanitizer did result in decreased susceptibility to that sanitizer. Additional experiments indicated that the resistance was stable. Results of these studies have implications to the food industry and human health.

P3-24 Simultaneous Determination of Synthetic Steroids Using Biochip Array Technology

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The use of a multi-analyte approach represents an advantage to monitor the illegal use of synthetic steroids in food-producing animals in view of consumer protection. In this context, the aim of this study was to develop sensitive immunoassays based on the biochip array technology to measure simultaneously the following steroid residues: 17-alpha-boldenone, 17-beta-boldenone, methylboldenone, methyltestosterone, CLAD, 17-beta-clostebol, stanozolol, ethinylestradiol, 17-alpha-19-nortestosterone, 17-alpha-trenbolone, 17-beta-trenbolone, medroxyprogesterone acetate, chlormadinone acetate, megestrol acetate and melengestrol acetate. In house polyclonal antibodies against the different steroids were bound to the biochip surface in defined positions, generating microarrays. The biochip is also the vessel where the competitive immunoassays take place, in a multi-analyte format, between the steroid molecules and the HRP-labelled steroids conjugates. Signal detection, data processing and storage were carried out using the semiautomated Evidence Investigator™. The system automatically produces and stores valid calibration curves for each analyte and reports quantitative sample results based on them. Initial intra-assay precision evaluation for the calibration range 0–10 ng/ml (n = 20) expressed as % CV was found to be between 1 and 12%. Typical IC50 values were for all the analytes < 0.5 ng/ml, as, for example, for the generic antibody for gestagens: 0.05 ng/ml (medroxyprogesterone acetate), 0.07 ng/ml (chlormadinone acetate and megestrol acetate), 0.13 ng/ml (melengestrol acetate). In the present format the assay applied to Evidence Investigator™ has the capacity to generate 675 results in less than 2 h. Employing the same methodology, the sample throughput can be significantly increased with the use of the fully automated Evidence R analyzer. Further development will address the increase in result output with the introduction of additional steroids in the panel as well as the application to different matrices. The performance characteristics evaluated so far indicate feasibility of simultaneous measurement of synthetic steroid compounds with the biochip array technology.

P3-25 Rapid Automated Method for the Detection of *Alicyclobacillus*

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Alicyclobacillus species cause a medicinal off-flavoring of fruit juices, acidic beverages and other health beverages. *Alicyclobacillus* species are gram positive rods, spore formers, capable of growth at high temperature and low pH. Guaiacol is the principal off-aroma compound produced by spoilage strains of *Alicyclobacillus*. An *Alicyclobacillus* vial has been developed to be used in the Soleris optical system, for rapid automated detection of this organism. CO_2 production by the metabolizing organisms is combined with KOH and result in a color change of the liquid pH indicator located at the bottom of the vial. After heat shock (70°C for 20 min) the vials were incubated at 43°C for 48 h. The developed method was tested by adding 1.0 ml of the sample to ready to use vials, or after filtration by the immersion of the whole filter into the vial. Guaiacol production was verified directly in the test vial, after the end of the assay by adding the appropriate reagents and observing a color change from amber to reddish brown. Fifty commercially available juices were purchased at local markets and tested in the system. There were no *Alicyclobacillus* species detected in these juices by either the developed method or K Agar plate. In samples inoculated with *Alicyclobacillus*, colonies were detected in the *Alicyclobacillus* vials within 2 days while the agar plates methodology required 3–5 days for the colonies to appear, with confirmation of giaiacol production requiring two additional days. A total of 42 strains of *Alicyclobacillus* were tested within the system and all detected within 48 h, even at very low levels (<1–10 CFU/vial). The developed method had excellent reproducibility with low limits of detection. The *Alicyclobacillus* species capable of Guaiacol production reacted correctly in the vial, resulting in the color change from amber to reddish brown.

P3-26 Rapid Concentration Method for Enteric Virus Detection on Berries

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Several hepatitis A virus (HAV) and norovirus (NV) outbreaks due to consumption of berries have been reported in recent years. To facilitate detection of enteric viruses that may be present on berries, we developed a rapid and sensitive concentration method for rotavirus (RV), NV and HAV in different types of berries. Initial studies focused on optimization of various extraction parameters, including elution buffer composition, viral concentration method and choice of extraction kit. Viruses were extracted from the berry surfaced by a direct elution method in a glycine/Tris pH 9.5 buffer followed by ultrafiltration. Since pectin often led to invalid results due to the presence of PCR- inhibitory substances, a pectinase treatment was included in the final protocol. However, occasionally residual inhibitors were still present in the processed samples, which gave relatively poor detection limits. To overcome this limitation, DMSO or BSA was added to the real-time RT-PCR reaction, which markedly improved the sensitivity of the method. After standardization, this concentration method was applied in combination with real-time RT-PCR, using specific primers in various types of fresh and frozen berries. The average detection limits were 1.2 TCID50, 54 PCRU and 0.5 TCID50, for HAV, NV and RV, respectively, per 25 grams of berries. Based on our results it is concluded that this procedure is suitable to detect and quantify enteric viruses within 6 h and can be applied to surveillance of enteric viruses in fresh and frozen berries. Future work will focus on methods for vegetables and herbs and on inactivation studies under specific manufacturing processes.

P3-27 Development and Comparison of Primers and TaqMan Probes for Hepatitis A Virus Detection and Quantification by Real-time RT-PCR

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Transmission of hepatitis A and E viruses may occur from ingestion of contaminated food or water and represents an increasing concern worldwide. A real-time quantitative RT-PCR method using TaqMan probe technology was developed to detect and quantify hepatitis A virus (HAV). Two new real-time RT-PCR systems targeting the protease and the polymerase regions of HAV genome were designed. These systems were both found able to detect HAV HM-175, HAS-15 and LSH/S strains. No amplification product or detection signal were observed with other human foodborne pathogens such as *Campylobacter jejuni*, *C. coli*, *Escherichia coli* O157:H7, *Salmonella* DT104, norovirus GgI, adenovirus type 40 and hepatitis E virus. Comparison between the viral titre determination by

cytopathic effect ($TCID_{50\%}$ - 50% of tissue culture infectious dose) and real-time RT-PCR with both systems was also performed with the HM-175 HAV strain. For a viral stock produced in vitro, a titre of 6.3 – 10^5 TCID50%/ml was estimated, while an average of 1.94 – 10^8 copies of viral RNA/ml were detected by real-time RT-PCR with the protease system and an average of 2.18 – 10^9 copies of viral RNA/ml with the polymerase system. Based on the standard curves for the two proposed systems, 1 copy (genomic equivalent)/ml could be detected. The analytical sensitivity, the quantitative abilities and the specificity of the primer and TaqMan probe systems will be useful for the detection of HAV in food commodities.

P3-28 Comparison of Methods for the Detection of Norovirus in Stool Samples

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Noroviruses are the most commonly identified foodborne pathogen causing acute gastroenteritis. They are associated with 40% of food outbreaks and 96% of all reported outbreaks of viral gastroenteritis. Electron microscopy, reverse transcriptase (RT) -PCR, or enzyme immunoassays (EIA) are currently used for routine diagnosis of norovirus infection. With the advance of molecular detection methods, RT-PCR is used for the diagnosis of norovirus in laboratories around the world. In this study, different methods were compared for the detection of norovirus in stool samples from norovirus outbreaks that had occurred in Labrador/New Foundland in Canada in the 2004–2005 season. Among those methods, commercial EIA kit, routine conventional RT-PCR, and real-time quantit-ative TaqMan RT-PCR were used. Among the 33 clinical samples tested, 31 samples (93.9%) tested positive by real-time RT-PCR, 27 (81.8%) by conventional RT-PCR and 29 (87.9%) by EIA. In general, the molecular methods used were more sensitive and detected stains non-reactive by the EIA test. The real-time RT-PCR system targeting the junction region of the norovirus genome detected more strains. Two samples were negative by EIA but were positive using both molecular methods. Two samples tested negative with all methods used. The majority of the samples analyzed were strains from genogroup II, confirming the high prevalence of GII strains (60–80%) in these norovirus outbreaks.

P3-29 Evaluation of the Compact Dry YM for the Enumeration of Yeasts and Molds

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Yeasts and molds (Y/M) can cause various degrees of food decomposition. Substantial economic losses may be sustained by the producer, processor, and consumer. Fruits can be invaded before harvesting as well as during storage. The FDA bacteriological analytical manual (BAM) method for enumeration of Y/M recommends spread plating on dichloran rose bengal chloramphenicol agar using a sterile bent glass rod. However, this technique is troublesome. The Compact Dry YM (CD-YM) is a ready-to-use test for the enumeration of Y/M in food. The plates are pre-sterilized, contain nonwoven material incorporating nutrients supplemented with antibiotics and a chromogenic enzyme substrate, (5-bromo-4-chloro-3-indoxyl phosphate, p-toluidine salt), to facilitate counting, and a cold water-soluble gelling agent. The medium is re-hydrated with 1 ml of diluted sample inoculated onto the center of the self-diffusible medium and the solution is allowed to diffuse immediately by capillary action. The plate can be incubated at 25°C for 7 days. The CD-YM was compared with the BAM method in enumerating colony forming units (CFU) of Y/M from four different fruit products. Samples were diluted in standard solution such as 0.1% peptone water. The difference of mean log CFU was compared by using a one way analysis of variance (ANOVA) for the yeast and mold counts. No apparent differences were observed ($P > 0.05$). The correlation coefficients for yeast and mold counts in fresh apples, frozen blueberries, orange juice, and dried banana chips by both methods was 1.00, 1.00, 1.00, and 0.93, respectively. The results show that these two methods performed equally well. Therefore, the CD-YM could be a convenient alternative method for routine microbiological testing of food for the enumeration of Y/M in fruit-based products.

P3-30 Yeast and Mold by PCR: Minimizing Time to Result

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Yeasts and molds are fungi, the most diverse kingdom in the tree of life, with over 1,000,000 species. By contrast, there are fewer than 10,000 species of bacteria. The food and beverage industry routinely tests for yeast and mold, usually by plating methods that require 5 to 7 days for a reliable result. This long wait creates an economic burden for the producers of short shelf life/refrigerated products. Using PCR-based technology and a unique tube for enrichment with disrupter beads, we have developed a new assay for the DuPont Qualicon BAX® system that detects and enumerates yeasts and molds. For foods with low cutoff levels (as low as 10 CFU/g), enrichment protocols allow for results in just two days. And for foods with higher cutoff levels (> 500 CFU/g), a direct test without enrichment provides results the same day (about 6 h). A highly repeated genomic target and controlled fractionation of DNA in the process allow for detection with no replication of the organism if high levels of fungal material are present, and minimal rounds of replication for lower starting levels of fungal organisms. The assay demonstrated 100% inclusivity against a test panel of culture collection isolates spanning the breadth of the fungal genetic tree, as well as 172 naturally occurring yeast and mold isolates obtained from 500 grocery store items. Food studies on spiked and naturally occurring contamination indicate that this test will allow substantially faster product release of items that are currently subject to test and hold. In addition, the direct test opens the possibility of monitoring the fungal burden in short shelf-life products, such as fresh produce, where the time to result using plate methods has been an insurmountable barrier.

P3-31 Direct Quantification of *Campylobacter* in Poultry Rinses by the Warnex™ Rapid Pathogen Detection System

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Campylobacter spp. is a major cause of bacterial gastroenteritis worldwide. However, very few microbiological methods exist to quantify this pathogen despite a growing need to monitor *Campylobacter* levels in poultry flocks and finished products. A recently developed Warnex assay was designed for the direct quantification of the three most prevalent *Campylobacter* species: *C. jejuni*, *C. coli* and *C. lari*. The quantification assay is based on direct DNA extraction from rinses of whole poultry carcasses, followed by PCR. The

system automatically generates CFU/g counts based on the fluorescence observed during the PCR reaction. On pure cultures, the system had a sensitivity and specificity of 98.6% and 100%, respectively. The system was also compared against a direct plating method on a series of 60 chicken carcasses, inoculated with varying concentrations of different *C. jejuni*, *C. coli* and *C. lari* strains. The coefficient of correlation between the two methods was found to be 0.95 over a 3-log range of target cell concentrations. The detection limit of the PCR assay was 20 CFU/g. Overall, the PCR system was reliable and easy to use, and the entire procedure could be completed in less than 4 h.

P3-32 Counting *Campylobacter* spp.: Performance Comparison of Two Selective Agars

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There is currently no consensus on the selective agar to be used for *Campylobacter* enumeration from contaminated non-clinical samples. In this study, two media, modified charcoal cefoperazone deoxycholate (mCCDA) and CampyFood ID (CFA) agars were compared. Six different sample-types, collected from various locations in South West England, were analyzed between November 2005 and January 2006. These samples comprised unpasteurized milk (RM); natural surface water (NSW); fresh chicken carcass rinses (CRW); fresh chicken neck skin (CS); freshly voided chicken feces (CFS) and frozen chicken carcass rinses (FRW). Samples were prepared and enumerated according to ISO/CD 10272-2, and both agars were incubated in microaerobic conditions at 41.5°C for up to 48 h. Suspect *Campylobacter* colonies were confirmed by biochemical and PCR-based tests. In total, *Campylobacter* spp. were detected in 34% (45/132) of samples, and a high level of positivity was identified in CRW (54%) and CS (56%) samples. No *Campylobacter* spp. were detected in RM samples. The predominant species isolated was *C. jejuni*, with *C. coli* being rarely detected. There was no significant difference ($P > 0.05$; McNemar's test) in the number of *Campylobacter* spp. positive samples detected using the two agars. The colored colonies produced on CFA assisted in the identification of *Campylobacter* spp.

P3-33 Resuscitation of Non-stressed or Stressed *Campylobacter jejuni* in Different Enrichment Broths

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Campylobacter jejuni is the most frequently identified cause of acute diarrheal infections in developed countries, for which poultry products constitute the main transmission route. To establish an enrichment system of high efficiency for recovery of *Campylobacter jejuni* ATCC 35921 in broth under microaerobic atmosphere, the effects of media (*Brucella*, Nutrient No.2, blood-free enrichment (BFEB), Hunt and *Campylobacter* blood-free (CCD without charcoal), inoculum levels (1 to 3 log CFU/ml), cell state (non-stressed and stressed cells), and stress conditions (cold or heat temperature, starvation and acid treatment) were evaluated. Lag-phase duration, physiological status (non-stressed, stressed, viable-but-nonculturable and dead cells), and percentage of coccoid form for each stress condition were determined. No significant differences in lag phase duration for recovery of non-stressed or stressed *C. jejuni* were observed in *Brucella*, Nutrient No. 2, BFEB and Hunt broth for any of the stressors ($P > 0.05$). However, Nutrient No.2 was the best enrichment broth because it exhibited the shortest lag phase duration for recovery of *C. jejuni* and the shortest incubation time to decrease the population of stressed cells, as well as the lowest percentage of cells showing a coccoid form. Lag phase duration was affected by inoculum levels, cell state and stress conditions.

P3-34 A Combination of Enrichment Broth and Immunomagnetic Separation for the Detection of *Campylobacter jejuni* in Chicken under Aerobic Conditions

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The foodborne pathogen *Campylobacter jejuni* is the primary causative of gastroenteritis in humans. Two immunomagnetic separation (IMS) systems (Pathatrix and Dynabeads) were tested for detection of non-stressed or stressed *C. jejuni* ATCC 35921 in artificially inoculated chicken breast rinse and pure culture. Pathatrix kits (Matrix Bioscience) for *Campylobacter* detection were used with the Pathatrix system. For Dynabeads, polyclonal anti-*Campylobacter jejuni* antibodies conjugated with biotin were used to label streptavidin C1-coated beads. After incubating the beads of both the Pathatrix kit and Dynabeads with chicken breast rinse and culture suspension, *Campylobacter*-bead complexes were separated from other components with a magnet. The capture efficiency was tested by plating bead-captured cells and unbound cells in the supernatant onto CCD agar. The effects of different beads, incubation time (30 and 60 min), number of immunomagnetic beads (10^6 to 10^7 beads/ml), inoculum levels (10^3 to 10^7 CFU/ml of culture suspension or CFU/225 ml for chicken breast rinse) and washing buffers (phosphate buffer or Nutrient No. 2) on the capture efficiency were determined for both pure culture and chicken breast rinse. Without pre-enrichment, Pathatrix and Dynabeads system could detect 10^5 and 10^4 CFU/225 ml of chicken breast rinse, respectively. Following pre-enrichment in modified Nutrient No. 2 under aerobic conditions for resuscitation of stressed cells, initial counts 10^0 to 10^1 CFU/225 ml of chicken breast rinse could be detected after 24 and 12 h of incubation using the Pathatrix and Dynabeads, respectively. Furthermore, with naturally contaminated chicken and chicken inoculated with non-stressed cells at a level of 10^1 CFU/225 ml of chicken breast rinse, IMS using Dynabeads allowed detection after 9 h of incubation whereas 24 h of incubation were required for detection by the conventional method.

P3-35 Rapid Automated Detection of *Salmonella* Organisms

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Salmonellosis caused by contaminated foods continues to be a serious problem. Detection of the pathogen is vital to ensure food safety, but since conventional methods require several days before results are obtained, rapid screening methods for presence/absence of the pathogen are of importance. We previously reported a rapid automated detection method for the pathogen using the Biosys system (recently changed to Soleris™). It utilizes a liquid selective medium consisting of two carbohydrates (dulcitol, xylose), two amino acids (lysine, ornithine), ferric ammonium citrate, thiosulfate, and inhibitors. The colorless medium turns black in the presence of salmonellae during incubation at 37–42°C. Detection time, which is inversely proportional to cell numbers, is generally <18 h for 10 cells. In the present study, 140 salmonellae were tested in vials containing the *Salmonella* selective broth. Sixty non-salmonellae were also included in the study, consisting of forty *Enterobacteriaceae*, 11 other Gram negatives, and 9 Gram-positive organisms. All organisms were also tested in vials containing non selective broth to verify that the cultures were growing. Of 50 *Salmonella* strains representing serovars that contribute to 80% of human salmonellosis (Typhimurium, Enteritidis, Newport, Heidelberg, Javiana, Montevideo, Muenchen, Hadar, Oranienburg, Agona, Thompson, Infantis and Saint Paul), three (6%) were false negative. Other false negative strains (21 of 80) belonged to serovars Arizonae, Choleraesuis, Gallinarum, Bovis, Cerro, Typhi and Paratyphi. Of the 60 non-salmonellae tested, two (*Proteus mirabilis* and *Morganella mirabilis*) were false positive (3%). The selected medium appears to have very good selectivity.

P3-36 RAPID'*Salmonella*: New EN ISO 16140 Validated Rapid Chromogenic Detection Method for *Salmonella* spp. in Food and Feeding Stuffs

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Sensitive and rapid detection methods for life-threatening *Salmonella* are valuable analytical tools that contribute to food safety, from farm to fork, in both developing and industrial countries. In this context, a new rapid and convenient chromogenic detection method, RAPID'*Salmonella*, has been validated according to the EN ISO 16140 standard versus the reference EN ISO 6579 method. Starting from an overnight Buffered Peptone Water pre-enrichment (18 h +/- 2 h) and after either a short (8 h +/- 2 h) or an overnight incubation time (24 h +/- 2 h) in Rappaport Vassiliadis Soja (RVS) broth, plating on a single RAPID'*Salmonella* plate provided similar results to those obtained with the EN ISO 6579 reference method for seafood, vegetable, dairy and egg product categories. A RVS 24 h incubation period was required for meat products and feeding stuffs. Results were obtained within 2 – 3 days for both negative and positive samples. Two options of confirmation are proposed to the users, the standard or a convenient method based on rapid serological and biochemical identification tests. The exclusivity and inclusivity of the RAPID'*Salmonella* method were assessed using, respectively, pure cultures of 42 non-targeted strains and 52 *Salmonella* strains belonging to 26 major serotypes including *S.* Typhi and *S.* Paratyphi A, B and C. Characteristic colonies were observed on RAPID'*Salmonella* agar plates for all *Salmonella* serotypes. The relative detection limit was investigated by spiking pure culture serial dilutions into 5 food types. The accuracy was determined by testing 408 food and feed samples, including 50% positive and 50% negative ones, most of the positive samples being naturally contaminated. The values of the relative detection limit, exclusivity, inclusivity and accuracy clearly show that RAPID'*Salmonella* gave comparable results to the EN ISO 6579 standard. In conclusion, the RAPID'*Salmonella* method has been demonstrated to be a rapid and convenient alternative method for *Salmonella* spp. detection in food and feed samples, according to the EN ISO 16140 validation requirements. The RAPID' *Salmonella* chromogenic plating agar could also be of interest as an appropriate second selective medium in the EN ISO 6579 reference method, notably to detect atypical lactose-positive *Salmonella* strains.

P3-37 The Use of Lateral Flow Immunoassay Devices with Serotype-specific Monoclonal Antibodies in the Development of *Salmonella* Enrichment Media

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Detection of *Salmonella* contamination in food depends largely on the results of enrichment, which is to selectively grow low levels of *Salmonella* (~1–10 cells per 25 g sample) to detectable concentrations (10^4 to 10^6 CFU/ml). Thus, enrichment media and protocols play a key role, especially for rapid detection methods. The usefulness of lateral flow devices (LFD) has been clearly demonstrated in our media development program for rapid detection of *E. coli* O157 and *Listeria* in foods. For *Salmonella* media development, there are significant challenges, including high-level natural contamination of *Salmonella* in some foods (~20%), cross-reacting strains, e.g., Citrobacters, and the complexity of food matrices. Here, we report the significance of using LFDs, which employ monoclonal antibodies and detect only serotype specific *Salmonella* strains, including serotypes B, C1, C2/C3, D1, D1/D2, D2, F and G2. After testing 87 non-*Salmonella* strains, LFDs with C1, D2 and G2 monoclonal antibodies showed no reactivity with any of them, and natural *Salmonella* contamination by these three serotypes are also rarely seen. Thus, *Salmonella* cells of strains C1, D2 and G2 were spiked in foods like raw ground turkey, beef, pork, and chicken carcass wash, enriched with various media and protocols, and detected with these LFDs. After extensive studies, a highly selective and productive *Salmonella* pre-enrichment media, and selective enrichment media have been developed. A comparison was made with buffered peptone water (BPW) and the new pre-enrichment media with spiked *Salmonella* C1 samples, after overnight growth, 13 out of 20 were LFD C1 positive after the new media, while none were positive after BPW. The advantages of using these LFDs for media development include specific results, fast, high efficiency, and ease of use, which make evaluation of large numbers of media formulations feasible. This method could also be used for media and protocol validation and qualification.

P3-38 Multistate Outbreaks of *Salmonella* Typhimurium Infection Associated with Cake Batter Ice Cream

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During May and June of 2005, 24 persons in Minnesota, Washington, Oregon and Ohio states were infected by *Salmonella* Typhimurium after eating cake batter ice cream. The cake mix used to prepare the cake batter in the ice cream was suspected as the source of *Salmonella* contamination. Initial tests did not detect *Salmonella* in cake mix collected during or immediately after the outbreak. The objective of this study was to evaluate different procedures to isolate *Salmonella* from the implicated cake mix, cake, and ice cream. All outbreak-associated food samples (total of 14 samples) were collected during or immediately after the outbreak by health departments of the states involved. Different combinations of sample sizes, preenrichment broths, enrichment broths, enrichment temperatures, and isolation media were used. *Salmonella* Typhimurium matching the PFGE profile of outbreak isolate was isolated from two cake mix samples. Universal preenrichment broth was substantially better than lactose broth for preenrichment, and Tetrathionate broth (TT) was slightly better than Rappaport-Vassiliadis broth (RV), for isolation of *Salmonella* from the two positive cake mix samples. Although more typical *Salmonella* colonies were observed on plates from enrichments at 35°C, more confirmed *Salmonella* isolates were obtained from plates of enrichment cultures at 42°C. Brilliant green agar (BGA), Xylose lysine Tergitol™ 4 agar (XLT4), Xylose lysine desoxycholate agar (XLD), Hektoen enteric agar (HE), and Bismuth sulfite agar (BS) plates were equally effective in isolating *Salmonella* from cake mix. The best combination of preenrichment-enrichment conditions for isolating the outbreak strain of *Salmonella* was preenrichment of cake mix samples in universal preenrichment broth at 35°C for 24 h followed by enrichment at 42°C for 24 h in TT broth.

P3-39 Comparison of Reveal® for *Salmonella* Enteritidis, FDA Culture Method and Selective Media for Recovery of *Salmonella* Enteritidis from Broiler Flock Environments

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Salmonella Enteritidis (*SE*) is the second most frequently isolated serovar among *Salmonella*. Egg products are most often associated with *SE* outbreaks. To prevent *SE* contamination of eggs, many producers are implementing *SE* flock inspections at their facilities. A rapid and simple method for detecting *SE* in poultry environmental samples is critical for effective control of *SE*. In this study, the Reveal® test for *SE* was compared to the conventional FDA culture method for detecting *SE*. In addition, the efficacy of two enrichment media — tetrathionate (TT) and Rappaport-Vassilliadis (RV) broth, and three selective plating media (brilliant green agar with novobiocin (BGN), xylose lysine tergitol 4 agar (XLT4) and bismuth sulfite (BS) agar) — were compared for efficacy of SE isolation. A total of 128 environmental drag swab samples were collected from chicken flocks in two US states and analyzed in parallel using Reveal® for *SE* and the FDA culture method. Twenty-five samples (19.5%) were positive for *SE* using Reveal®, whereas 23 (18.0%) were positive by the FDA culture method. No significant difference (P=0.527) was seen between the two methods. The Reveal® test for *SE* had a sensitivity, specificity and overall accuracy of 83, 94, 92%, respectively. Overall, a significantly greater number of positive samples were obtained by enriching in RV compared to TT broth. XLT4 and BGN were more efficient than BS for isolating *SE*. However, no single method or medium could recover *SE* from all positive environmental samples.

P3-40 Evaluation of a New Chromogenic Plating Medium for the Isolation and Presumptive Identification of *Salmonella*

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A novel chromogenic agar medium, Oxoid *Salmonella* Chromogenic Medium II (OSCM II), has been developed for the isolation and presumptive identification of *Salmonella*. The combination of chromogens provides improved color definition and contrast, allowing the clear discrimination of *Salmonella* strains from competing organisms. The medium also incorporates novel Inhibigen™ technology targeted at non-*Salmonella* organisms, providing enhanced selectivity and sensitivity. A total of over 100 *Salmonella* strains, including non-H_2S producers, lactose fermenters and non-motile strains, and 80 non-*Salmonella* organisms (including *Escherichia coli*, *Citrobacter freundii*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp. and *Pseudomonads*) were evaluated on OSCM II, AES Laboratoire ASAP (ASAP), Xylose-Lysine-Deoxycholate Agar (XLD) and Hektoen Enteric Agar (HE). After 24 h incubation on OSCM II at 37°C, colonies of *Salmonella* were coloured purple, whereas non-*Salmonella* spp. appeared blue, green or colourless on the white background. The media could be ranked according to sensitivity (highest first) as OSCM II>ASAP>XLD>HE; and as OSCM II + ASAP> XLD>HE for specificity. These data show OSCM II provides improved sensitivity and selectivity compared with conventional media and can be considered as an alternative medium for isolation and presumptive identification of *Salmonella* as well as providing an opportunity for the development of a single plate *Salmonella* isolation procedure.

P3-41 DSC Sensitive and Specific Detection of *Salmonella* from Ground Beef and Potato Salad Samples within Eight Hours

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There is still a need for rapid detection methods that are both sensitive and specific for *Salmonella* in food. This study investigates flow through immunocapture (FTI), using the Pathatrix, followed by real-time PCR for the specific and sensitive detection of *Salmonella* in ground beef and potato salad within 8 h. Food samples were inoculated with an appropriate dilution of a 5-serovar *Salmonella* cocktail and enriched for 5 h. Following enrichment, *Salmonella* were separated and concentrated from the food samples for 30 min on the Pathatrix, and aliquots from the recovered bead-bacteria complex were analyzed for *Salmonella* by spread plating on XLD (FTI-XLD) and by real-time PCR (FTI-PCR). Food samples were also analyzed by the FDA's Bacteriological Analytical Manual (BAM) culture method for *Salmonella* for comparison. For potato salad and ground beef samples inoculated at 10^1 CFU/25 g, all three methods resulted in positive detection in 10 out of 10 samples. For potato salad samples inoculated at 10^0 CFU/25 g, the BAM

and FTI-PCR methods resulted in positive detection in 9 out of 10 samples, while FTI-XLD was positive for *Salmonella* in 8 out of 10 samples. For ground beef samples inoculated at 10^0 CFU/25 g, the BAM method detected *Salmonella* in 9 out of 10 samples, the FTI-PCR method detected *Salmonella* in 8 out of 10 samples, and the FTI-XLD method detected *Salmonella* in 4 out of 10 samples. The FTI-XLD obtained presumptive colonies on XLD plates 24–48 h faster than the BAM culture method.

P3-42 Multi-plex Detection of *Salmonella* spp., *Escherichia coli* O157 and SEB Using Bio-nanotransduction

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Bio-nanotransduction is a biological detection scheme that allows recognition of multiple targets and target types in a sample and the ultimate detection and differentiation using various detection platforms. In this system, a biological recognition event is transduced into an RNA nano-signal specific for the particular interaction. From a single sample it is possible produce a specific RNA nano-signal for numerous targets. A nano-signal profile can then be compiled using RNA detection techniques such as specific hybridization. We have used Bio-nanotransduction to specifically recognize and discriminate *Salmonella* serovar Typhimurium, *Escherichia coli* O157, and *Staphylococcal enterotoxin* B in a single sample. Polyclonal antibodies recognizing *Salmonella* spp., *E. coli* O157 and SEB were used for both capture and detection of the targets. Capture from pure samples was accomplished with the use of antibody functionalized magnetic beads. RNA nano-signals profiles were produced by covalently attaching T7 RNA specific DNA templates to the antibodies. Recognition of the captured target with the antibody DNA conjugate was followed by an in vitro transcription reaction. A specific RNA nano-signal was produced for each of the three recognition events. The nano-signal profile of the sample was initially determined using a standard ELONA (enzyme linked oligonucleotide assay) in combination with a fluorescence plate reader. After optimization, an electronic biosensor system was used as the detection system through the use of a gold electrode array as the hybridization surface. Analysis of the nanosignals was accomplished using a handheld electrochemical analyzer. Using either system, we were able to detect heat killed target organisms (less than 10^5 cells/ml) and SEB (less than 5 ng/ml) singly or in varying combinations with the other targets. Continued optimization of the process together with application to emerging technologies should provide an important tool for biosensor development and food system monitoring.

P3-43 Evaluation of Two Real-time PCR Systems for the Detection and Confirmation of *Escherichia coli* O157:H7 and *Salmonella* in Sprout Irrigation Water

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Sprouts contain high numbers of background flora which can make the isolation of a target pathogen difficult. In addition, confirmation using conventional methods can take at least two days. Two real-time PCR systems, the Roche LightCycler and the DuPont Qualicon BAX® system, with the accompanying kits were evaluated for rapid detection and confirmation of *E. coli* O157:H7 and *Salmonella* in sprout irrigation water. Both 100-fold concentrated and unconcentrated sprout irrigation water (25 ml) were inoculated with 0, 1, 10 or 100 cells of *E. coli* O157:H7 or *Salmonella* and enriched in 225 ml of Reveal® 8-h medium and modified buffered peptone water with acriflavin, cefsulodin and vancomycin (mBPW+ACV) for *E. coli* O157:H7, and BPW and tetrathionate broth for *Salmonella*. After enrichment, samples were subjected to the protocol for each real-time PCR method. For *E. coli* O157:H7, similar detection limits were observed for both real-time PCR methods. Levels of 10 CFU/25 ml concentrated water and 1 CFU/25 ml unconcentrated water enriched in mBPW+ACV were confirmed. Levels of 100 CFU/25 ml concentrated water enriched in Reveal® could not be confirmed while 10–100 CFU/25 ml of unconcentrated water enriched in Reveal® were confirmed. Both real-time PCR systems were able to confirm *Salmonella* at 1 CFU/25 ml of concentrated and unconcentrated water enriched in tetrathionate broth. Levels of 10 and 1 CFU/25 ml of concentrated and unconcentrated sprout water, respectively, enriched in BPW were detected by the LightCycler *Salmonella* kit while the BAX® *Salmonella* kit had a slightly lower sensitivity. The higher level of background in concentrated water may have an effect on the sensitivity of detection; however, concentrating the irrigation water allows for testing a larger sample with greater likelihood of finding the target pathogen. This study suggests that sample concentration with selective enrichment followed by real-time PCR can confirm the presence of *E. coli* O157:H7 at levels of 10 CFU and *Salmonella* at a level of 1 CFU in 2.5 L of sprout irrigation water within one day.

P3-44 Application of a Biosensor for Rapid Detection of *Escherichia coli* O157:H7 Contamination in Food

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Development of rapid, sensitive methods for detecting *E. coli* O157:H7 is of significant interest because it offers the opportunity to increase the efficiency of food testing and decrease risk to the public. We offer a novel biosensor-based system that allows for simple, sensitive, real-time detection of pathogens in a variety of food matrixes. The sensor, based on the CANARY™ technology, allows for the detection of as few as 50 CFU *E. coli* O157:H7 and requires only 5 min to perform. The current CANARY™ *E. coli* O157:H7 assay enables detection of low levels of target (~1CFU/25 g sample) after 7 h enrichment using standard media. In this study our objective was to determine if we could shorten the enrichment time by making modifications to the sample handling process. Ground beef samples (25 g) obtained from a commercial outlet were placed in sterile sample bags and inoculated with low levels of *E. coli* O157:H7 (1-0CFU/sample). Samples were diluted in standard enrichment media, including mEC and TSB, and agitated using a Stomacher device, then incubated at 37°C for various times. Sample volumes of 1 ml-100 ml were then processed and analyzed using the CANARY™ biosensor assay. Our results confirm that the CANARY™ biosensor assay is capable of detecting as few as 220 CFU *E. coli* O157:H7 in ground beef samples post-enrichment, enabling the detection of low levels of contamination in ground beef samples after 7 h enrichment times. Optimizing enrichment conditions and increasing the volume of the sample analyzed allows one to decrease the enrichment time, leading to a shorter time to result. The combination of speed, sensitivity, and specificity offered by this assay system makes it an effective tool for routine screening of food samples.

P3-45 Withdrawn

P3-46 A Preliminary Study of Environmental *Escherichia coli* Source Tracking by Microarray

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On-farm hazard analysis and systems risk assessments are aided by microbial monitoring. Reliance on indicators, such as generic *Escherichia coli*, remains a major tool for critical, economic decision-making. Effective management of food safety risks is dependent on accurate identification of the indicators' source(s) to support the development of corrective action(s) that avoid setting microbial limits that cause economic hardship. A recent evaluation of bacterial source tracking (BST) techniques found false-homology in over half the samples and almost 10% incorrect source determination by pulsed field gel electrophoresis, repetitive element PCR, and ribotyping (Myoda et al., 2003). This project evaluated the potential of a DNA microarray to enhance sub-species differentiation between strains for BST as a consequence of the greater degree of discriminatory genetic variation and accuracy offered by this technique. The GeneChip® *E. coli* Genome 2.0 Array [EcG2 (Affymetrix, Inc.; Santa Clara, CA)] evaluates 20,366 genes and more than 700 intergenic regions from four different *E. coli* strains. A preliminary assessment of the EcG2 in discriminating presumptive clonal and non-clonal *E. coli* isolates recently recovered from water sediment and fresh produce was performed. Fifteen *E. coli* isolates were selected from sources of known common or unique temporal and geographic collection events. Pairwise comparisons (Cyber-T, UC Irvine, CA) and hierarchical clustering (ArrayAssist, Stratagene, La Jolla, CA) were done with the generated array data to assign relatedness among the isolates. These results were compared to BOX PCR and PFGE [analyzed by GelCompar (Applied Maths, Austin, Texas)] patterns for the same isolates, and variation is evident among all methods. Array analysis revealed that closely linked geographically, but temporally separated, isolates were homologous within a 3% divergence limit. Based on this preliminary *E. coli* library, microarray technology offers advantages in a more definitive assignment of relatedness that is highly desirable in specific forensic investigations.

P3-47 An Independent Comparison of the USDA/FSIS Reference Method to the USDA/FSIS Reference Method Incorporating the VIDAS® Immuno-Concentration *Escherichia coli* O157 Procedure for the Isolation and Recovery of *Escherichia coli* O157:H7 from Raw Ground Beef

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The current USDA/FSIS Reference Method for the detection of *E. coli* O157:H7 uses an immunomagnetic separation method along with chromogenic agar for the isolation of the target organism. The purpose of this study was to compare the results of the traditional immunomagnetic separation isolation procedure in the USDA/FSIS reference method to the USDA/FSIS reference method with the VIDAS® Immuno-Concentration *E. coli* procedure (ICE) for the isolation and recovery of *E. coli* O157:H7 from raw ground beef. For both the USDA/FSIS reference method and the USDA/FSIS reference method ICE procedure, the primary enrichment and confirmation procedures were the same. The use of the ICE procedure replaced the immunomagnetic separation step (IMS) used in the USDA/FSIS method. Additionally, all immunoconcentrated samples from the IMS procedure and the ICE procedure were streaked onto Rainbow chomogenic agar and O157:H7 ID agar. A total of 80 inoculated ground beef samples and 15 uninoculated ground beef samples were evaluated by both methods. Twenty replicate samples of ground beef were inoculated at a high level (2 CFU/25 g), forty replicate samples of ground beef were inoculated at a medium level (1 CFU/25 g) and twenty replicate samples were inoculated at a low level (0.73 CFU/25 g). At the high level of inoculation, there were 3/20 confirmed positive results from the USDA/FSIS reference method compared to 5/20 confirmed positive results from the USDA/FSIS + ICE procedure. At the medium level of inoculation, there was 1/40 confirmed positive results from the USDA/FSIS reference method compared to 15/40 confirmed positive results from the USDA/FSIS + ICE procedure. At the low level of inoculation, there were 2/20 confirmed positive results from the USDA/FSIS reference method compared to 3/20 confirmed positive results from the USDA /FSIS + ICE procedure. The ICE procedure compared favorably with the IMS procedure in the USDA/FSIS reference method. The use of O157:H7 ID chromogenic agar increased the number of confirmed positive samples at all three inoculation levels compared with the Rainbow agar used in the USDA/FSIS reference method.

P3-48 An Independent Comparison of the bioMérieux TEMPO® EC Method to the Petrifilm™ *Escherichia coli*-Coliform Count Plate Method (AOAC Official Method 991.14) for the Enumeration of *Escherichia coli* in Food Products

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The TEMPO® EC (*E. coli*) method is an automated system for the enumeration of *E. coli* in foods. The test consists of a vial of dehydrated culture medium and an enumeration card containing 48 wells of three different volumes. Inoculated cards are incubated for 24 h and then placed in an automated reader where the fluorescence in the wells is detected, and depending on the number and location of the positive wells, the system reports the number of microorganisms present as the most probable number (MPN). Naturally contaminated and artificially contaminated food products were tested. A total of 224 samples representing 6 food categories (poultry, meat products, vegetables, dairy, fish and seafood) were tested. A miscellaneous category included the following foods: fresh fruit, salad dressings and dry pet foods. In this study, 25 g of product was added to 225 ml of Butterfield's phosphate buffer and stomached for 2 min in a TEMPO stomacher bag. For the TEMPO method, 1 ml of the sample was added to the culture medium. Cards were filled and sealed in the automated filler and then were incubated for 24 h at 35 ± 1°C. After incubation, the cards were placed in the automated TEMPO reader and an MPN result was generated. For the Petrifilm™ *E. coli*-Coliform Count Plate Method (AOAC Official Method 991.14), decimal dilutions were prepared from the same homogenized sample described above and transferred onto Petrifilm™ *E. coli*-Coliform Count Plates. Plates from meat, poultry and seafood were incubated at 35 ± 1°C for 24 ± 2 h. Plates from all other foods were incubated at 35 ± 1°C for 48 ± 2 h. The percentage agreement between the two methods for the food groups were: dairy products-100%, fish and seafood-80%, meat products-96.8%, poultry-85.7%, vegetables-93.4% and miscellanous-97.3%. Overall agreement between the two methods was 94.2%. In terms of overall accuracy, the TEMPO EC method is equivalent to the Petrifilm™ *E. coli*-Coliform Count Plate Method (AOAC Official Method 991.14) for the enumeration of *E. coli* in foods.

P3-49 A Comparative Evaluation of the MPN Method with Plating Methods for the Enumeration of *Escherichia coli* in Spiked Cold Smoked Salmon Fillets

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The Canadian Food Inspection Agency (CFIA) uses the multiple tube fermentation method (MPN) for the enumeration of *E. coli* in processed fish product, to assess compliance with the Bacteriological Guidelines for Fish and Fish Products. There are alternative methods for the enumeration of *E. coli* in foods which provide a more accurate and timely result, but many of these do not provide the required sensitivity of < 4 CFU/gram for Ready-to-Eat fish products. The purpose of this study was to evaluate three methods in parallel with the MPN method, for the testing of spiked cold smoked salmon: Hydrophobic Grid-Membrane Filtration (HGMF) using Chromocult® Coliform Agar (CCA), Direct Plating onto CCA, and 3M PETRIFILM™ *E. coli* Plate (Petrifilm) method. The fillets were spiked at four *E. coli* concentrations ranging from 0 to 80 CFU/g, and then tested by each method. To increase the sensitivity of the Direct Plating and the Petrifilm methods, the initial dilution of 1 to 1 (w/w) was used on multiple plates, resulting in a sensitivity of 2 CFU/g and 1 CFU/g respectively. Variance analysis (ANOVA, $P < 0.01$) along with a multiple comparison procedure (Tukey HSD, $P < 0.01$) were performed to identify significant differences. The HGMF on CCA method resulted in mean *E. coli* values that were significantly lower than the 5-tube MPN method ($P < 0.01$), and is therefore not suitable. The direct plating on CCA and the Petrifilm method are both easy to use, are less labor intensive, provide results within 48 h, and show no significant difference (ANOVA, $P < 0.01$) from the MPN method.

P3-50 Comparison of Results between Two International Standard Methods (ISO 16649) and the TEMPO EC Test for the Quantification of *Escherichia coli* from Chilled and Frozen Foods

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Rapid and accurate quantification of *E. coli* in foods remains important, especially recovery and isolation of sub-lethally injured cells post storage. This study compared the recovery and quantification of *E. coli* in 50 foods (7 food types) using two published standard methods (ISO 16649-1 and 16649-2) and the TEMPO EC test (bioMérieux). Part 1 (ISO 16649-1) involves direct plating suitable dilutions onto a cellulose acetate membrane (0.45µm) on a recovery medium (MMGA) incubation at 37°C/4 h, followed by transfer to typtone bile X-glucuronide (TBX) medium (44°C/20 h). Part 2 (ISO 16649-2) permits direct plating onto TBX (44°C /24 h) without a recovery step, unless injury is suspected, but many do not favor this additional step. *b*-Glucuronidase-positive *E. coli* colonies are calculated from plate counts. The TEMPO EC test, is a 3 × 16 well most probable number (MPN) style test comprising a plastic card with clear optical window. With the aid of the TEMPO instrument, each card is automatically filled with a diluted sample (containing a fluorogenic substrate), sealed and after 37°C/24 h incubation off-line, the fluorescence in each well is read and the MPN/g calculated by the TEMPO software. Each sample, previously contaminated (range 10^1 to 10^4 CFU/g) with a strain of *E. coli* (NCTC 12241) were stored (2–8°C or –20°C/48–72 h) diluted (serial decimal) in maximum recovery diluent. Appropriate dilutions were used for the ISO methods and 1/40 by the TEMPO EC test. The results revealed that the TEMPO EC test gave equivalent results to ISO 16649-1 (R= 0.93), indicating good recovery of injured cells. Comparison between the two ISO methods (R= 0.87) and TEMPO EC test and ISO 16649-2 (R= 0.88) showed that without resuscitation this ISO method under-recovers injured *E. coli* cells and that TEMPO EC test and iso16649-1 are superior for the enumeration of *E. coli* from foods likely to contain injured cells.

P3-51 Evaluation of the TEMPO System to the FDA/BAM Reference Method and an Alternative Plating Method for the Enumeration of Total Viable Count, *Escherichia coli* and Coliforms in Foods

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The increasing emphasis on total quality approach in food production, HACCP plans and risk assessment procedures highlight the role that microbial quality indicators such as Total Viable Count, Coliforms and *Escherichia coli* have in monitoring the hygienic and commercial quality of food. Quality indicators represent almost 80% of the analyses performed by the routine food microbiology laboratory. Although numerous rapid microbiological methods have been developed to introduce a degree of automation for pathogen detection in food, the focus on automated methods for the enumeration of quality indicators has introduced only a limited standardization of the analysis. TEMPO® is an automated enumeration method based on an innovative miniaturized Most Probable Number (MPN) card requiring only a single sample dilution step. This work evaluates the performance of the TEMPO TVC (Total Viable Count) compared to the conventional FDA/BAM reference plate count method (PCA 35°C 48 h) and the TEMPO EC (*Escherichia coli*) and TEMPO CC (Coliform Count) to the alternative Petrifilm™ *E. coli*-Coliform method (35°C 24–48 h) for the testing of a wide range of naturally contaminated food products (raw and cooked meats, poultry, egg products, RTE foods, seafood, raw and processed vegetables and dairy products). A total of 150 results were obtained for Total Viable Count, 150 for *Escherichia coli*, and 100 for Coliform Count. The statistical analysis based on log comparison and paired *t*-test at a 5% level demonstrated that the TEMPO method performs as well as the FDA/BAM reference plate count method and the Petrifilm™ *E. coli*-Coliform. Results show a good correlation with a percentage agreement of 95.0%, without any significant deviation (*P*-values < 5%). TEMPO TVC, EC and CC methods are viable alternatives to FDA/BAM and Petrifilm™ *E. coli*-Coliform methods, while reducing time, labor and providing a complete traceability of the analysis.

P3-52 Identification and Quantification of Unknown Enterohemorrhagic *Escherichia coli* (EHEC) Isolates by Multiplex Real-time PCR Assay: A Multi-laboratory Study

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Use of Real-time PCR technology in identification of foodborne pathogens provides rapid and sensitive detection of potentially harmful bacteria. However, the PCR assay must be easy to perform while providing reliable results. Adequate training and follow-up assessment of aptitude are critical to ensure both personnel and assay perform exceptionally well. A lyophilized, multiplex real-time PCR assay has been developed to detect both Shiga toxin-producing *E. coli* and *E. coli* O157:H7, a human pathogen capable of causing serious illnesses such as hemorrhagic colitis and hemolytic-uremic syndrome. The PCR assay targets the *stx*1, *stx*2 and conserved +93 SNP in the uidA gene. Forty-two sets of reagents were provided to trainees representing 23 different state and federal laboratories.

Each set of reagents consisted of lyophilized components in the form of STEC/*E. coli* O157:H7 beads (primers, probes, and Internal Control) and OmniMix, PCR beads. Participants calculated PCR efficiencies from dilutions of a supplied control DNA template (*E. coli* O157:H7 containing both *stx*1 and *stx*2 genes), in addition to dilutions prepared by the participant. Also, four unknown templates supplied at two concentrations (10^3 and 10^5 CFU/ml) were tested by each laboratory. Total average PCR efficiency was 85.2%, 97.2%, and 79.5% for *stx*1, *stx*2, and uidA, respectively. Unknown templates were correctly identified by participants in 42 of 42 test samples (100%) across both dilutions and all gene targets (0% false positive, 0% false negative). Cycle threshold (Ct) values were consistent for the concentrations in the unknown templates, permitting relative quantification of pathogen from a standard curve using any of the three gene targets. No major differences were observed between deriving unknown concentrations from provided dilutions and dilutions prepared by testing laboratory. Hands-on training, robust PCR methods, and follow-up practice samples ensured proficient use of real-time PCR technology in multiple laboratory environments.

P3-53 Rapid and Effective Method to Improve Detection and Isolation of *Escherichia coli* O157:H7 from Fresh Produce

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Consumption of fresh produce has been increasingly implicated in *E. coli* O157:H7 outbreaks in the US. Low numbers of organisms in Ready-to-Eat salad have been associated with illnesses. Low-level contamination with high background of competing microorganisms is a major limiting factor for detection and isolation of the *E. coli* O157:H7 in food particularly in fresh leafy produce. We used the Pathatrix immuno-magnetic capture system coupled with a real-time PCR to detect and isolate low-levels of *E. coli* O157:H7 from Ready-to-Eat leafy produce with a short enrichment. The Pathatrix system recirculates enrichment culture of 150 grams of romaine lettuce or spinach previously enriched in modified buffer peptone water past immuno-magnetic beads to capture the *E. coli* O157:H7. The real-time PCR specific for *stx*1, *stx*2 and mutation of uidA genes was used to identify the presence of the organisms. Real-time PCR analysis showed increased detection of *E. coli* O157:H7 in whole leaves of romaine lettuce or spinach compared to those in rinsates when both were artificially contaminated with 0.1–2 CFU per gram after a five-hour enrichment. Cultivation at 42°C for 5 h of pre-packed romaine lettuce or spinach seeded with 0.07–0.1 CFU per gram promoted better growth of the *E. coli* O157:H7 than those enriched at 37°C. Isolation of positive colonies based on the US FDA Bacteriological Analytical Manual method and real-time PCR analysis indicated a significantly increased recovery of *E. coli* O157:H7 following the Pathatrix immuno-magnetic capture system. Pathatrix and real-time PCR with 5-h enrichment at 42°C of whole leafy produce improves detection and isolation of *E. coli* O157:H7. This rapid method increased the chances of recovery for *E. coli* O157:H7 from fresh produce and it can be used to expedite foodborne outbreak investigations and prevent further occurrences of illnesses.

P3-54 *Escherichia coli* O Antigen Typing Using DNA Microarrays

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Traditional serotyping for *Escherichia coli* is laborious, time consuming, and often generates equivocal results due to cross-reactions among different serogroups. The objective of this study was to develop DNA microarrays for rapid identification of different serogroups of *E. coli* in a single platform. Oligonucleotides as well as PCR products from genes in the O-antigen gene clusters of *E. coli* serogroups O7, O104, O111, and O157 were spotted onto glass slides. This was followed by hybridization with labeled long PCR products of the entire O antigen gene clusters of these serogroups. Results demonstrated that microarrays consisting of either oligonucleotides or PCR products generated specific signals for each serogroup. In related studies, the O antigen gene clusters of *E. coli* serogroups O28, O117, O119, O126, and 146 were sequenced, and the genes were annotated. There were 7, 11, 9, 10, and 11 open reading frames in *E. coli* O28, O117, O119, OO26, and O146, respectively, and unique sequences in these serogroups were identified. The *E. coli* typing arrays can be expanded to include unique sequences from these and additional serogroups. Thus, DNA microarrays spotted with either oligonucleotides or PCR products, followed by hybridization with labeled long PCR products are feasible approaches for identifying *E. coli* strains based on O types, which could replace serogrouping using antisera.

P3-55 Evaluation of the Envisio™ *Escherichia coli* O157 Test System for the Detection of *Escherichia coli* O157:H7 from Ground Beef

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A newly developed detection system, using the Envisio™ magnetic detection technology (Centrus International, Inc.), was evaluated for the detection of *E. coli* O157:H7 in ground beef. The system performance was compared to RapidChek® *E. coli* O157 (Strategic Diagnostics, Inc.), a widely used commercial lateral flow immunoassay, and to a *E. coli* O157:H7 reference method (Health Canada Compendium of Analytical Methods). The limits of detection were estimated at 10^5 CFU/mL for the RapidChek O157 test kit and 10^3 CFU/mL for the Envisio *E. coli* O157 assay for the three *E. coli* O157:H7 strains tested. Detection rates of 88% (21/24) were achieved by the Envisio system at <1 CFU/25g inoculum level after 7.25 h of enrichment, compared to a 78% (21/27) detection rate by the RapidChek® test kit after 8 h of enrichment. Equivalent detection rates of 96% (22/23) were obtained by both tests systems when samples were inoculated at a 1 to 5 CFU/25 g level and 100% (18/18) detection rate in samples containing >10 CFU/25 g level. Testing of 100 natural, uninoculated retail samples by both detection systems in their respective enrichment media showed 100% specificity for the Envisio *E. coli* O157 system, and 99% specificity for the RapidChek *E. coli* O157 test kit. These results demonstrate that the Envisio *E. coli* O157 test system is suitable for rapid detection of *E. coli* O157:H7 at low levels in ground beef.

P3-56 Optimization of *Escherichia coli* O157:H7 tRNA Extraction for Microarray Analysis

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In this work, we describe an optimized protocol for total RNA (tRNA) extraction from *Escherichia coli* O157:H7 for microarray analysis. Two common extraction methods (RNeasy mini kit [Qiagen] and TRIzol Reagent [Gibco]) were evaluated on four sample groups of *E. coli* O157:H7 grown to 7 log CFU/mL in Luria-Bertani broth as follows: control (pH 7; 37°C), refrigeration (pH 7; 10°C),

acid shocked (pH 7 for 6 h at 37°C; then pH reduced to 3.5 for up to 60 min), and acid adapted (pH 5 for 12 h at 37°C; then pH reduced to 3.5 for up to 60 min). Cultures were centrifuged and tRNA was extracted initially according to manufacturers' protocols, which were then modified as necessary. tRNA quality and quantity were determined spectrophotometrically (expressed as absorbance) using the 260/280 absorbency ratio; organic carryover (impurities) was determined using the 260/230 absorbency ratio. Our results demonstrated that extracted tRNA (260/280 ratio) of 1.8 to 2.0 were optimal for microarray analysis. Lower ratios indicate that extracted tRNA is not pure, resulting in poor microarray hybridization; 260/230 ratios < 1.8 indicate that organic compounds from the extraction are carried over. Extracting using TRIzol reagent yielded low levels of tRNA, with a 260/230 ratio consistently less than 1.8 from all sample groups. The RNeasy mini kit extracted optimal tRNA levels with optimal 260/280 and 260/230 ratios from control and refrigeration groups, but no tRNA was extracted from the acid shocked and acid adapted groups. Microscopic and culture analyses revealed that acid shocked and acid adapted cells were intact and viable after extraction. Modifying the extraction protocol to include RNA stabilizing reagents, increased lysozyme concentration, increased extraction incubation time, and cell lysis columns was necessary to provide optimal tRNA yield for microarray analysis. These results demonstrate the need for optimization of RNA extraction methods when culturing conditions deviate from optimal.

P3-57 Duplex Fluorescence Real-time PCR for Detection and Quantification of *Escherichia coli* Harboring Heat-stable Enterotoxin Genes in Foods

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Since the discovery of enterotoxigenic *Escherichia coli* (ETEC) O169:H41 in 1991, this emerging pathogen has spread not only throughout Japan but also the USA. However, the source and routes of infection have not been clarified because detecting and identifying this organism among many coliform bacteria is difficult. To facilitate ETEC identification, we developed and evaluated duplex real-time fluorescence PCR assays using the ABI Real-Time PCR System against genes encoding type I heat-stable enterotoxins (ST I) that comprise STp and STh according to their nucleotide sequences. Primers and TaqMan probes labeled with the fluorescent markers FAM or VIC were designed to amplify and quantify the STp and STh genes in a single reaction. Specificity was confirmed using 10 ST-ETEC that produce ST I and 20 non-ETEC strains of human origin. The fluorescence threshold cycle and the number of CFU per milliliter directly correlated in this assay. A total of 150 food samples including 30 of minced pork spiked with ST-ETEC were enriched according to the bacteriological analytical manual of the FDA, and ST-ETEC were assayed in the enrichment media by real-time PCR. The detection limit was about 1.0×10^4 CFU/ml of enrichment broth and below 10 CFU/g with the enrichment. We also compared the abilities of conventional and real-time PCR to detect ST-ETEC. Duplex real-time assays detected 27 spiked and 3 naturally polluted samples, whereas conventional PCR detected only 7 spiked samples. Although the conventional PCR assay showed amplicons of a similar size to the predicted ST I gene fragment in 3 samples, they seemed to be false positives since they were negative in the real-time PCR assay with the probes. The rapidity, sensitivity and specificity of duplex real-time PCR allow efficient, quantitative detection of ST-ETEC in foodstuffs.

P3-58 The Application of Acid Shock as a Selective Step to Isolate Enterohemorrhagic *Escherichia coli*

DSC

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Escherichia coli O157:H7 is the primary cause of outbreaks due to enterohemorrhagic *E. coli* (EHEC) in the US, but recent epidemiological studies have suggested that 25% of EHEC-related illnesses are caused by non-O157 serotypes such as O26 and O111. However, no official method exists that allows differentiation between non-O157 EHEC and commensal *E. coli* strains, mainly due to the lack of distinguishing characteristics. The objective of this project was to determine the selectivity of a protocol for the detection and isolation of a variety of EHEC serotypes based on the ability of *E. coli* to withstand exposure to acidic conditions and a selective media containing potassium tellurite. Cultures of EHEC strains and other enterobacteria were grown overnight in complex media, and they were either plated directly onto potassium tellurite-containing agar or subjected to an acid shock in glutamic acid solutions (2 h at pH 2.0). Acid-shocked cells were then plated onto the same tellurite agar. More than 81% of EHEC and commensal *E. coli* strains had 10% or greater recovery rates after acid shock, but only 5% of other enterobacteria had similar survival. From a total of 190 EHEC and 151 commensal *E. coli* isolates, approximately 95 and 5%, respectively, were capable of growing on tellurite-agar. Based on these results a 3-step protocol was established: enrichment in EC broth, acid shock treatment, and plating on tellurite-containing media. Competition studies in mixed cultures of EHEC strains vs. enterobacteria and commensal *E. coli*, inoculated at different levels showed that with this protocol EHEC could be recovered from an inoculation level as low as 10 CFU/ml. These findings indicated that a combination of an acid shock with selective plating could be used in the detection and isolation of EHEC including serotypes O157, O26 and O111.

P3-59 Cloth-based Hybridization Array System for the Identification of *Escherichia coli* O157:H7

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A simple macroarray system based on the use of polyester cloth as the solid phase for DNA hybridizations has been developed for the identification of various virulence, toxin and strain-specific genes in bacteria. This approach, termed Cloth-based Hybridization Array System (CHAS), was adapted as a tool for the definitive identification of presumptive *E. coli* O157:H7 colonies isolated from foods using standard culture techniques. Key indicator genes for this organism, rfbO157, fliCH7, vt1 and vt2 (encoding the O and flagellar antigenic determinants and verotoxin genes, respectively) were amplified in a multiplex PCR incorporating digoxigenin-dUTP, followed by hybridization of the amplicons with an array of specific oligonucleotide probes immobilized on polyester cloth, and subsequent immunoenzymatic assay of the bound digoxigenin label. This system includes a simple internal amplification control (IAC) to gauge PCR inhibition based on the incorporation of a primer pair with complementary 3' ends, resulting in the generation of a unique "primer-dimer" detectable by hybridization with a specific capture probe immobilized on polyester cloth. The CHAS

provided sensitive and specific detection of the *E. coli* O157:H7 gene markers, exhibiting the expected patterns of reactivity with a panel of target and non-target organisms. The result was a simple and effective confirmatory tool for *E. coli* O157:H7, permitting the detection of the key identifying markers for this pathogen.

P3-60 Comparison of the TEMPO® EC with the Traditional MPN Method for Enumeration of *Escherichia coli*

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The TEMPO system (bioMérieux) provides a new and streamlined approach to enumeration of quality indicator organisms. Although, in principle, the TEMPO method is based on the MPN technique (3 × 16 tubes), the tedious and labour intensive steps of the traditional method, such as multiple tube dilutions, multiple transfers, plating, media preparation and glassware washing, have been eliminated. It is only necessary to prepare a tenfold dilution of the food sample for testing in the automated system. A study comparing the TEMPO EC with the 3 tube MPN method (AOAC 966.24) was undertaken as part of the AOAC approval process. A variety of foods (both naturally and artificially contaminated) were tested at 3 different contamination levels: 10–100, 100–1000, and 1000–10,000 *E. coli*/g. For each level 5 replicates were tested, as well as 5 uninoculated control samples, for artificially contaminated foods. For the majority of foods and levels there was no significant difference between the 2 methods using a paired *t*-test at the 5% level. However, the TEMPO appeared better for detection of stressed *E. coli*, especially in acid products, and detection of weak gas producing strains. The TEMPO method gave an *E. coli* count within 24 h of sample set up, compared to 5 days for a presumptive count using the reference method. The TEMPO system provides very significant savings in labour especially when large numbers of positive samples are processed, as well as complete traceability of samples, which is important in today's testing environment.

P3-61 Comparison of Commercial Test Kits to Screen for *Escherichia coli* O157:H7 in Media

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Follow-up analyses of food and meat samples that have false-positive screening results consumes laboratory resources. The present study was undertaken to determine the performance characteristics of five lateral flow devices (LFD) and a PCR test for screening *E. coli* O157:H7. A total of 1550 enrichment broths were spiked with various levels of *E. coli* O157:H7 (10^1–10^9) to directly compare the Reveal (Neogen), RapidChek (SDI), SinglePath (Merck), Transia (Diffchamb),VIP (BioControl), and the BAX MP (Qualicon) PCR assay. Reveal and SinglePath had the highest number of false positives ($P < 0.05$) when compared to the other 3 LFD tests. The BAX MP had fewer false positives ($P < 0.05$) when compared to the LFD tests. The BAX MP detected *E. coli* O157:H7 in spiked enrichment broths at 10^3 CFU/ml, whereas the LFD tests detected the pathogen at 10^5 CFU/ml. There was no difference in the false negative rate ($P < 0.05$) for the BAX MP, Reveal, RapidChek or Transia tests. The costs of the four LFDs and BAX are within 10% of each other. Implementing the BAX MP for screening *E. coli* O157:H7 in meat and food could reduce the number of false-positive screening results, thus decreasing additional use of resources, decreased sample throughput, and increased analytical turnaround times.

P3-62 Comparison of an Automated Method, TEMPO® CC for the Enumeration of Coliforms in Food with the Reference Method (FDA/BAM) and Petrifilm™ Method

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The enumeration of quality indicators is increasing in the food industry due to the focus on food microbial quality. A widely used indicator is the coliform count, a group of enteric bacteria known as an indicator of sanitary conditions in the food-processing environment. In this study, we compared TEMPO® CC (Coliform Count), a new automated coliform enumeration method, to the Food and Drug Administration —Bacteriological Analytical Manual (FDA/BAM) method, and to an alternative method, Petrifilm™ *E. coli*-Coliform (AOAC Official Method 991.14). TEMPO® CC is a method for coliform enumeration, based on the familiar format of the MPN procedure. The TEMPO CC method utilizes a selective dehydrated culture media and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the MPN. TEMPO® CC provides results within 24 h compared to 2–4 days for the reference method. In this study, more than 200 naturally contaminated products were tested from different food categories such as raw and cooked meat and poultry products, fish and seafood products, vegetables, and Ready-to-Eat (RTE) foods. A combination of regression analyses, difference log distributions and t-tests at the 5% level were used to analyse the data and compare performance. The TEMPO method showed similar performance to the reference and the alternative method with an agreement level greater than 90%. Regression analysis gave coefficients of variation close to 0.9 for the different comparisons. The TEMPO® CC method compared favorably to the FDA/BAM reference method and the Petrifilm™ *E. coli*-Coliform method. The TEMPO® CC method offers an alternative rapid automated enumeration system for food laboratories conducting coliform counts.

P3-63 The Recovery of *Enterobacter sakazakii* Using a New Enrichment Broth

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The recovery of 63 strains of *Enterobacter sakazakii* previously grown in brain heart infusion broth at 30°C for 24 h, 4 yeasts, and 39 bacteria other than *E. sakazakii* was determined in a new enrichment broth (R & F *Enterobacter sakazakii* Enrichment Broth [ESEB]), in EE broth (FDA recommended procedure), and in tryptic soy broth (TSB), incubated for 24 h at 35°C. Potato dextrose agar (PDA), APT agar, and tryptic soy agar with 0.06% yeast extract (TSAYE) were used to enumerate, respectively, the yeasts, lactic acid bacteria, and *E. sakazakii*. Duplicate PDA, APT, and TSAYE plates were incubated at 25°C for 5 days, 30°C for 48 h, and 35°C for 24 h, respectively. Log values of the growth responses for each strain growing in ESEB and EE were related to growth in TSB using the following categories: 4+ (0.5 log or less), 3+ (between 0.5 and 1.0 logs less), 2+ (between 1.0 and 2.0 logs less), 1+ (greater than 2 logs less), and 0 for no growth or death. In ESEB, 87.3% (55/63) of the strains were 4+, 11.1% (7/63) were 3+, and only 1.6% (1/63) was at 2+ of the total. In comparison, the first three growth categories in EE were nearly equally distributed between 4+ (28.6%),

3+ (31.7%), and 2+ (31.7%); with 1+ (4.8%), and 2 strains (3.2%) at 0. Both ESEB and EE broths did not allow for growth of *Bacillus, Listeria,* and *Staphylococcus* spp. For the 10 genera of enteric bacteria tested, both broths supported good growth in comparison with TSB having 4+ and 3+ distributions. For the enterococci, yeasts, and lactic acid bacteria, EE broth was more inhibitory than ESEB. The increased efficiency of ESEB versus EE broth on the recovery of uninjured *E. sakazakii* requires that its effect be established as well on the recovery of injured *E. sakazakii* cells from artificially inoculated food and environmental sources.

P3-64 A Multi-Chromogenic Agar for the Dual Detection of Nonpathogenic and Pathogenic *Listeria* Species

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A selective and differential chromogenic plating medium (R & F *Listeria* spp./*Listeria monocytogenes* Plating Medium [LSPM]) has been developed that simultaneously differentiates presumptive colonies of both the nonpathogenic *Listeria* species (*L. innocua, L. seeligeri. L. welshimeri, and L. grayii*) and the pathogenic species (*L. monocytogenes* and *L. ivanovii*) on a single plate in 24–48 h at 35°C. Unlike chromogenic media that produce only a single color in detecting the presence of all *Listeria* species on the basis of -glucosidase activity, or those that specifically detect the two pathogenic species by phosphatidylinositol-specific-phospholipase C activities (PIPLC), LSPM contains a combination of indoxyl-derivative chromogenic substrates, wherein colonies of nonpathogenic *Listeria* species are pink due to their -glucosidase activity, and pathogenic species are blue-green to blue-violet depending on the strain-specific balance of -glucosidase (pink) and PIPLC (blue) activities on an agar with an opaque white background. On LSPM, 39 pure culture strains of *L. monocytogenes* yielded blue-green to blue-violet colonies 1–2 mm diameter with or without surrounding precipitates in 24–48 h at 35°C, and 4 strains of *L. ivanovii* yielded dark blue-green colonies with dark precipitates, whereas all of the nonpathogenic *Listeria* strains yielded pink colonies 1–2 mm diameter without precipitates. The high selectivity of LSPM was evidenced in the lack of growth by common species of five gram-positive genera (*Bacillus, Staphylococcus, Lactobacillus, Pediococcus, and Enterococcus*), and eight gram-negative genera (*Escherichia, Enterobacter, Citrobacter, Shigella, Morganella, Providencia, Pantoea, and Klebsiella).* Two yeast genera, *Zygosaccharomyces* and *Candida,* also failed to grow.

P3-65 A Comparison Study of the VIDAS® *Listeria* Species Xpress (LSX) with Ottaviani Agosti Agar (OAA) Method to the USDA/FSIS and AOAC Official Methods for the Specific Detection of *Listeria* Species in Meat and Dairy Products

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The VIDAS® *Listeria* species Xpress (LSX) test is an enzyme-linked fluorescent immunoassay (ELFA) designed for use with the automated VIDAS® or mini-VIDAS® instruments for the specific detection of *Listeria* species. The test method was validated according to AOAC Official Method guidelines for use with meat and dairy products, using a 30-hour proprietary enrichment broth. The alternative method also included the use of chromogenic medium, Ottaviani Agosti Agar (OAA), for confirmation of LSX presumptive results.The alternative rapid method was compared to the USDA reference method for the detection of *Listeria* species in artificially contaminated raw pork, frankfurters, roast beef, raw ground beef and ham, and was also compared to the AOAC Official Dairy Method (993.12) for the detection of *Listeria* species in artificially contaminated yogurt, camembert cheese, pasteurized whole milk, cheddar cheese and vanilla ice cream. The number of samples found positive by VIDAS and negative by the reference methods vs. number of samples positive by the reference methods and negative by VIDAS was compared using McNemar's method for paired analysis. A X2 value of > 3.84 was indicative of a significant difference at the 5% probability level. The VIDAS LSX method was significantly more sensitive than the USDA method for raw pork, and was significantly more sensitive than AOAC Official Method for Camembert cheese. For all other products tested, VIDAS LSX was statistically equivalent to the reference methods. Confirmation of presumptive LSX results with the chromogenic OAA medium was shown to be equivalent to the reference method agar (modified Oxford agar [MOX] for USDA method and Oxford Agar [OXA] for AOAC 993.12).

P3-66 Use of 1-ply Composite Tissues in an Automated Optical Assay for Recovery of *Listeria* from Stainless Steel, High-density Polyethylene, and Environmental Samples

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A novel 1-ply composite tissue (CT) method using the Soleris (formerly BioSys) optical analysis system was compared to the conventional USDA environmental sponge enrichment method for recovery of *Listeria* spp. from food contact surfaces and naturally contaminated food processing environments. Stainless steel (SS) and high-density polyethylene (HDPE) plates inoculated to contain a 6-strain *L. monocytogenes* cocktail at 1 to 10 CFU/plate as well as naturally contaminated food contact surfaces and floor drains from a food processing facility were sampled using the CT Soleris and USDA environmental sponge methods. CT samples were transferred into Soleris 32 system vials and incubated at 35°C, after which positive vials were streaked to Modified Oxford plates for *Listeria* confirmation, whereas environmental sponge samples were processed according to the USDA *Listeria* enrichment protocol. The CT Soleris 32 and USDA environmental sponge methods had detection sensitivities of 73% and 57% for SS, and 75% and 48% for HDPE, respectively. *Listeria* spp. were detected in 9.0% and 11.0% of environmental samples using the CT Soleris and USDA environmental sponge methods, respectively. No significant differences ($P > 0.05$) were seen between the two methods for inoculated food contact surfaces or naturally contaminated environmental samples with *Listeria* detected 2 to 3 days sooner using the CT Soleris method. These findings indicate that environmental samples from food processing facilities can be rapidly and reliably screened for *Listeria* spp. using the newly developed CT Soleris method.

P3-67 Evaluation of Chromogenic Media for the Isolation and Identification of *Listeria monocytogenes* and Other *Listeria* Species

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Methods for the detection of *Listeria monocytogenes* in foods include an enrichment procedure, which promotes the growth of all *Listeria* species. It has been documented that other *Listeria* species, specifically *Listeria innocua*, replicate at a faster rate and may have an inhibitory effect on the growth of *L. monocytogenes*. Since it is not possible to distinguish between these species when streaked onto the traditional selective agars, the probability of selecting a *L. monocytogenes* colony may be reduced. Optimizing a method for the selection and identification of *L. monocytogenes* is advantagous because compliance action is based solely on the detection of this species. The objective of this study was to evaluate two chromogenic agars, Oxoid Chromogenic *Listeria* Agar (OCLA) and Bio-Rad Rapid'L.mono Agar® (RLm) for use in the *Listeria* methods Isolation of *Listeria monocytogenes* from all Food and Environmental Samples [MFHPB-30], and The Detection of *Listeria* spp. in Foods and Environmental Samples Using Palcam Broth [MFHPB-07]. These methods use Oxford and Palcam as selective agars. Five *Listeria* species were used individually and in mixtures to inoculate seven food samples. The data demonstrates that both chromogenic agars were equivalent to Oxford and Palcam agars for both methods. Colonies of *L. monocytogenes* on these chromogenic agars were distinctive and easily selected from other *Listeria* species for confirmation. Both chromogenic agars supported the growth of *L. ivanovii*, which Oxford agar did not do consistently. MFHPB-30 detected all species of *Listeria* from the test samples, while MFHPB-07 detected only *L. innocua* in all but one sample. This could indicate a potential problem with the Palcam selective enrichment broth in MFHPB-07 which may select for the growth of *L. innocua* over *L. monocytogenes*.

P3-68 One-step Enrichment for Detection of *Listeria* spp. in Environmental and Food Samples by DNA Hybridization

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A one-step enrichment protocol for use with a microwell-format DNA hybridization assay has been validated for environmental surfaces including stainless steel, plastic, cast iron, ceramic tile, sealed concrete and painted wood. Pilot studies have been conducted with five foods including deli turkey, brie cheese, lettuce, smoked salmon and cooked crab meat. Enrichment was conducted in *Listeria* Enrichment Single-Step (LESS) broth. Environmental surfaces and foods were inoculated with *Listeria* spp. at a level intended to produce fractional positive results. The environmental surfaces were allowed to dry for a minimum of 16 h before testing, which resulted in a several log reduction of the inoculum and produced the intended fractional recovery. Food samples were inoculated at two levels and refrigerated for at least 72 h prior to testing. Following enrichment for 30 h at 30°C, samples were tested with the GeneQuence DNA hybridization assay, which employs *Listeria*-specific oligonucleotide probes labeled with horseradish peroxidase (HRP) and results in a photometrically determined endpoint. A duplicate set of inoculated surfaces or foods was tested using the FDA/BAM or USDA-FSIS culture method. For environmental surfaces, out of a total of 175 inoculated samples and controls, the DNA hybridization method detected 110 positives versus 62 positives by the USDA-FSIS culture method. Only on sealed concrete did the USDA-FSIS method produce more positives than the hybridization method, although the difference was not significant. For the remaining 5 surfaces, the DNA hybridization method produced more positives than the USDA-FSIS method, and for stainless steel, plastic, cast iron and painted wood the differences were statistically significant. For the pilot study with foods, out of a total of 50 samples and controls, the DNA hybridization method detected 32 positives versus 34 by the FDA/BAM or USDA-FSIS culture methods. The DNA hybridization method produced no false positive results.

P3-69 DSC Use of Procedures Incorporating a Repair Step Results in Improved Detection of *Listeria* in Food Processing Plant Environmental Samples

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Colonization of *Listeria monocytogenes* in food processing plants and establishment of niches can lead to continuous contamination of food during processing. We evaluated four commercially available rapid detection procedures against a standard and a modified cultural procedure for the detection of *Listeria* spp. in environmental samples. A total of 420 environmental sponge samples were collected from an infant formula manufacturing facility from processing/production areas, entrances and sites external to the facility. Sponges were composited (6 sponges per composite) and stomached with neutralizing buffer yielding 70 samples. Sample aliquots were equally divided and enriched/analyzed for *Listeria* spp. following recommended protocols. Analysis was conducted using: (a) a PCR based detection method (BAX); (b) a visual-immunoassay (VIA); (c) a visual-immuno-precipitate method (VIP); (d) 3M™ Petrifilm™ Environmental *Listeria* Plates (Petrifilm); (e) the USDA standard culturalprocedure (USDA); and (f) a modified USDA procedure (mUSDA) which combined University of Vermont/*Listeria* Repair Broth enrichment. Of 70 samples tested, 23 samples were positive by one or more methods, limited to entrances and sites external to the facility. Positive samples and (sensitivity/specificity) of the methods were found as follows: Petrifilm 10 (43%/72%); VIP 8 (35%/68%); BAX 7 (30%/66%); USDA 8 (35%/68%); and VIA 1 (4%/53%). The USDA method was significantly better ($P < 0.05$) at recovering *Listeria* spp. in samples than all other methods tested, identifying 17 of 23 positive samples (74% sensitivity/87% specificity). These results indicate the importance of a repair step for resuscitation of stressed cells in environmental samples as highest levels of recovery were achieved by procedures incorporating repair steps (Petrifilm and mUSDA).

P3-70 DSC Comparison of *Listeria monocytogenes* Recovery from Hot Dogs Using the Pulsifier and Stomacher Sample Processors

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Effective microbiological sampling equipment is needed to determine the quality and safety of foods. The Stomacher sample processor was introduced to laboratories in 1972. However, its "crushing action" caused a significant amount of debris to remain in diluents. The Pulsifier was developed for sample processing with less debris and greater ease of sample manipulation. By using a combination of shock waves and stirring action, the Pulsifier has provided clearer suspensions for vegetables and lean meat tissues. Our objective was to use hot dogs to compare the recovery of *Listeria monocytogenes* using the Stomacher and Pulsifier. A 5-strain cocktail of *L. monocytogenes* was inoculated on hot dogs (commercially treated with =2% sodium lactate, potassium lactate, and sodium diacetate). Twenty-five hot dogs were pulsified (Microgen Bioproducts Ltd., Surrey, England). Another 25 hot dogs were stomached (A. J. Seward and Co. Ltd., London, England). Each hot dog was processed for 30 s with 100 ml of 0.1% peptone water. One mL of each sample was serially diluted (1:10) and dispensed on Thin Agar Layer (TAL)-Modified Oxford (MOX) agar. After incubation at 37°C for 24 h, viable cell count data were collected. Diluents from the Pulsifier and Stomacher were also used to analyze turbidity (Klett spectrophotometer), pH, and carbohydrates (Molisch reaction). These experiments were repeated with 25 repetitions for each method. Results indicated that the Pulsifier was effective for recovering *L. monocytogenes* (3.94 log CFU/cm^2) as compared to the Stomacher (3.60 log CFU/cm^2). Comparison of both methods resulted in a bacterial recovery ratio of 1.09. Furthermore, the diluents from pulsified samples were clearer, with fewer carbohydrates, and a slightly higher pH (7.4 versus 7.0) than stomached samples. These results suggest that the Pulsifier was more effective than the Stomacher for the recovery of *L. monocytogenes*, and provided a more favorable sample for further microbiological evaluation.

P3-71 DSC Evaluation of 3M™ Petrifilm™ Environmental *Listeria* Plates and Three Enrichment Broths for Recovery of *Listeria monocytogenes* Injured by Acid

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The USDA/HHS Joint *Listeria* Risk Assessment (2001) has identified certain acidic foods (fermented sausage, deli salads, soft cheeses, etc.) as high risk. Current detection procedures using selective enrichment broths for isolation of *Listeria* often underestimate injured *Listeria* cells which persist after acidic exposure. These injured cells are sensitive to selective agents and may escape detection. Low levels of injured cells can grow, repair, and regain pathogenicity, thus posing a serious threat to public health, especially in products with extended refrigerated storage times. Lactic acid, a weak organic acid often used as a preservative in the food industry, has the ability to injure *Listeria*. The efficacy of enrichment media to facilitate repair of injured cells is a vital step in *Listeria* detection. Three enrichment broths: UVM (used by FSIS-USDA), *Listeria* Repair Broth (LRB) and Peptone water (3M) along with 3M™ Petrifilm™ Environmental *Listeria* Plates were evaluated for their capacity to recover lactic acid injured cells. *Listeria* (serotype1/2a ribotype DUP-1053) was exposed to acid [lactic acid, pH 3.0, for 25 min] resulting in an injury level of >83%. Cells were resuspended in the broths and incubated at 35°C. Percent repair was evaluated using Tryptose Phosphate Agar (TPA), TPA w/ 4% NaCl (TPAN) and 3M™ Petrifilm™ Environmental *Listeria* Plates by plating at hourly intervals for 4 h. Results indicated that both LRB and Peptone Water media supported repair of acid injured cells during 4 h, compared to UVM media which failed to support repair. Plating on 3M™ Petrifilm™ EL Plates indicated a higher R2 (0.89) than repair in Peptone water plated on TPAN (0.82). When repaired in Peptone water for 4 h, 3M™ Petrifilm™ EL Plates facilitated higher recovery than TPAN. These data indicate the efficacy of 3M™ Petrifilm™ EL Plates for recovery of *Listeria* in acidified foods or food environments cleaned with acid-based cleaners.

P4-01 Evaluation of Differences among Guaiacol Producing and Non-Guaiacol Producing *Alicyclobacillus* spp.

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Alicyclobacillus spp. are spoilage bacteria implicated in pasteurized juice products, especially apple juice. Spoilage is characterized by guaiacol production. Recent studies indicate that only *Alicyclobacillus* spp. are capable of producing guaiacol. In this study, the differences among guaiacol producing *Alicyclobacillus* spp. and non-guaiacol producing *Alicyclobacillus* spp. are investigated to evaluate the possible differentiation parameters. Growth conditions, including incubation temperature (25°C, 32°C, 37°C, 43°C, and 55°C), medium pH (pH 3.0, 3.5, 4.0, 4.5), oxygen concentration (microaerophilic, aerobic), and carbon utilization profiles were compared between the two groups. All tested guaiacol and non-guaiacol *Alicyclobacillus* spp. grew most rapidly at 43°C and did not exhibit significant ($P > 0.05$) growth differences at 25°C, 32°C, 37°C, and 55°C. No growth was observed at pH 3.0 and no significant ($P > 0.05$) differences were evident in the other pH tested. Results for growth under microaerophilic and aerobic conditions were similar. For carbon source utilization, api 50 CHB were used and pH reduction was evaluated after 7 days. Differences in carbon utilization was observed between the two types of *Alicyclobacillus* spp. for L-sorbose, n-acetylglucosamine, d-maltose, d-saccharose, d-trehalose, inulin, d-melezitose, d-raffinose, glycogen, and xylitol. Guaiacol producing *Alicyclobacillus* spp. could not utilize these carbon sources, while non-guaiacol producing isolates could. Our results indicate that the two types of *Alicyclobacillus* spp. are similar and cannot be differentiated depending on growth characteristics. Carbon utilization, on the other hand, is a possible basis of differentiation.

P4-02 Effect of Background Microflora and Temperature on the Behavior of *Salmonella enterica* on Cilantro (*Coriandrum sativum* L.)

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Fresh vegetables, including cilantro, have been implicated in recent outbreaks of foodborne illness in the US. Ionizing radiation can effectively eliminate human pathogens from leafy vegetables. The objective of this study was to determine the effect of background microflora and storage temperature on the behavior of *Salmonella enterica* on fresh cilantro (*Coriandrum sativum* L.). Gamma irradiated (2 kGy) and non-irradiated bunches of fresh cilantro were inoculated with *Salmonella enterica* (10^5 CFU/ g) and kept at 22°C

for one hour to allow the inoculum to dry. Inoculated samples were stored at 4 and 22°C, for 10 and 2 days, respectively; relative humidity was maintained at 100%. At pre-selected time intervals, the levels of *S. enterica* were determined by pour-plating samples on tryptic soy agar supplemented with rifampicin. Growth parameters were calculated for each storage condition. Irradiation reduced the background flora (total plate count) by 3 log CFU/g relative to the untreated control. During storage at 22°C, no differences were observed ($P < 0.05$) in *S. enterica* growth parameters between irradiated and non-irradiated products. Growth rates of *S. enterica* in irradiated and non irradiated cilantro were 0.165 and 0.078 log CFU h^{-1}, respectively. Maximum population density was not significantly influenced by the level of background microflora (range: 8.2 to 9.1 log CFU/g). Similarly, lag phase duration was ~4.2 h in both products. In contrast, at 4°C there was no growth but survival of the pathogen at all studied conditions. These findings indicate that levels of background microflora do not affect the growth of *S. enterica* on cilantro and that common storage temperature may allow the growth and survival of *S. enterica*. This information could be applied to develop mathematical models that can be used by industry and regulatory agencies to implement new control measures for cilantro.

P4-03 Role of *Escherichia coli* O157:H7 O Side Chain on Cell Hydrophobicity, Charge and Attachment to Lettuce

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The effect of extracellular appendages of *Escherichia coli* O157:H7 on attachment to lettuce surfaces was examined. Curli producing and non-producing strains of *E. coli* O157:H7 (43894, 43895, 0018), and three strains of *E. coli* O157:H7 86–24 were used: 86-24 (wild-type), F12 (mutant lacking the O-antigen) and pRFBE (O157 gene reintroduced on plasmid). Cultures were surveyed for their hydrophobicity and cell charge using HIC, and ESIC techniques. Iceberg lettuce squares (2 x 2 cm) were inoculated with *E. coli* O157:H7 (~7 log CFU/g), dried in a laminar flow hood and rinsed twice with sterile de-ionized water. Strips of each cut edge of lettuce were aseptically removed. Cut and whole samples were homogenized and spiral plated onto Luria-Bertani Agar, supplemented with nalidixic acid (50 ppm), to assess levels of bacteria remaining on lettuce after rinsing. Strains 43894, 43895 and 0018 attached preferentially to cut leaf surfaces ($P < 0.05$). Neither the expression of curli, O-polysaccharide or leaf surface (cut vs. whole) influenced *E. coli* O157:H7 attachment to lettuce with the exception of strain 0018. Curli producing 0018 attached significantly greater ($P < 0.05$) to both leaf surfaces. Strain F-12 (lacking O157-antigen) was significantly more hydrophobic than the wild-type or pRFBE strains ($P < 0.01$). All curli producing strains were significantly more hydrophobic than curli non-producing strains ($P < 0.01$). Surface charge did not differ between 43894, 43895 and 0018 strains, but did differ among the 86-24 strains ($P < 0.01$). Results indicate that hydrophobicity and cell charge differences among strains of *E. coli* O157:H7 may not influence attachment to iceberg lettuce surfaces.

P4-04 Cell Surface Charge and Hydrophobicity of Sixteen *Salmonella* Serovars on Attachment to Cantaloupe Rind and Decontamination with Sanitizers

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Cantaloupe melon has been associated with several outbreaks of salmonellosis. Several recent outbreaks have been due to contamination with strains of *Salmonella* Poona. In this study, we compared the surface charge and hydrophobicity of two cantaloupe-related outbreak (CRO) strains of *S.* Poona (RM2350 and G-91-1595) to those of fourteen additional *Salmonella* strains using electrostatic and hydrophobic interaction chromatography. Also, the relative ability of the 16 strains to attach to cantaloupe surfaces and resist removal by washing with water, chlorine (200 ppm) or hydrogen peroxide (2.5%) for 5 min after a storage period of up to 7 days at 5 to 20°C was determined. Whole cantaloupes were inoculated with 8.36 log CFU/ml of each pathogen, dried for 1 h inside a biosafety cabinet, stored and then subjected to the washing treatments. Only with regards to the positive surface charge were the two CRO strains of *S. Poona* significantly different (higher) ($P < 0.05$) than the other strains. Initial bacterial attachment to cantaloupe surfaces ranged from 3.68 to 4.56 log CFU/cm^2 (highest values for *Salmonella* Michigan, Newport, Oranienburg and Mbandaka). Washing inoculated melons with water did not cause a significant ($P > 0.05$) reduction of the pathogens. Chlorine and hydrogen peroxide treatments caused an average 3 log reduction when applied 20 to 40 min postinoculation. However, sanitizer treatments applied 60 min or more post-inoculation were less effective (an approximate 2.5 log reduction). No significant ($P > 0.05$) differences were noted in sanitizer efficacy against the individual strains. In conclusion, the two CRO Poona strains did not significantly differ from the other *Salmonella* strains tested in negative cell surface charge or hydrophobicity, were not more effective in attaching to whole melon surfaces and were not more resistant to the various washing treatments when present on rinds.

P4-05 Moisture, Seed Coat Characteristics, and Disinfection of Artificially Inoculated Alfalfa Seeds

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Vegetable sprouts can be a vehicle for foodborne illness. The seeds used for sprouts are considered the probable source of the pathogen contamination. Since naturally contaminated seeds have a very low pathogen level, artificially inoculated seeds are used to test decontamination methods. There are research reports on the efficacy of decontamination procedures using seeds inoculated by several different methods. A study using these published inoculating conditions was done to compare 15 different inoculation procedures as it impacts the % moisture of alfalfa seeds. The % moisture after drying was similar for 14 procedures, verifying that the inoculation method did not matter. Further investigation was done to examine the physical characteristics of seed coats from different alfalfa varieties. The studies determined the effect of wetting and drying on broken or cracked seed coats and the efficacy of a reformulated peracetic acid sanitizer to disinfect *Salmonella* inoculated alfalfa seeds. Wrinkled, broken and cracked alfalfa seed coats were observed for all varieties studied. During inoculation, the cracks or breaks in the seed coats became more pronounced and curled upward away from the cotyledon, allowing bacteria cells in the inoculums to become trapped under the seed coat when dried. After drying, the cracks or breaks in the seed coats did not return to their original condition. Disinfection of *Salmonella* inoculated seeds with 3%, as opposed to 1%, peracetic acid, resulted in an additional 2 log reduction of the pathogen. This 3 to 4 log reduction of *Salmonella* is greater than any reported reduction in previously published reports for disinfection of seeds with peracetic acid.

P4-06 Misting Effects on Microbial Growth of Retail Produce

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Research on the practice of misting fresh produce in retail grocery stores is scant. This preliminary study examined aerobic plate count (APC), yeasts (Y), and molds (M) on green leaf lettuce and broccoli in three retail grocery stores that misted produce and three retail grocery stores that did not mist produce. APC, Y, and M were analyzed by use of standard methods given in the Compendium of Methods for the Microbiological Examination of Foods. APC for lettuce ranged from 8.5×10^2 CFU/g to 2.81×10^8 CFU/g ; Y ranged from 4.1×10^3 CFU/g to 1.82×10^6 CFU/g; and M ranged from 1.6×10^2 CFU/g to 2.8×10^5 CFU/g . APC for broccoli ranged from 1.0×10^3 CFU/g to 3.7×10^6 CFU/g; Y ranged from 5.6×10^5 CFU/g to 1.7×10^6 CFU/g; and M ranged from 1.4×10^2 CFU/g to 7.0×10^4 CFU/g. For green leaf lettuce, higher average APC and Y counts were found in the misted produce and average M counts approached significance ($P = 0.073$). Misted lettuce that were at higher levels in the produce display did have a significantly higher levels of M ($P = 0.016$). For broccoli, season had a significant effect. Higher levels of M were found in the spring ($P = 0.019$). Based on these preliminary results, additional research is necessary to make specific recommendations for misting practices.

P4-07 Factors Affecting the Recovery of *Salmonella* spp. and *Escherichia coli* O157:H7 from the Surface of Cantaloupe

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The purpose of this study was to evaluate factors that can be adjusted to develop a reliable method for the recovery of *Salmonella* and *Escherichia coli* O157:H7 on cantaloupe. A total of 288 melons were inoculated by dipping in a bacterial suspension of a mixture of 4 rifampicin-resistant (Rif^r) *Salmonella* serotypes (*S.* Poona, *S.* Agona, *S.* Gaminara and *S.* Typhimurium) and 4 rifampicin-resistant strains of *E. coli* O157:H7 containing ca. 7.0 log CFU/ml of each pathogen. This resulted in initial inoculum levels of 3.2–5.5 log CFU/cm^2 and 3.6–5.7 log CFU/cm^2 respectively. Inoculated melons were analyzed for variations in levels of Rif+ *Salmonella* and *E. coli*, using methods that included factors to evaluate, such as rind sampling method (rinsing, swabbing or excising), mixing method (hand rubbing or stomacher for 30 or 60 s), type of diluent (0.1% peptone water or buffered peptone water), and time between inoculation and analysis (1 or 24 h). The addition of surfactants to the 0.1% peptone water was also studied, with excision and homogenizing with stomacher for 60 s and testing 1 h after inoculation of melons. Peptone water with added tween 80, sodium lauryl sulfate or niaproof 08 at 0.1, 0.5 or 1.0%, respectively, was used as medium for sample and dilution preparation. Simultaneous enumeration of both pathogens was performed by spread plating on lactose-sulfite-phenol red-rifampicin (LSPR) agar plates, and incubating at 35°C for 27 h. Regardless of type of diluents, mixing method or time between inoculation and sampling (1 or 24 h), excision produced significantly ($P < 0.05$) higher counts of both pathogens (5.2 log CFU/cm^2), compared with rinsing (4.9 log CFU/cm^2) and sponging (4.1 log CFU/cm^2). Addition of surfactants to the 0.1% peptone water did not improve the recovery of either pathogen ($P < 0.05$). The sensitivity of this plating method for recovering *Salmonella* and *E. coli* O157:H7 was 0.20 ± 0.7 and 1.7 ± 0.8 log CFU/cm^2, respectively. Recovery of *Salmonella* and *E. coli* O157:H7 from cantaloupe surface was affected by sampling technique, diluent composition, time and procedure used to homogenize the sample.

P4-08 Survival of *Salmonella* spp. on Whole and Minimally Processed Mangoes

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Mangoes have recently been associated with salmonellosis. The objective of this study was to evaluate the ability of *Salmonella* spp. to survive on whole mangoes and on cubed and pureed mangoes. Whole mangoes (*Manguifera indica* Var. Kent) were surface-inoculated at levels of ca. 2.0 and 4.0 log CFU/cm^2. Cubes and puree were inoculated at levels of ca. 4.0 and 6.0 log CFU/cm^2 or CFU/g. The inoculum consisted of a cocktail with 6 strains of rifampicin-resistant *Salmonella*. Inoculated mangoes were placed on sanitized racks at room temperature (25 ± 5°C) and sampled daily over 13 days. Mango cubes were placed on racks and inoculated with the appropriate bacterial concentration. Ten-gram portions of mango puree were dispensed in sterile stomacher bags and inoculated with the appropriate bacterial concentration. Both mango cubes and puree were stored at room temperature (25 ± 5°C) and sampled every 4 h during the first 2 days and then daily over a 4 day period (6 days total). Serial dilutions were surface spread on tryptic soy agar supplemented with 100 µg/L of rifampicin, and incubated at 37°C for 24 h. Colony counts were transformed into log values before data analysis. *Salmonella* survived on the surface of mangoes over the 13-day storage period, and no effect of the inoculum level was observed when ANOVA was performed ($P > 0.05$). In minimally processed mangoes, *Salmonella* survived on cubed mangoes only when inoculated with 6 log CFU/cm^2, but decreased significantly over the 6 days of storage on cubed mangoes with 4 log CFU/cm^2. In puree, *Salmonella* decreased at the same rate regardless of the inoculum level. Data analysis using DMFit program indicated that *Salmonella* decreased on the mango surface and minimally processed fruit at both inoculum levels. The survival of *Salmonella* observed on whole mangoes may be of concern since these are commonly sold commercially at room temperature.

P4-09 Internalization of *Salmonella* ser. Typhimurium into Mango Pulp and Its Prevention by Chlorine and Copper Ions

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Outbreaks of *Salmonella* infections associated with fresh mangoes consumption has occurred in recent years. The purpose of this study was to evaluate the effectiveness of chlorine and copper ions applied to cool water treatment to reduce *Salmonella* ser. Typhimurium and prevent mango pulp internalization. Immature Tommy Atkins mangoes were water-immersed at 46.1°C for 100 min and at 25°C water containing 6 log CFU/ml of *S.* ser. Typhimurium with or without chlorine (2, 5 and 8 mg/l) or copper ions (2, 5 and 8 mg/L) for 30 min. Also, the experiment was performed using 25°C water containing raspberry red dye for 30 min. Vascular elements

by stem-end segment and lenticels by middle-side were measured. Chlorine at 2, 5 and 8 mg/L reduced 6 log CFU/ml of *Salmonella*. Whereas copper ions at 2, 5 and 8 mg/L reduced 2.38, 3.26 and 4 log CFU/mL, respectively on the cooling treatment water. *S.* ser. Typhimurium penetrated into mango pulp at a concentration of 2 log CFU/g, when disinfectants were applied to a 25°C water; both chlorine and copper ions, at 2, 5, and 8 mg/X, were effective in prevent the *Salmonella* internalization to mango pulp. 76.8 and 89.0 mg/kg of residual chlorine were detected in mango pulp after the treatment of 5 and 8 mg/L. Copper ions (5 and 8 mg/L) showed a residual content of 0.3 and 0.6 mg/kg, respectively, residual chlorine an copper levels were down compared with control reference. *S.* ser. Typhimurium entered mango pulp through vascular elements and lenticels, both naturals structures measured at an average of 21 and 112 μm dm, respectively. The results illustrates the potential for pathogen penetration if heated-mangoes are cooled using contaminated water. Also, appropriate application of chlorine or copper ions prevent the penetration-potential of *S.* ser. Typhimurium into mango fruit.

P4-10 Interaction of *Salmonella* with Pre- and Post-Harvest Tomato Fruit

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The persistence of a range of *Salmonella* serovars on/within pre- and post harvest tomatoes has been studied. Serovars were selected on the basis on previous association with tomatoes (Javiana, Montevideo, Newport) and those of animal/clinical origin (Dublin, Enteritidis, Hadar, Infantis, Senftenberg, Typhimurium). Serovars were introduced onto the flowers of growing tomato plants and the subsequent fruit screened for the presence of *Salmonella*. From the ten serovars tested, Montevideo was more adapted to persist on and within tomatoes. In contrast, Enteritidis and Typhimurium were only sporadically recovered from tomatoes at the time of harvest. All the serovars could persist and grow when introduced onto un-ripened (green) tomato fruit that was subsequently stored at 10°C or 25°C under different relative humidities (75% or 95%). In general, growth (internal and external) was promoted at the higher incubation temperature, with relative humidity having a less significant affect. The growth/persistence of *Salmonella* on and within ripened (red) tomatoes exhibited serovar dependency similar to that observed with pre-harvest fruit. Specifically, Montevideo, Newport and Hadar all persist on ripened tomatoes than did the other serotypes tested. All the *Salmonella* types studied exhibited cellulose production and similar biofilm forming abilities. However, serovars were found to differ with respect to *RpoS* –mediated gene expression and attachment strength, but neither attribute could be correlated to persistence on tomatoes. Microbial ecology of pre-harvest tomatoes was studied using a combination of Denaturing Gradient Gel Electrophoresis (DGGE) and Biolog plates. Evidence to date indicates that tomatoes contaminated with *Salmonella* had a different microflora composition compared to controls. The results may suggest that the micro-ecology of tomatoes is modified by the presence of *Salmonella*, which may explain the enhanced persistence of certain serovars.

P4-11 Potential Sources of *Salmonella* Contamination on Tomatoes Grown in Hydroponic Greenhouses in Mexico

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A study was conducted to determine the microbiological quality of tomatoes grown in hydroponic greenhouses in Mexico over the period of 2002 to 2004. The farm consisted of 13 harvest greenhouses, one nursery greenhouse, and a packaging shed. Contamination of tomatoes with enteric bacteria occurred during normal working conditions, and also during two natural and unexpected events: (1) a flood in 2003 that introduced runoff into four greenhouses, and (2) the entry of wild animals into five greenhouses in 2004. *Salmonella* and *E. coli* were found on tomatoes, working and personal shoes, vehicle wheels, sponges, greenhouse soil, and feces from livestock, wild, and domestic animals. The most frequently isolated *Salmonella* serotypes were Montevideo (51.6%), Newport (6.8%), Connecticut (5.2%), and strains of serogroup F (13.2%). At least one strain from each positive sample was selected (n = 250) for analysis by pulsed-field gel electrophoresis (PFGE) to elucidate potential sources of contamination. PFGE was performed employing a CDC protocol for *Salmonella* using *XbaI* and *AvrII* restriction enzymes. Gel images were analyzed and dendograms were constructed using BioNumerics software. *AvrII* detected differences in strains belonging to *S.* Montevideo and F serogroups that were not distinguished by *XbaI*. *XbaI* and *AvrII* distinguished 8 and 10 pulsotypes for Montevideo, and 5 and 7 for serogroup F, respectively. Pulsotypes C (*XbaI*) and b (*AvrII*) from S. Montevideo were more frequently isolated from tomatoes, possum feces, puddles and soil. Pulsotypes I (*XbaI*) and iii (*AvrII*) from serogroup F were isolated from tomatoes, cow feces and personnel shoes. All *S.* Newport and *S.* Connecticut pulsotypes were associated with greenhouse floods and goat feces. Detection of sources of contamination provides baseline information for implementing effective control measures to prevent the presence of enteropathogens in produce grown in greenhouses.

P4-12 Survival and Growth of *Salmonella* Enteritidis PT 30 in Almond Orchard Soils

DSC

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Extended environmental persistence of *Salmonella* Enteritidis Phage Type (PT) 30 has been reported in almond orchards. Factors that may contribute to long-term survival and growth of *Salmonella* Enteritidis PT 30 in almond orchard soils were evaluated. Artificial microcosms were established to evaluate effects of soil type (sandy or clay loam), moisture level (high or low), and temperature (20 ± 2 or 35 ± 2°C) on survival of nalidixic acid-resistant *Salmonella* Enteritidis PT 30 inoculated at approximately 6 log CFU/g dry weight. For additional clay loam soil microcosms stored at ambient temperature, an aqueous extract of almond hulls (1.62% mono and disaccharides) or equivalent volume of water was sometimes added 7 days after inoculation at 2, 3, and 5 log CFU/g dry weight. Levels of *Salmonella* Enteritidis PT 30 decreased rapidly at 35 ± 2°C and were significantly different ($P < 0.05$) from counts at 20 ± 2°C from 14 to 150 days of storage regardless of soil type or moisture level. *Salmonella* was detected by enrichment of 10-g from all microcosms held at 20 ± 2°C and no microcosms held at 35 ± 2°C after 180 days of storage. Significant ($P < 0.05$) growth was observed within 8 or 24 h of adding hull extract but not water to soil inoculated at 5 or 2 and 3 log CFU/g dry weight, respectively. Opportunities may exist for *Salmonella* Enteritidis PT 30 to survive for extended periods of time and to grow within almond orchard soils where hull nutrients are released.

P4-13 Fate of Vancomycin-resistant *Enterococci* during Active Composting on Farm

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Enteroccoci are well-known for either their intrinsic ruggedness or having acquired, with relative ease, resistance to many antibiotics that are essential for maintaining public health. Animal waste is loaded with nutrients, antibiotic residues, human pathogens, and high numbers of fecal bacteria, including *Enterococci* spp. The objectives of this study were to determine the fate of vancomycin-resistant *Enterococci* (VRE) during active composting, and to further characterize the antibiotic resistance in VRE isolates. Duplicate dairy compost heaps were constructed on an outdoor, fenced site. All heaps were ca. 1.5 m in height by 2 m in width, with a conical shape. Samples of the composting mixture were taken from the surface and at three locations (top, center, and bottom) within each heap. At selected intervals, populations of *Enteroccoci* spp. and *E. coli*/coliforms were enumerated, and temperature, pH, moisture content, and oxygen levels were determined. *Enterococci* spp. were enumerated on both bile esculin agar (BEA) and BEA containing 6 µg/ml of vancomycin. *Enterococci* populations inside the composting heaps decreased rapidly, from ca 7.0 to 5.0 log CFU/g within 3 days of active composting, followed by a slow reduction of cell numbers during a 60-day composting trial. Vancomycin-resistant *enterococci* accounted for 75% of total *Enterococci* in initial dairy compost, and were inactivated inside the compost heaps at a rate similar to that of total populations of *Enterococci*. However, on the surface, vancomycin-resistant *Enterococci* survived very well, with a slight reduction in cell population at the end of the experiment. Isolates of those VRE were further analyzed for the presence of vanA, vanB, and vanC genes. Our results revealed that a significant number of vancomycin-resistant *Enterococci* cells survived the active composting, and the existence of these VRE could serve as a potential source for disseminating vancomycin resistance among microbes on the farm.

P4-14 DSC Cryotolerance, Attachment, and Recoverability of *Escherichia coli* O157:H7 and Selected Surrogates from Romaine Lettuce Leaf Surfaces

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Using non-pathogenic surrogates in place of pathogens when evaluating commercial food processing operations offers safety advantages, but surrogates must exhibit characteristics similar to the pathogen. Non-pathogenic *Escherichia coli* strains were compared with *E. coli* O157:H7. Cryotolerance of all *E. coli* strains was compared at 0, 1, 2, 4, and 8 days after holding at -18°C in sterile deionized water (SDW). Populations were reduced after 1 d storage (1.3–1.7 log CFU/ml) for all strains, with little change thereafter. Each strain was inoculated (6.5–7.0 log CFU/cm^2) onto 3 × 3 cm^2 pieces of Romaine lettuce. After 1 h of drying, attachment was determined, using selective agar. Pieces were then rinsed with 200 ppm free chlorine and survivors enumerated. *E. coli* ATCC 25922 exhibited the greatest attachment rate (75.8% compared to *E. coli* O157:H7). The *E. coli* ATCC 25922 population decreased 2.5 log CFU/cm^2 after the chlorine treatment, which was similar to that of *E. coli* O157:H7 (2.2 log CFU/cm^2 reduction). *E. coli* ATCC 25922 also had similar hydrophobicity and cell surface charge compared to *E. coli* O157:H7. Cryotolerance and survival of starved organisms were measured after *E. coli* ATCC 25922 and *E. coli* O157:H7 were held in SDW to induce starvation (37°C for 4 h, 20°C for 24 h, or 4°C for 7 d). Starved cells exhibited greater cryotolerance than nonstressed control cells. There was no difference in cryotolerance regardless of starvation conditions. *E. coli* ATCC 25922 attached to lettuce pieces more than *E. coli* O157:H7, with the highest rate (117%, compared to *E. coli* O157:H7) after starvation at 4°C for 7 d. Populations of both were reduced (1.6–3.2 log CFU/cm^2) after washing with chlor-inated water, regardless of starvation conditions. *E. coli* ATCC 25922 is a useful surrogate for *E. coli* O157:H7 for studies involving attachment and recoverability on chilled produce.

P4-15 Dry Heat Treatment for Non-pathogenic Surrogate Cultures for *Salmonella* Enteritidis on Whole Almonds

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Recent outbreaks of *Salmonella enterica* serotype Enteritidis infections have been associated with raw almonds.The objective of this study was to evaluate the thermal inactivation rate of non-pathogenic surrogate cultures for *Salmonella* Enteritidis on whole almonds when treated by dry heat. *Pantoea agglomerans* SPS2F1 and *Pediococcus* spp. NRRL B-2354 were selected as surrogate cultures. Whole almonds were inoculated with cultures ca. 7 log CFU/g. The inoculated samples were exposed to dry heat at 250, 265, 280 and 300°F in an isotemp oven. Almonds were analyzed by the pour plate technique using Tryptic Soy Agar for *S.* Enteritidis and *P. agglomerans,* incubated at 35°C for 2 days and MRS agar for *Pediococcus* spp., incubated at 30°C for 5 days. Thermal death curves were used to determine the heat resistance of test microorganisms. Thermal death curves of surrogate cultures were compared to that of *Salmonella* Enteritidis using the slope of the best-fit line. The slope of the best-fit line is graphically determined by plotting the log of survivors of the test organism per gram versus the time of dry heat exposure at each temperature. The negative slope of the best fit lines showed that *P. agglomerans* was slightly more dry heat resistant than to *S.* Enteritidis, and *Pediococcus* spp. was comparable to *S.* Enteritidis at test temperatures. Counts of *S.* Enteritidis and *Pediococcus* spp. were reduced by 2.12 and 1.62 log CFU/g at 250°F after 32 min, 2.76 and 2.33 log CFU/g at 265°F after 24 min, 3.73 and 2.97 log CFU/g at 280°F after 20 min and 5.47 and 4.54 log CFU/g at 300°F after 16 min, respectively. These results suggested that *P. agglomerans* could be used as surrogate for *S.* Enteritidis for evaluating the efficacy of dry heat conditions in processing environments where *S.* Enteritidis cannot be introduced.

P4-16 Reduction of *Salmonella* Enteritidis PT 30 on In-shell Almonds Using Gaseous Propylene Oxide

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A small but significant market exists for almonds sold in the shell. While almond kernels may be pasteurized by a number of heat treatments, these options are unacceptable for in-shell almonds. The gas propylene oxide (PPO) is commonly used to treat raw almond kernels sold in the US, and a standard process was previously shown to reduce *Salmonella* Enteritidis Phage Type (PT) 30 by 6.6 ± 1.0 log CFU/g almond kernels. However, the impact of almond shells on the efficacy of the PPO treatment was unknown. Hard shell (Peerless) and soft shell (Nonpareil) almonds were inoculated with *Salmonella* Enteritidis PT 30 to approximately 9.7 log CFU/g after

drying. Inoculated almonds were placed in bags designed for gaseous sterilization (Steris) and prewarmed to 37°C for 18 to 24 h. Tempered almonds were inserted into the center of a 22.7-kg bag of the same variety of tempered in-shell almonds at the approximate center of a five-layer pallet, prior to applying a standard PPO treatment (0.5 l/m^3, 4 h). Duplicate pallets of each variety were treated and the experiment was replicated three times. Almonds were sampled directly after treatment and after subsequent storage for 2 and 5 days at 15–18°C for off gassing of PPO. Almonds (50 g) were blended in 450 ml of Butterfield's phosphate buffer, plated onto tryptic soy and bismuth sulfite agars, and incubated at 37°C for 24 and 48 h, respectively. PPO treatment of soft or hard shell almonds resulted in a 3.7 ± 0.7 or 4.9 ± 0.6 log reduction of *Salmonella* after PPO treatment and a 6.1 ±1.0 or 6.8 ± 0.8 log reduction after 5 days of post-PPO storage, respectively. PPO is an effective treatment for reducing populations of *Salmonella* Enteritidis PT 30 on in-shell almonds.

P4-17 DSC The Effect of Pre-treatments on the Reduction of *Salmonella* Enteritidis PT 30 on Almonds during Dry Roasting

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Almonds may be roasted by immersing in oil or by applying hot air (dry roasting). Dry roasting is significantly less effective than oil roasting in reducing *Salmonella* on almonds. Our objective was to determine if standard or novel pretreatments could improve the efficacy of the dry roasting process. Whole Nonpareil almonds were inoculated to approximately 8 log CFU/g with *Salmonella* Enteritidis Phage Type (PT) 30. Inoculated almonds (40 g) were pretreated by spraying with 2.5 ml of sterile deionized water, a saturated salt solution, citric acid (10% w/v, pH 1.9), or lactic acid (10% w/v, pH 2.1), and holding for 10 min. A standard heat treatment of 135°C for 40 min in a laboratory oven was applied. Samples were transferred to 80 ml of cold (4°C) tryptic soy broth, stomached for 2 min, plated onto tryptic soy and bismuth sulfite agars, and incubated at 37°C for 24 and 48 h, respectively. *Salmonella* concentrations did not change during pretreatment with either water or a saturated salt solution. After heating, reductions of approximately 3.6 log CFU/g *Salmonella* were observed on control samples that did not receive a pretreatment and samples pretreated with a saturated salt solution. Significantly ($P < 0.5$) greater decreases (4.7 log CFU/g) were observed when almonds were pretreated with water. *Salmonella* concentrations decreased by approximately 1 log CFU/g during pretreatment with citric or lactic acid, and additional reductions of 4.7 and 5.3 log CFU/g, respectively, were observed during heating. Pretreatment of almonds with water or lactic or citric acids may provide a simple means of improving the antimicrobial efficacy of the dry roasting process, but further studies are needed to determine the effect of pretreatments on the quality of the finished product.

P4-18 Effects of Sanitization Treatments and Storage Temperature on Survival and Growth of *Listeria* and *Escherichia coli* on Fresh-cut Vegetables

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Sanitization of fresh-cut vegetables to reduce microbial load is a common commercial practice. Fresh-cut vegetables (shredded lettuce, dry coleslaw mix) were inoculated with *L. monocytogenes*, *L. innocua* or *E. coli* prior to sanitization and were subsequently packaged and stored at 3°C or 8°C. The effects of sanitization treatments (100 ppm chlorine solution, 1% citric acid solution, 1% ascorbic acid) were determined by comparison of treated with unwashed produce. Survival and growth patterns for *Listeria* and *E. coli* were dependent on vegetable type, sanitization treatment, package atmosphere and storage temperature and time. Populations of *Listeria* and *E. coli* on unwashed lettuce increased ($P < 0.05$), while populations on unwashed coleslaw mix gradually decreased ($P < 0.05$) during a 12 day storage period at 8°C. The efficacy of the sanitization treatments were dependent on vegetable type; they were generally more effective on lettuce than on coleslaw. Dipping lettuce or coleslaw mix in a chlorine, citric acid or ascorbic acid solution reduced initial *Listeria* and *E. coli* populations but resulted in enhanced survival and/or growth during extended storage at 8°C. Reducing the storage temperature from 8°C to 3°C reduced ($P < 0.05$) microbial growth on packaged produce, and the beneficial effects of the sanitization treatments persisted for the duration of the storage life. In conclusion, the sanitization treatments did not eliminate *Listeria* or *E. coli* from vegetables but resulted in a reduction in initial population densities. However, mild temperature abuse, in particular when combined with over-extension of the shelf life, allowed significant growth on sanitized produce.

P4-19 Effectiveness of a Simple Chlorine Dioxide Method for Controlling *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, *Staphylococcus aureus*, and *Yersinia enterocolitica* on Blueberries

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Chlorine dioxide (ClO_2) has been studied for use as a sanitizer because of its strong oxidation capacity. The generation of ClO_2 traditionally needs either reaction with acid or on-site instruments such as an applicator or generator. For application of ClO_2 in the food industry, sanitizing procedures need to be simple and inexpensive. Recently, a simple pouch method for generating ClO_2 was developed. We studied the bactericidal effectiveness of this method against five pathogens inoculated on blueberries. A sachet (2 g size) containing all necessary chemicals was used to generate approximately 320 ppm of ClO_2 in 7.6 liter of distilled water. *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, *Staphylococcus aureus*, or *Yersinia enterocolitica* was spot-inoculated on blueberries. The efficacy of different concentrations (0, 1, 3, 5, 10, and 15 ppm) of ClO_2 and various times of contact (10 s, 1, 5, 10, 15, 20, 30 min, 1 h, and 2 h) were studied. Reductions of all pathogens on blueberries by ClO_2 treatments were achieved. *S.* Typhimurium was reduced ($P < 0.05$) by 3.99 log and 4.33 CFU/g when treated with 10 ppm of ClO_2 for 1 h and with 15 ppm for 30 min, respectively. Treated with 5 ppm of ClO_2 for 20 min, 30 min, 1 h, and 2 h, *Y. enterocolitica* declined by 1.67, 1.4, 3.23, and 3.82 log CFU/g, respectively. *P. aeruginosa* reduced by 2.21 log CFU/g ($P < 0.05$) even after 5 min when treated with 15 ppm of ClO_2. The simple ClO_2 method showed promise as a sanitizer for controlling foodborne pathogens, although the efficacy of ClO_2 treatments varies among different pathogens. The strong bactericidal efficacy of ClO_2 provides advantages to producers who do not want significant changes in the appearance of the produce after the sanitization procedure. This ClO_2 method has much potential to be an effective microbial decontaminant for produce.

P4-20 Comparison of Treatment of Fresh-cut Produce with Sodium Hychlorite and Calcium Hypochlorite for Effects on Microbiological and Sensory Quality

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Treatment of some types of fresh and fresh-cut produce with calcium hypochlorite can result in firming of tissue texture, thereby reducing the amount of tissue fluid released, with a consequent preservation of sensory quality and shelf life. Theoretically, treatment solutions containing the same concentration of free chlorine at a particular pH and temperature should be equally effective in killing microorganisms on produce, regardless of the type of hypochlorite salt, i.e., sodium hypochlorite (NaOCl) or calcium hypochlorite [$Ca(OCl)_2$], used to achieve that concentration. A potential benefit of using $Ca(OCl)_2$ may be the retention of sensory quality resulting from the presence of calcium in the treatment solution that may not be achieved using NaOCl. A study was done to test this hypothesis. Fresh-cut iceberg lettuce, Romaine lettuce, mesclum lettuce, and tomatoes were treated with 0 (control), 50, 100, and 200 µg/ml chlorine, using NaOCl and $Ca(OCl)_2$. Produce was stored at 4°C and analyzed for populations of mesophilic aerobic microorganisms (total counts) and yeasts and molds at 3- to 4-day intervals for 13 days. Treatment of lettuce with chlorinated water caused significant ($P \leq 0.05$) reductions in both groups of microorganisms, but these reductions were unaffected by the type or concentration of hypochlorite salt in the treatment solution. Treatment of diced tomatoes with up to 200 µg/ml chlorine did not cause reduction in either total counts or yeast and mold counts. Appearance, color, aroma, texture, and overall quality of treated fresh-cut lettuce and diced tomatoes were likewise unaffected by the type of hypochlorite salt used to prepare chlorine treatment solutions. The calcium concentration in treatment solutions containing $Ca(OCl)_2$ was apparently too low to cause a detectable effect on shelf life.

P4-21 Comparison of Lactic Acid and Hypochlorite Treatments for Reducing *Listeria monocytogenes* on the Surface of Fresh Mangoes

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Because of the wide environmental distribution of *Listeria monocytogenes*, controlling this pathogen in the fresh-cut produce industry seems to pose a greater challenge than the control of other bacterial pathogens. Fresh produce disinfection may be one more hurdle to prevent the presence of *L. monocytogenes* in the end product. The objective of this study was to compare the reduction of *L. monocytogenes* populations on the surface of fresh mangoes after application of lactic acid (L-lactic 2, 4, 5 or 6% solutions applied at 25 or 55°C for 5, 10 or 30 s), sodium hypochlorite and calcium hypochlorite (for each hypochlorite compound, 50, 100 or 200 mg/L at pH 6 or 10 applied for 5, 10 or 30 s) spray treatments. Mangoes (*Mangifera indica*, var. Kent) were inoculated by submerging them in a suspension containing a mixture of 6 strains of *L. monocytogenes*, allowed to drain at room temperature for 20 min, and separated into treatment groups, after which the treatments listed above were applied. Mangoes were sampled by placing in a stomacher bag with 100 ml sterile 0.1% peptone water and hand rubbing for 1 min. The resulting suspension was then subjected to *L. monocytogenes* count. The difference between log counts on control mangoes and log counts on treated mangoes was considered to be log reduction. The greatest log reduction (4.2 log cycles) was obtained by spraying 6% lactic acid at 55°C for 30 s. This reduction was significantly different from the reductions produced by all other treatments. In general, reductions after applying sprays of calcium hypochorite were greater than with sodium hypochlorite ($P < 0.05$). The reductions produced by combined treatments, using lactic acid followed by calcium or sodium hypochlorite, were no greater than the reductions obtained by warm (55°C) 6% lactic acid spray alone. Use of validated pathogen interventions to reduce *L. monocytogenes* in fresh produce may minimize the transfer of the pathogen to the fresh-cut product.

P4-22 Comparison of Treatments for Reducing *Salmonella* and *Escherichia coli* O157:H7 on the Surface of Fresh Fruits

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The objective of this study was to compare the effect of lactic acid, sodium hypochlorite and calcium hypochlorite treatments on the reduction of *Salmonella* and *Escherichia coli* O157:H7 on fresh cantaloupes, oranges, avocados and mangoes. Sixty units each of cantaloupe, avocado (*V.* Hass), orange (*V.* Valencia) and mango (*V.* Kent) were inoculated by immersion in a mixture of 4 rifampicin-resistant strains of *Salmonella* (*S.* Agona, *S.* Poona, *S.* Montevideo and *S.* Typhimurium) and 4 rifampicin-resistant strains of *E. coli* O157:H7 (932, 994, H1750, 6157) for 1 min and then separated into 6-units treatment groups. Groups were subjected to no treatment (control), washing with distilled water for 15 s (WW), WW followed by spraying sodium hypochlorite solution ((Na-CL, 200 mg/L free chlorine, pH 6) for 15 or 30 s, WW followed by spraying calcium hypochlorite solution (Ca-CL, 200 mg/L free chlorine, pH 6.0 for 15 or 30 s, or WW followed by spraying 2 or 4% lactic acid (LA) solution at 55°C for 15 or 30 s. After treatment, the fruits were sampled from the stem and skin areas, and estimated log reductions (ELR) on each area were calculated by subtracting the log count for each treatment from the control log count. No differences were observed based on the time of contact with the sanitizer, nor were differences observed between treatments on mangoes, where ELR was 1.4–1.9 for *E. coli* O157:H7 and 2.1–2.5 for *Salmonella* when initial levels at control group were 3.3–4.2 and 4.1–5.4 respectively. On cantaloupes, oranges and avocados, the treatment that produced the greatest ($P < 0.05$) ELR was 4% LA spray (1.8 - >2.5 log cycles for *E. coli* O157:H7 and 2.9 - >3.2 log cycles for *Salmonella*, when initial levels were 2.9–5.3 and 3.1–5.6 respectively). Fruit disinfection with lactic acid may be implemented as one more hurdle in the production of fresh and fresh-cut fruits and vegetables.

P4-23 Evaluation of Ionizing Radiation for the Inactivation of *Salmonella enterica* in Cilantro (*Coriandrum sativum* L.)

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The presence of biofilms on the surface of fruits and vegetables could interfere with the efficacy of common disinfection treatments. Ionizing radiation has been applied as a decontamination measure for produce. The efficacy of gamma irradiation in inactivating *Salmonella enterica* on cilantro, as affected by temperature and relative humidity, was studied. A 10-g bunch of cilantro was submerged in a cell suspension of *Salmonella enterica* (ca. 10^6 CFU/ml) and held at 22°C for 60 min to dry the inoculum. Inoculated cilantro was then stored at different conditions: 22°C and 100% RH, 22°C and 50% RH, 4°C and 100% RH, 4°C and 70% RH. After storage, samples were irradiated at 1 and 2 kGy. The number of survivors was determined by pour plating on trypic soy agar supplemented with rifampicin; negative samples were subjected to pre-enrichment on lactose broth. Irradiation at either 1 or 2 kGy eliminated the initial population of *S. enterica* (6 log CFU/g) on cilantro leaves. During storage at 22°C and 50% RH and 4°C and 70% RH, the pathogen was not recovered after irradiation. In contrast, when relative humidity was adjusted to 100%, *S. enterica* was recovered from samples irradiated at 1 kGy after 1 day at 22°C (3 log CFU/g) and 5 days at 4°C (1.5 log CFU/g). A dose of 2 kGy resulted in the recovery of 2 log CFU/g at 22°C after one day of storage, while no regrowth was observed at 4°C. Enriched samples were positive after irradiation and during storage. Results indicate that the effectiveness of ionizing radiation decreased when storage conditions allowed for growth and possibly biofilm formation on the surface of cilantro.

P4-24 DSC Fate of *Listeria monocytogenes* and *Salmonella* sp. on Irradiated Minimally Processed Organic Watercress during Refrigerated Shelf Life

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Demand for organic food has increased in the last 10–15 years because of consumer trends. The word "organic" is applied only to guarantee crops grown under special conditions, such as no use of chemical fertilizers. Therefore, manure is widely used in this kind of agriculture. Pathogenic microorganisms such as *Salmonella* and *L. monocytogenes* may be present in manure, thus contaminating the vegetables. These microorganisms have been involved in foodborne disease outbreaks associated with the consumption of minimally processed vegetables. Irradiation may be used to improve the safety of organic vegetables since no residues are detected after its application. The aim of this study was to evaluate the fate of *L. monocytogenes* and *Salmonella* sp. on irradiated minimally processed organic watercress during its shelf life. Organic watercress inoculated with ca. 10^6 CFU/g of both microorganisms was exposed to doses of 1 kGy, 2kGy and 3 kGy and stored at 7°C during 16 days. Non-irradiated samples were used as controls. Populations of *L. monocytogenes* and *Salmonella* were determined on days 0, 9, 12, 14 and 16. The behavior of both microorganisms was very similar. When exposed to 1 kGy and 2 kGy, there was a 5 log reduction, however, the populations increased to 1.37 log and to 2.65 log for *Salmonella* and Lm, respectively, by the end of the storage period. Samples exposed to 3 kGy showed reductions of 6 log for both bacteria, with no population being detected during storage. The safety of organic watercress can be improved using irradiation without growth of surviving microorganisms during storage at 7°C.

P4-25 Effect of Irradiation on Flavonoid Content and Radio-resistance of *Listeria monocytogenes* on Arugula

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Foodborne disease outbreaks involving Ready-to-Eat vegetables have indicated that the minimal processing used may not be sufficient to guarantee their safety. *Listeria monocytogenes* is one of the microorganisms responsible for those outbreaks. The aims of this study were to determine the radio-resistance of *L. monocytogenes* in minimally processed arugula, as well as the effect of irradiation on the content of flavonoids present in this vegetable. Minimal processing of arugula for the study consisted of cleaning, disinfection by immersion in ozone-treated water, and packaging in normal atmosphere. Immersion in ozone-treated water reduced psychrotrophic, mesophilic, lactic acid bacteria, *Pseudomonas* and fecal coliform levels in the argula by 1 log CFU/g. *L. monocytogenes* was not isolated from the samples. Minimally processed arugula was inoculated with *L. monocytogenes* (10^6 CFU/g) and exposed to doses of 0.5 kGy, 1.0 kGy, 1.5 kGy, 2.0 kGy, and 2.5 kGy. D10 values for *L. monocytogenes* on minimally processed arugula varied from 0.37 kGy to 0.48 kGy. The combination of immersion in ozone-treated water and exposure to doses of 2 kGy provides a reduction of 5 log CFU/g to the population of *L. monocytogenes*, improving the safety of this type of vegetable. Irradiation also caused a very significant increase of flavonoid content. Kaempferol glycoside levels were 4 and ca. 3 times higher in samples exposed to 1 kGy and 2 kGy, respectively, than in control samples. An increase of quercetin glycoside was also observed, mainly in samples exposed to 1 kGy. An increase in flavanoid compounds in these products may be of benefit because of the known anti-oxidant capacity of these compounds.

P4-26 Comparative Inactivation of Foodborne Viruses on Fresh Produce

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The ability of ultraviolet (UV) light to inactivate viruses is well established; however, to our knowledge research on inactivation of foodborne viruses on fresh produce is not yet well understood. This study investigates the UV inactivation of three viruses: feline calicivirus (FCV), a surrogate for norovirus; and hepatitis A virus (HAV) and Aichi virus (AiV), two human foodborne picornaviruses. Three produce types were analyzed. Green onions, lettuce, and strawberries have varying surface topography and have been previously involved with viral outbreaks. Produce samples were individually spot inoculated with 10^7 PFU in 10µl cell culture media containing 2% serum (pH 7.8) to mimic the potential contamination by environmental conditions or food handlers. Produce samples were exposed to UV light at varying doses (40–240 mWs/cm^2) and eluted using an optimized recovery strategy for each produce type. Virus

infection was quantified by TCID50 in mammalian cell culture and compared with results from untreated recovered virus. Reduction of 6 and 4 logs of HAV was observed for green onions and lettuce, respectively, with 120 mWs/cm^2. At this same dose, treatment of green onions, lettuce, and strawberries reduced AiV by 5, 6, and 2 logs, respectively, whereas FCV had log reductions of 3, 3.5, and 4.5 for green onions, lettuce, and strawberries. On strawberries, FCV inactivation continued at 240 mWs/cm^2 in constrast to AiV which was not significantly different from treatment at 120 mWs/cm^2 ($P < 0.05$). It is obvious that produce type and surface along with virus type play a role in UV inactivation of viruses.

P4-27 Reduction of Salmonellae Inoculated onto Different Tomato Surfaces by Gaseous Chlorine Dioxide

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Consumers and restaurateurs are increasingly purchasing pre-cut and packaged melons, tomatoes and other produce. Sales of fresh-cut produce have increased nearly four times over the past decade. Recent foodborne outbreaks associated with raw tomatoes have promoted the industry and regulatory agencies to seek alternative intervention treatments. Our objective was to determine survivability of *Salmonella* spp. (*Salmonella* Montevideo, *Salmonella* Javiana, and *Salmonella* Baildon) on hydroponic tomatoes after treatment with chlorine dioxide gas. Our goal was to achieve a 5 log reduction of *Salmonella* to meet the recommendations set by the National Advisory Committee on Microbiological Criteria for Foods. The mixed culture of *Salmonella* was inoculated onto tomato skin, stem, and stem scar areas. The tomatoes were then treated with 0.0, 0.3, 0.5, 0.7, 0.9 and 2.0 mg/l chlorine dioxide gas for 10 min at 22°C and 85% relative humidity. Stem and stem scar areas were also treated with higher concentrations (5 and 8 mg/l) chlorine dioxide gas. A centrifuge coupled with a membrane transfer plating method (using plate count agar) was incorporated into the direct surface plating procedure (using XLD agar) for resuscitation and enumeration of surviving salmonellae. A >8 log reduction in *Salmonella* ($P < 0.05$) was observed on the tomato skin surface after treatment with 0.3 mg/l chlorine dioxide gas for 10 min. For the stem and stem scar areas, pathogen reduction was far more challenging. Even at high concentrations of chlorine dioxide (up to 8 mg/l) less than 3 log reduction was observed, which is consistent with results obtained by previous researchers. Thus chlorine dioxide gas continues to be a promising technology for use as an antimicrobial for fresh produce.

P4-28 Development of a Pilot-scale Continuous Flow Process for Sanitizing Lettuce by Aqueous Ozone

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Minimally processed fresh-cut produce (FCP) is implicated in foodborne disease outbreaks. Chlorine is currently used by the FCP industry to reduce microbial contamination; however, the use of this sanitizer raises safety and environmental concerns. Ozone could be an alternative to chlorine because of its high sanitization power and environment-friendly attributes. The objective of this study was to design and evaluate a pilot-scale washer that uses ozone to sanitize fresh-cut lettuce. A pilot-scale continuous washing system, allowing adjustments to optimize the mechanics of the process, was designed. The system consisted of a corona-discharge ozone generator, produce treatment chamber, vibratory draining conveyor, water collection and buffer tanks, water chillers, off-gas exhaust system, flow control valves, recirculation pumps, and additional auxiliary components. The capability of ozone to sanitize fresh-cut lettuce was tested with a two-step washing procedure. Whole lettuce heads (150 lbs) were cut manually into pieces (2 × 3 cm) and pre-washed with water containing 0.1% tetra-sodium pyrophosphate (TSPP) at 5 lbs-lettuce/min feeding rate and 13.2 gal/min water flow rate. Samples were drained and divided into equal amounts for subsequent washings with either tap water or ozonated tap water (10 ppm maximum delivered concentration), with sample collections at 5, 10, 15 and 20 min. Processed lettuce was tested for mesophilic aerobic and psychrotropic microbial counts. Results indicated that pre-washing lettuce with TSPP, followed by washing with ozonated tap water, effectively decreased an initial population (~10^5 CFU/g lettuce) of mesophilic aerobic and psychrotrophic microorganisms by >1 log for all treatment times. No changes were observed in the natural microflora of cut-lettuce treated with TSPP followed by a tap water wash.These experimental trials satisfactorily simulate an industrial sanitizing procedure and demonstrate the effectiveness of ozone.

P4-29 Eliminating *Salmonella enterica* on Alfalfa and Mung Bean Sprouts by Acid and Heat Treatments

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Contaminated alfalfa and mung bean sprouts have caused numerous salmonellosis outbreaks in recent years. The focuses of this study were to evaluate the efficacy of acid and heat treatments in eliminating *Salmonella enterica* from artificially contaminated sprouts. Seeds were inoculated with a mixture of four *S. enterica* strains and air-dried for 24 h to simulate a worst-case scenario, with *Salmonella* contamination at 7.2 and 6.1 log CFU/g on alfalfa and mung bean seeds, respectively. After seed germination for 6 days, pathogen contamination and elimination were analyzed before and after treatments, by use of selective plating, enzyme-linked immunoassay, and scanning electron microscopy techniques. The inoculated *S. enterica* formed biofilms and multiplied to 7.6 log CFU/g on alfalfa and 6.9 log CFU/g on mung bean sprouts during germination. Immersing the contaminated alfalfa and mung bean sprouts in 2% acetic acid for 24 and 48 h, respectively, eliminated *S. enterica* on the sprouts (<1 cell/25g). With 5% acetic acid, *S. enterica* elimination was achieved by immersing alfalfa sprouts for 4 h or mung bean sprouts for 16 h. However, immersing alfalfa and mung bean sprouts in 5% citric acid for 48 h failed to achieve elimination. Dipping alfalfa sprouts in hot water at 70, 80, 90 and 100°C for 10, 5, 3, and 3 s, respectively, eliminated *S. enterica*. For mung bean sprouts, the elimination was achieved by dipping in hot water at 70 or 80°C for 20 s, 90°C for 10 s, or 100°C for 5 s. In conclusion, acetic acid or heat treatments could be utilized to enhance the microbial safety of seed sprouts and to prevent sprout-related *Salmonella* outbreaks.

P4-30 DSC Efficacy of High Pressure Processing in Combination with Antimicrobials for the Reduction of *Escherichia coli* O157:H7 and *Salmonella* in Apple Juice and Orange Juice

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The effect of pressure on the log reduction of six strains of *E. coli* O157:H7 and five serovars of *Salmonella enterica* were investigated in tryptic soy broth, sterile distilled water and commercially sterile orange and apple juice. Samples were subjected to high hydrostatic pressure (HHP) at 300 and 550 MPa for 2 min at 6°C, and then held for 24 h at 4°C following treatment. *E. coli* O157:H7 strain E009 was the most pressure resistant, having a decrease of only 0.77 log CFU/ml directly after pressurization in TSB. *S.* Agona was the most pressure resistant *Salmonella* serovar tested, with a decrease of 3.79 log CFU/ml in TSB at 550 MPa. The two most pressure resistant cultures were then used in a subsequent study that used HHP in conjunction with antimicrobials (dimethyl dicarbonate [DMDC] at 62.5 and 125 ppm, hydrogen peroxide at 150 and 300 ppm, cinnamic acid, potassium salt at 125 and 250 ppm, potassium sorbate [KS] at 500 and 1000 ppm and sodium benzoate [NaB] at 500 and 1000). For both *E. coli* O157:H7 and *Salmonella*, the most effective antimicrobial was DMDC, causing a 5.79 and 5.96 log CFU/ml decrease directly following pressurization, respectively. Other treatments that were significantly different from the samples with no antimicrobial added were hydrogen peroxide at 150 and 300 ppm, NaB at 500 ppm for *E. coli* O157:H7, and a treatment of NaB at 1000 ppm for *S.* Agona. After 24 h at 4°C, *S.* Agona samples with added antimicrobials had a reduction close to or above 5-log CFU/ml. DMDC should be further investigated as an antimicrobial agent that can work in conjunction with HHP.

P4-31 DSC Inactivation of *Escherichia coli* O157:H7 in Apple Juice as Affected by Cranberry Juice Concentration and Holding Temperature

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Cranberry juice concentration and holding temperature were evaluated for reducing *Escherichia coli* O157:H7 populations in pasteurized apple juice. Pasteurized, 100% cranberry (CJ) and apple juices were combined to yield mixtures containing 0 (control; pH 3.83), 10 (pH 3.55), 20 (pH 3.35), 30 (pH 3.20), 40 (pH 3.07), and 50% (pH 2.95) CJ. *E. coli* O157:H7 (5-strain mixture) was inoculated into the juice mixtures to obtain an initial population of approximately 7 log CFU/mL. Juices held at 4 and 25°C were sampled at 24-h intervals for up to six days, while juices held at 45°C were sampled at 0.5- or 1-h intervals for up to 8 h. Samples (in phosphate buffer, pH 6.4 – 6.63) were plated in duplicate on tryptic soy agar (TSA) and sorbitol MacConkey agar (SMAC). After six days storage at 4°C, *E. coli* O157:H7 populations were reduced < 1 log CFU/mL in 0 – 30% CJ mixtures, but were reduced by > 2 and 4 log CFU/mL in 40 and 50% CJ, respectively. In juices held at 25°C, *E. coli* O157:H7 populations were undetectable in 10% CJ after 120 h, in 20 and 30% CJ after 48 h, and in 40 and 50% CJ after 24 h. The population in 0% CJ was reduced by 5 log CFU/mL after 120 h. At 45°C, *E. coli* O157:H7 was reduced to non-detectable levels in 30, 40, and 50% CJ after 6, 4.5, and 4 hours, respectively. Reductions of about < 1, 2, and 6 log CFU/mL were observed in 0, 10, and 20% CJ, respectively. In all samples, substantial proportions of populations were sublethally injured during holding as indicated by poorer recover on SMAC in comparison to TSA; injury development was more pronounced at higher holding temperatures. At 45°C, 100% injury was observed in 20, 30, 40, and 50% CJ after holding for 7, 5.5, 4, and 3.5 h, respectively. When combined with temperatures of 25 or 45°C, with minimal holding time, concentrations of 30–50% pure CJ could serve to effectively reduce *E. coli* O157:H7 populations in juice.

P4-32 Microbiological Safety of Retail Pre-packaged Mixed Salads for *Listeria monocytogenes*

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The increasing availability of pre-packaged salads reflects consumer demand for fresh, healthful, and convenient foods that are safe and nutritious. European Commission Recommendation 2005/175/EC required member states to undertake a program of sampling pre-packaged mixed salads containing raw vegetables and meat or seafood ingredients from retail premises in 2005 in order to determine the prevalence of *Listeria monocytogenes* in such products. Samples of mixed salads were collected in the UK from May to June 2005 by Environmental Health Departments and examined by Official Food Control Laboratories for *L. monocytogenes* in accordance with EN/ISO standards 11290-1,2. The majority of mixed salads (95.2%; 2556/2686) did not have *L. monocytogenes* present. However, two (0.07%) samples were found to have adverse *L. monocytogenes* results (≥ 100 CFU/g) compared with microbiological criteria stipulated in Commission Regulation No 2073/2005. A further 4.8% of samples had *L. monocytogenes* present <10 CFU/g; 4.7%, or at levels below 100 CFU/g; 0.07%. Most (93%) salad samples were displayed at or below 8°C. *L. monocytogenes* were present more often in salad samples displayed above 8°C (5.7%), compared to those at or below 8°C (4.7%). Most salad samples (91%) had a durability date on the packaging and most (81%) samples had remaining shelf lives ranging from 0 – 12 days. *L. monocytogenes* was present in samples, with remaining shelf life ranging between 0 and 12 days. One of the two samples that contained *L. monocytogenes* at over 100 CFU/g had a remaining shelf life of 0 days, while the other sample had no expiration date. The presence of high levels of *L. monocytogenes* is unacceptable. Mixed salads are not heat treated, and the implementation of specific hygiene measures, including appropriate shelf life and temperature control, are essential to avoid growth of pathogenic bacteria eventually present in such products and to protect public health.

P4-33 DSC Hygienic-sanitary Conditions of Minimally Processed Fruits and Vegetables Marketed in Campinas, São Paulo, Brazil

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Minimally processed vegetables can be defined as products that, although physically modified, keep the characteristic freshness of food "in-natura" and many times do not need subsequent preparations for consumption. The minimum processing of fruits and vegetables includes activities such as selection and classification of the raw material, daily pre-washing, processing (cutting, slicing),

disinfecting, rinsing, centrifuging and packing. Many of these operations are executed manually, making possible the contamination of these products by pathogenic and spoilage microorganisms, which can be a risk to consumers' health and reduce the product's shelf life. The analysis of coliforms, aerobic total count, moulds and yeasts are hygienic-sanitary quality and shelf-life indicators of these products, and the determination of *Salmonella* aims to prevent the consumer from food poisoning that could be acquired by ingestion of this pathogen. The objective of this study was to monitor these parameters through representative sampling lots (n=180) of Ready-to-Eat fruits and vegetables. The analysis procedures followed the rules of officially recognized international organizations and Brazilian legislation for microbiological standards in foods. The results showed that counts of total mesophilic aerobic organisms were between 3.09 and 8.94 log CFU/g; moulds and yeasts between 3.19 and 6.61 log CFU/g and total coliforms between <1 and 7.73 log CFU/g *Salmonella* was not detected. However, 37.2% of the samples were not in agreement with the maximum limits for *E. coli* established by the current legislation (RDC n°12/2001-ANVISA). Based on the obtained results, it can be concluded that the hygienic-sanitary conditions of minimally processed fruits and vegetables marketed in Campinas need to be improved, which may increase the shelf life of this kind of product that, in Brazil, is shorter (from 5 to 7 days) when compared to that sold in markets abroad.

P4-34 *Salmonella* Surveillance in Mexico, 2002–2005: Results from a Four-state Network

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We report the results for *Salmonella* surveillance conducted in four states (Yucatan, Sonora, San Luis Potosi, Michoacan) in Mexico from 2002 to 2005. The prevalence of *Salmonella* in humans, raw retail meat and food-animals from slaughterhouses was the following: children with diarrhea, 12.5%; asymptomatic children, 5.3%; retail chicken, 21.3%; chicken intestine, 13.7%; retail pork, 36.3%; swine intestine, 42.3%; retail beef, 30%; and bovine intestine, 21.3%. The main serotypes in ill humans were: (Typhimurium (15.2%), Enteritidis (8.8%), Agona (7.2%), Anatum (5.4%), Meleagridis (4.4%), Newport (4.3%), Oranienburg (3.9%) and Muenchen (3.8%)) were all present in food animals and/or retail meats. Swine was the major source of *S.* Typhimurium (ST) and chicken, for *S.* Enteritidis. Of all serotypes, ST (n=276) presented the highest frequency of antimicrobial resistance: ampicillin, 73.1%; chloramphenicol, 77.5%; trimethoprim-sulfamethoxazole, 64%; nalidixic acid, 57.1%; gentamicin, 42.9%; kanamycin, 36%; and extended-spectrum cephalosporins (ESC), 50.5%. ESC- resistance was found in lower frequency in *S.* Newport (4/58), *S.* Reading (2/77), *S.* Kentucky (1/45), *S.* Anatum (3/340), *S.* Uganda (1/42) and *S.* Bredney (1/14). ST also presented a high prevalence of multidrug resistance. Eighteen per cent of isolates were resistant to 0–3 antimicrobials, 36% were resistant to 4–6 antimicrobials and 46% were resistant to 7 or more antimicrobials. Pulsed-field gel electrophoresis analysis of all *S.* Typhimurium isolates showed that in each state several clones recovered from humans were identical to those found in retail meat and/or food–animal intestines, with two clones present in all four states. Our data shows that in Mexico multidrug resistant, ESC-resistant *Salmonella* is an emerging problem that requires immediate attention. The finding of identical clones in food-animals, meat and humans in all four states suggests that *Salmonella*, including antimicrobial resistant variants, is likely to be transferred to humans through the food chain.

P4-35 Enumeration of *Salmonella* in Raw Retail Meat in Yucatan, Mexico

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Salmonella is a frequent cause of enteric infection and may cause severe invasive infection in infants, the elderly and immunosupressed patients. Due to the impact of this pathogen on human health, national legislation in Mexico established a zero tolerance for *Salmonella* in fresh and frozen retail meat. In the state of Yucatan, *Salmonella* is present in 30% to 100% of the raw meat destined for domestic use. Current socioeconomic conditions make compliance with zero tolerance unfeasible in the short term. This study was conducted to determine the level of *Salmonella* contamination in raw meat from different retail outlets. Enumeration of *Salmonella* was performed by the Most Probable Number technique by inoculation of 25 g of meat in 250 ml of buffered peptone water, which was divided into 3 aliquots of 4 serial dilutions. Each aliquot was subcultured to tetrathionate broth and subsequently to XLT4 agar. The number of *Salmonella* was calculated using the MPN table of de Man. Seventy-seven samples of retail meat were collected from 7 open markets, 3 supermarkets and 6 butcher shops. Mean CFU/100 g in chicken, pork, and beef in open markets were: 169 (range >6–1500), 4302 (<30–>22,000), and 6364 (<6–>22,000), respectively. In butcher shops, mean counts from these sources were: 21(>6–60), 2836 (<6–22,000), and 996 (<6–4800), respectively. In supermarkets, mean counts were all 33 or less with a range from <30–40 CFU/100g. This study showed that in Yucatan raw retail meat from open markets and butcher shops was significantly more contaminated than that from supermarkets. Since 90% of the state population purchases their meat from open markets and butcher shops, we can assume that humans are continuously exposed to *Salmonella* via the food chain. More research is needed to correlate levels of *Salmonella* contamination in raw retail meat with the development of human illness.

P4-36 Isolation of *Enterobacter sakazakii* from Sunsik (Traditional Korean Ready-to-Eat Food)

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Enterobacter sakazakii has been implicated in severe forms of neonatal infections such as meningitis and sepsis. This organism has been isolated from a wide range of foods, including cheese, meat, vegetables, grains, herbs, and spices, but the major habitat is still doubtful. Sunsik (powdered mixture of roasted grains and other foodstuffs) is widely consumed in Korea as a side dish and energy supplement. Because dried infant milk formula (IMF) has been epidemiologically identified as the source of *E. sakazakii*, investigation of *E. sakazakii* in Sunsik has been required in Korea. Sunsik is consumed without heat treatments; therefore it has no additional chance for inactivation of foodborne pathogens. In this study, the prevalence of *E. sakazakii* was monitored in 23 different Sunsik powders according to FDA recommended methods (2002). But OK medium and Chromogenic *Enterobacter sakazakii* medium (Oxoid) were used as selective media. In total, 24 presumptive strains were isolated and 15 strains were confirmed as *E. sakazakii* by API 20E biochemical test and oxidase test. The 15 strains were further confirmed by PCR amplification, using three different primer sets (tDNA sequence, ITS sequence and 16S rRNA sequence) and compared to ATCC strains (12868, 29004, 29544, 51329). Enzyme activities and

carbohydrate utilization were also checked by API ZYM and 50CH kit. Not all of the isolates utilized D-sorbitol as a carbon source and the 3 strains isolated from dried anchovy powder did not produce alpha-glucosidase.

P4-37 Growth and Persistence of *Listeria monocytogenes* Strains on the Model Plant *Arabidopsis thaliana* and Prevalence of *Listeria* spp. on Plant Matter in Natural Environments

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The foodborne pathogen *L. monocytogenes* is found in diverse environments, including on vegetable and plant materials, especially following plant contact with untreated ruminant manure. Thus, an important food safety concern is understanding *L. monocytogenes* attachment to and survival on plant matter and its prevalence on plants. In this study, the abilities of four wild-type *L. monocytogenes* strains to attach to and grow on the model plant *Arabidopsis thaliana* were assessed. When exposed to 8.7 log CFU of *L. monocytogenes*, *L. monocytogenes* attached to leaves at densities from 2.04– 3.95 log CFU/mm 2 within 5 min of exposure. The lineage II, serotype 1/2a strain tested showed a statistically higher initial attachment to *Arabidopsis* as compared to the two lineage III (serotype 4a & 4c) strains tested, while the lineage I, serotype 4b strain tested did not statistically differ in initial attachment from the lineage II and III strains. Leaf tissues were also enumerated for bacterial presence at 10 days post initial exposure; all strains multiplied on the leaves, with increases in bacterial numbers between 1.3 and 2.0 logs. In addition, a survey of natural plant samples (n = 25) from four different sampling periods was performed to assess *L. monocytogenes* and *Listera* spp. prevalence in nature. While no *L. monocytogenes* was recovered, other *Listeria* spp. were isolated from 24% of plant samples. While these data suggest the possibility of a natural presence of LM on plant material, more extensive sampling is needed to define *L. monocytogenes* prevalence and lineage distribution on plant samples from natural and agricultural environments. Overall, our study demonstrates that *L. monocytogenes* is able to rapidly attach to and multiply on plant tissue. Therefore, plants may not only serve as a transmission source of *L. monocytogenes* to humans (e.g., through contaminated produce), but also may provide a vehicle for continuous dispersal of *L. monocytogenes* through the environment.

P4-38 Susceptibility of *Enterococci* Faecium and *Enterococcus* Faecalis Associated with Dairy Cattle: A Pilot Study

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The emergence of antimicrobial-resistant bacteria, including *Enterococci*, is of serious public health concern. Food animals have been incriminated as a possible reservoir for antimicrobial resistant organisms. In a pilot project, we used on-farm administered questionnaires and isolated *Enterococcus faecium* to evaluate commercial dairy cattle as potential reservoirs of antimicrobial-resistant bacteria. Fecal samples, collected from dairy cattle that were randomly selected from two cohorts in five commercial dairy herds, were cultured for *Enterococcus* spp. The first cohort was composed of immature non-lactating females (heifers, n = 28) that were initially housed away from but subsequently commingled with the second cohort, the lactating herd of mature cows (n = 20). The genotypes of 69 *E. faecium* isolated from fecal samples were determined by ribotype banding pattern and antimicrobial susceptibility patterns. Based on ribotype, a significantly different population of *Enterococci* was recovered from pre-lactational heifers versus lactating heifers. An on-farm administered questionnaire was used to gather data on antimicrobial use. The farms reported that beta-lactams were the most common class of antimicrobials administered to cattle. The two antimicrobials reported most commonly administered systemically were ampicillin and penicillin. All 69 *E. faecium* isolates tested were sensitive to both, suggesting metabolic or other non-antimicrobial selection pressures responsible for changes in the *Enterococci* of these dairy cattle. This research should be repeated in several different production systems in which a variety of indicator organisms and pathogens are examined.

P4-39 Comparison of Occurrence of Four Major Foodborne Pathogens on Swine Farms in Four States

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Human illnesses caused by foodborne pathogens have been linked to the consumption of pork products and most studies have focused on the occurrence of a single pathogen. Baseline data on the occurrence of four major foodborne pathogens, *E. coli* O157:H7, *Salmonella*, *Campylobacter jejuni*, and *Listeria monocytogenes*, is needed for control of the pathogens at the farm level. The objective of this study was to compare the occurrence of the four major foodborne pathogens in animals and their environments on swine farms in different states. Fecal swab samples (n = 480) and environmental samples (n = 144) were collected from swine farms in Tennessee, North Carolina, California, and Washington every 3 months over 22 months. The samples were analyzed for the presence of the four pathogens by use of modified FDA-BAM or USDA-FSIS protocols. Overall, the most frequently isolated foodborne pathogen was *C. jejuni* (31.7%), followed by *Salmonella* (10.9%), *E. coli* O157:H7 (8.2%), and *L. monocytogenes* (0.3%). Most of the positive samples were animal (fecal swab) samples. The pathogens were sporadically isolated from environmental samples, especially feeds. The occurrence of the pathogens tended to be higher in warmer months (in spring, summer or early fall, in October). The isolation rate of the pathogens was highly correlated with the number of animals on farms (r = 0.98). The results of this study indicated that swine continue to be a significant reservoir of *C. jejuni*. Swine were also a significant reservoir of *Salmonella* and *E. coli* O157:H7, particularly in warmer months of the year.

P4-40 The Effect of Swine Production System on Bacterial Prevalence and Antibiotic Resistance

DSC

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The current study examined the populations and prevalence of antibiotic resistance of *Enterococci*, *Enterobacteriaceae*, and *Campylobacter* species in swine finished in conventional (CON) and antibiotic-free (ABF) production systems. Carcasses were swabbed prior to scalding, in the bung cavity prior to evisceration, and in the pleural cavity after carcass splitting. Bacterial isolates from each species were tested for susceptibility and/or resistance to ampicillin, tetracycline, erythromycin and ciprofloxacin. Resistance was most prevalent against erythromycin. *Enterococci* isolated from ABF pigs were less resistant to ampicillin (8.62% vs. 20.90%) and tetracycline (20.16% vs. 32.35%) compared to CON pigs. *Enterobacteriaceae* isolates from bung cavities of ABF pigs were significantly

less resistant to ampicillin than the other two sampling areas. CON finished pigs had a higher percentage of resistant and susceptible *Enterobacteriaceae* isolates against ampicillin and tetracyclin, respectively, than ABF pigs. *Campylobacter* spp. isolated from ABF pigs were 22.86% resistant and 51.43% susceptible to ciprofloxacin. All antibiotic resistant isolates of *Campylobacter* spp. were identified as *C. jejuni* by the hippurate test. The presence of bacterial populations on ABF pigs that were highly resistant and/or susceptible to antibiotics not administered is a pivotal assessment that warrants future investigation.

P4-41 Dissemination of Clonal Strains of *Campylobacter* Resistant to Multiple Antimicrobial Drugs among Retail Chickens in Korea

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Antimicrobial resistant *Campylobacter coli* (n=63) isolated from chicken carcasses (n=111) from retail markets (n=37) were evaluated for the presence of disseminated clonal types based on highly similar composite pulsed-field gel electrophoresis (PFGE) patterns, shared plasmid profiles and resistance genotypes. Isolate species were identified with a combination of phenotypic tests, hipO colony blot hybridization, and multiplex lpxA PCR. Breakpoint resistance to four antimicrobials (ciprofloxacin, nalidixic acid, erythromycin, and doxycycline) was determined by agar dilution. All *C. coli* isolates were resistant to antibiotics, 59 (93.7%) of which were multi-drug resistant. The most frequently detected resistance was to nalidixic acid (96.8%), followed by ciprofloxacin (85.7%), doxycycline (73.0%), and erythromycin (34.9%). PFGE after *SmaI* and *KpnI* restriction identified 28 and 34 distinct composite PFGE patterns among *C. coli* isolates typeable (n=39). Eighteen (46%) of the resistant isolates exhibited one of five main composite PFGE patterns (clonal groups with >90% similarity). Resistance genotypes were largely homogeneous, including ciprofloxacin resistance (uniformly attributable to a Thr 86 Ile mutation in GyrA) and doxycycline resistance (uniformly attributable to tet(O) gene). Erythromycin resistance in 13 of 15 resistant isolates was attributable to an A to G point mutation at position 2075 in 23S rRNA gene which resulted in high-level resistance; the other erythromycin resistant isolates had lower MICs consistent with efflux pump activity based on metabolic inhibitor study results. Plasmid profiles were correlated to *C. coli* clonal groups and doxycycline resistance was invariably associated with plasmid carriage among the clonal isolates. The presence of clonal populations of multi-drug resistant *C. coli* from retail chickens indicates the need for development of effective control measures to prevent the introduction of such strains into the food chain.

P4-42 pVir Plasmid and Tetracycline Resistance of *Campylobacter jejuni* Isolates from Poultry, Meat and Humans in Korea

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The plasmid pVir may play a role in the virulence of *Campylobacter jejuni*, a major foodborne pathogen and leading cause of bacterial gastroenteritis. Tetracycline resistance in *Campylobacter jejuni* is encoded by the tet(O) gene and is usually associated with conjugative plasmids. Therefore, we investigated the presence of pVir and tetracycline resistance in 104 *Campylobacter jejuni* isolates from poultry, meat and humans. The pVir plasmids were identified from 3 of 104 (3%) *C. jejuni* isolates studied (1 isolate from human and 2 isolates from poultry). These isolates containing pVir were associated with the presence of tetracycline-resistant plasmids. High-level tetracycline resistance was observed (76%) from the isolates, ranging from 32 to >256g/ml. These data indicate that pVir plasmid and tet(O) gene, previously reported in *Campylobacter jejuni* strains throughout the world, is present also in Korean isolates. This study will lead to a better understanding of the contribution of *C. jejuni* plasmids to pathogenesis and antibiotic resistance.

P4-43 Phenotypic and Genotypic Characterization of *Enterobacter sakazakii*

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Enterobacter sakazakii, an emerging foodborne pathogen, is linked to a number of outbreaks, in many cases associated with consumption of powdered infant formula (PIF) and in most cases involving infants. The purpose of this work is to perform comprehensive phenotypic and genotypic characterization of *Enterobacter sakazakii* for a better understanding of the epidemiology of *Enterobacter sakazakii* infections. Very little research has focused on identification or molecular characterization of *Enterobacter sakazakii*. Current methods of identification involve observation of yellow colony pigmentation, testing for positive á-glucosidase activity and biochemical fermentation tests such as API 20E and ID 32E test strips. For regulatory agencies to make decisions with respect to the presence of *Enterobacter sakazakii* in PIF and other foods, and for health agencies to support their epidemiological studies in reliable databases, it is necessary to have in place accurate methods for confirming the presence and identity of this pathogen. In this study, phenotypic [API20E/API32E, chromogenic media (DFI, ESPM), pigment/coloration/morphology on TSA/DFI/ESPM media] and genotypic [ribotyping, BAX PCR, pulsed-field gel electrophoresis (PFGE), and 16s rDNA sequencing] methods were performed on 220 environmental, clinical and food isolates of *Enterobacter sakazakii*. Of 197 strains tested, 16s rDNA analysis has shown 94% identity among 184 isolates. Thirteen strains were shown not to be *Enterobacter sakazakii*. Interestingly, three strains negative by BAX PCR and API20/32E were identified as *Es* by 16s rDNA sequencing. Of the 184 *Enterobacter sakazakii* isolates identified by 16s rDNA sequencing, 46 and 7 were not correctly identified by API20E and API32E, respectively. PFGE patterns of 179 (5 strains had DNA degradation) strains tested showed 143 clusters with a base similarity of 52%, with 7 unrelated strains having identical pulsotypes. Automated ribotyping clustered 178 (6 strains had DNA degradation) into 125 ribogroups with a 20% base similarity, thus appearing less discriminatory than PFGE.

P4-44 Development of a Non-primate Animal Model for *Enterobacter sakazakii*

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Enterobacter sakazakii (*Es*) is an emerging opportunistic pathogen implicated in neonatal meningitis and in many cases involving consumption of powdered infant formula. There is at present very little known about the organism in terms of where and how often it is found in the environment and in foods. The mechanism(s) by which it causes disease in humans and how many cells of this organism must be ingested to cause illness remain unknown at present. Therefore, it is very difficult for regulatory agencies to set policies and for industry to develop control measures for this organism. In this study, we assessed five different animal species in order to find a suitable model that better mimicked human pathogenesis and clinical manifestations of *Es* infection. Three groups of young pigs, chicks, rabbits, gerbils and guinea pigs were challenged orally with 1 ml of reconstituted powdered infant formula containing 10^9 cells of either environmental, food or clinical isolates of *Es*. The animals were observed until days 7 and 14 post-inoculation and their tissues (brain, heart, liver, spleen, mesentery, kidney and intestines), blood, and fecal specimens were examined for the presence of *Es* and host immunological response. None of the animals presented the symptoms observed in *Es* infections in humans. *Es* was isolated from fecal samples of all animals challenged. Gerbils were the only species in which Es was recovered in high numbers from brain tissues. Similar results were obtained when using either oral dosing or intraperitoneal (i.p.) inoculation of guinea pigs. in parallel to oral inoculation did not show any different results. We are currently assessing other neonatal animal models in order to try to mimic more closely the responses seen in neonates.

P4-45 Detection of Norovirus in a Small Community Groundwater Source

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Norovirus (NoV) is the most common cause of acute nonbacterial gastroenteritis in humans worldwide. NoV is highly infectious and spreads by ingestion of contaminated food, such as oysters and water. The quality of water is a major issue in the food industry. Groundwater is a common transmission route for waterborne infectious disease around the world and not an unusual source of water for small municipalities in rural areas distributing water to business such as processing plants, retail stores and restaurants. NoV and hepatitis A virus have been the most frequently reported viral etiologic agents of groundwater-related outbreaks. We have tested 112 water samples from 12 different groundwater sources, among which 6 were under the direct influence of surface water, for the presence of enteric viruses, NoV, F-specific coliphages, somatic coliphages, total coliforms, *E. coli*, heterotrophic plate counts (HPC) and *enterococci*. The water samples were collected monthly from March through December 2004. NoV were found in one water sample from one site considered well protected from surface water and runoffs, where no other enteric viruses or microbiological indicators were present. The NoV strain was detected by nested RT-PCR after organic flocculation on a large volume of treated water. The strain was further characterized by sequence analysis of the PCR product. The NoV was identified as a GII.4 strain and showed a high homology (95%) with Norovirus Hu/NLV/Oxford/B6S6/2003/UK variant strain. However, no case of NoV infection was reported by the Quebec public health laboratory and no symptoms associated with viral infection were notified to the Quebec food inspection agency for the implicated region from July through September 2004. Thus the absence of indicator microorganisms does not seem to correlate well with the absence of NoV. Furthermore, the molecular detection of NoV in water samples should be interpreted with caution regarding health risk effects.

P4-46 Survival of Human Norovirus on Fresh Lettuce Using Real-time Quantitative RT-PCR and Two-step RT-PCR

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Enteric virus transmission due to the consumption of fecally contaminated foods is a significant public health concern. Among the enteric viruses, noroviruses remain the most widely incriminated agents of foodborne viral gastroenteritis. Norovirus outbreaks have been linked to fresh and frozen vegetables/fruits, seafood and water. The fresh produce may get contaminated with norovirus before harvesting, during harvesting, packing, handling, storage or preparation by infected food handlers, or from contaminated food surfaces. Survival studies of norovirus on fresh vegetables or fruits are lacking because of the difficulties in working with the virus, which cannot be cultured in vitro, and because of the questionable suitability of canine or feline calicivirus surrogates. To investigate the survival of human norovirus, fresh lettuces have been contaminated with a viral suspension of norovirus GII-4 strain (2602VR7) and incubated for 1, 4, 7, 10, 14, 20 and 24 days at 4°C. After elution of the viruses with glycine buffer in tryptose phosphate broth, RNA was extracted with a magnetic silica bead kit from bioMérieux. Five ul of extracted RNA were used for detection by two-step RT-PCR and TaqMan quantitative real-time RT-PCR. Norovirus was detectable after 24 days by real-time and after 20 days by conventional RT-PCR. The threshold cycle (Ct) of the amplification plots has shown a significance increase after a long period of time on lettuce. The increase of Ct implicates the decrease of RNA target or reduction of viral charge. We have estimated a reduction of 1–10% of virus particles on lettuce after 24 days by extrapolating the standard curve. The quantitative real-time method was more sensitive, and non time-consuming and can be routinely used for survival studies of human norovirus in food products. Studies of survival of norovirus on other food matrices are in progress.

P4-47 Characterization of *Salmonella* spp. Isolated from Pre- and Post-chill Whole Broiler Carcasses

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While several reports exist on characterization of *Salmonella* spp. isolated from processed poultry by phenotypic and genotypic methods, little is known about the characterization of *Salmonella* spp. isolated from pre- and post-chill carcasses by these methods. The objectives of this study were to characterize *Salmonella* spp. isolated from pre- and post-chill whole broiler carcasses by serotyping

and antimicrobial susceptibility testing and to ascertain the presence of class 1 intergrons and blaCMY beta-lactamase genes among isolates of predominant serotypes. A total of 414 *Salmonella* spp. obtained from pre- and post- chill carcasses were tested for serotyping by standard method. Thirteen serotypes were identified among *Salmonella* isolates recovered from pre- and post-chill carcasses, the most common being *Salmonella* Kentucky (51.4%; 67.3%) and *S.* Typhimurium (20.7%; 15.3%). Antimicrobial minimal inhibitory concentrations for all isolates were determined by use of the Sensititre system. A total of 76.1% and 83.2% of pre- and post-chill isolates were resistant to one or more antimicrobial agents tested, whereas approximately 50% of pre- and 43% of post-chill isolates exhibited resistance to five or more tested antimicrobials. Resistance was most often observed to tetracycline (69.3% of pre- and 78.7% of post-chill isolates). Sulfisoxazole and ceftiofur resistant *S.* Kentucky and Typhimurium isolates were tested by PCR, for class 1 integrons and blaCMY genes, respectively. Sixty-seven percent of the ceftiofur resistant *S.* Kentucky isolates possessed blaCMY genes; however, no class-1 integrons were detected. In contrast, thirty percent of *S.* Typhimurium isolates contained class-1 integrons; however, no blaCMY genes were found. The results of this study indicate that a large number of *Salmonella* spp. isolated from whole broiler carcasses displayed resistance to commonly used antibiotics. This research also suggests that class 1 integrons and blaCMY genes contribute to specific antimicrobial resistance phenotypes among *Salmonella* spp. recovered from poultry.

P4-48 Prevalence of Class 1 Integrons and Antibiotic Resistance Patterns of Enteric Bacteria in Broiler Chickens in Thailand and the United States

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To determine a genetic basis for antibiotic resistance and the prevalence of class 1 integrons, which are known to carry multiple antibiotic resistance (MAR) genes, in enteric bacteria from poultry, PCR analysis was performed on *Salmonella* spp., *Proteus mirabilis*, and *E. coli* from broiler chickens in the US and Thailand. Two thousand one hundred thirty-seven isolates were collected from broilers representing 7 farms at abattoirs in the US and 1,050 isolates were collected from broilers from 2 farms in Thailand. Bacteria were derived from the fecal samples by use of standard culture methods, and confirmation to genera (*Salmonella*) or species (*E. coli* and *Proteus mirabilis*) was accomplished by API20E biochemical analyses (bioMérieux, Inc. Hazelwood, MO). Isolates were subjected to a multiplex PCR analysis to detect class 1 integrons. Primers were designed to target the conserved genes *qacEΔ1*, *int1*, and *sul1*, coding for quaternary ammonium resistance, a specific integrase, and sulfonamide resistance, respectively, as reported to be associated with class 1 integrons. Antibiotic resistance patterns were determined by the standardized National Antimicrobial Resistance Monitoring System (NARMS) broth dilution analysis. Class 1 integrons were found in 40%, 26% and 0.7% of *E. coli*, *P. mirabilis*, and *Salmonella* spp., respectively, from the US, and 21%, 33% and 39% of *E. coli*, *P. mirabilis*, and *Salmonella* spp., respectively, from Thailand. Isolates demonstrated a number of MAR patterns, with most patterns being specific to bacterial type and no common patterns being found between isolates from the US and Thailand. Many MAR patterns were not associated with integron presence. These data indicate that class 1 integrons are common in some types of enteric bacteria, and that some, but not all, MAR are associated with the presence of class 1 integrons.

P4-49 Growth Characteristics and Susceptibility to 1% Lactic Acid of Nalidixic Acid Resistant Mutants of *Salmonella* Typhimurium Developed from a Single Wild-type Strain

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Antimicrobial drug resistant foodborne pathogens are used as research tools to inoculate foods and are easily isolated to examine the effects of processing or interventions on pathogen reduction. Multi-drug resistant pathogens are a concern for human and animal medicine and the food industry. Six nalidixic acid resistant (NAL) mutants, developed from a single wild-type strain and differing in multi-drug resistance, were examined (three replications) for growth characteristics and susceptibility to 1% lactic acid (LA). The objectives were to validate that NAL mutants represented the growth and survival of the wild-type strain and to examine the ability of LA to inhibit multi-drug resistant *Salmonella*. A separate, step-wise process was used to select NAL mutants from *Salmonella* Typhimurium ATCC 14028. A three-strain *Salmonella* Typhimurium DT-104 cocktail was used as a positive control for multi-drug resistance. The wild-type strain, NAL mutants and DT-104 cocktail were inoculated in tryptic soy broth (TSB) and TSB containing LA and were incubated at 37°C; samples were taken at incubation times 0, 1, 2, 3, 6, 8, 12 h and plated on duplicate tryptic soy agar plates. Area under the curve (AUC) was calculated and analyzed. In TSB, mutants 21B and 32B had lower ($P < 0.01$) AUC compared with the wild-type strain. In LA, the wild-type strain had a greater ($P < 0.01$ and $P = 0.069$, respectively) AUC than mutant 2A (resistant to 5 drugs) and the DT-104 cocktail (resistant to at least five drugs). In LA, mutant 2A had a lower ($P < 0.05$) AUC than the other NAL mutants. In-vitro development of nalidixic acid resistance in *Salmonella* resulted in some mutants that were significantly different in growth characteristics and survival in LA. Multi-drug resistant *Salmonella* were equally or more susceptible to LA than pan-susceptible *Salmonella*. Exposure to nalidixic acid can result in cross-resistance to other antimicrobial drugs and alter growth and survival characteristics.

P4-50 Insertional Mutagenesis of *Listeria monocytogenes* 568 Reveals Genes That Contribute to Elevated Thermotolerance

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Listeria monocytogenes is a gram positive foodborne pathogen that has garnered much attention from the food industry over the past quarter century. This is mainly due to the high mortality rate of the disease it causes and to its unique growth attributes. *L. monocytogenes* generally displays a relatively high tolerance to many inhospitable conditions that would normally incapacitate vegetative cells of most other foodborne pathogenic bacteria. However, there appears to be a wide degree of variability among *Listeria* strains to withstand environmental stresses. This strain-to-strain variance directed us to seek out potential mechanisms which may be responsible for increased tolerance to thermal stress. A library of 4300 Tn917 insertion mutants was constructed in *L. monocytogenes* strain 568 (serotype 1/2a) and screened for phenotypes possessing increased thermotolerance. Eighteen mutants consistently exhibited

increased tolerance to heat treatments at 52°C compared to the wild type strain. Examination of the survivor curves for these mutants demonstrated that increased tolerance was achieved by (1) extension of shoulder region of the thermal death time curve, (2) increased D-value, as determined from the slope of the logarithmic region of the curve, or (3) the combination of both attributes. Inverse PCR on several of these mutants isolated the regions flanking the inserted transposon. DNA sequencing of the amplicons revealed that the insertions were located in sites encoding for proteins involved in fatty acid, nucleic acid and carbohydrate metabolism, and general stress response, as well as transport mechanisms. Characterization of a mutant with a Tn917 insertion in the gene encoding for alpha, alpha phosphotrehalase demonstrated cross protection against high osmolarity (20% NaCl) in addition to tolerance to high temperatures. Further characterization of this mutant is in progress. The information obtained in this study may contribute to better understanding of the mechanisms involved in bacterial stress tolerance to factors important for the minimal processing of food products.

P4-51 DSC The Role of σ^B-dependent and σ^B-independent Mechanisms of *Listeria monocytogenes* during Cold Shock and Growth at Low Temperature

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Listeria monocytogenes is a foodborne pathogen that has the ability to multiply at low temperature. The global stress response regulator σ^B-(encoded by *sigB*) is activated under a variety of environmental stresses in *L. monocytogenes*. We evaluated the role of σ^B in directing expression of selected putative and confirmed cold response genes in a *L. monocytogenes* wild-type and in an isogenic *DsigB* null mutant, which were either cold adapted at 4°C in brain heart infusion (BHI) broth for up to 30 min or grown at 4°C in BHI broth for 12 days. Transcript levels of the housekeeping genes *rpo*B and gap, the σ^B- dependent genes *opu*CA and *bsh*, and cold response genes *ltrC*, *oppA* and *fri* were measured, using TaqMan quantitative real-time reverse transcriptase PCR (qRT-PCR). Statistical analysis was performed on all qRT-PCR data, using standard regression diagnostics and a general linear model with multiple comparisons. Transcriptional start sites were confirmed by use of RACE-PCR. Our data indicate that (i) *ltrC* and *fri* are transcribed from at least one σ^B-dependent promoter at 4°C as revealed by RACE-PCR, (ii) deletion of σ^B-does not significantly reduce expression of *ltrC*, *oppA* and *fri* within the first 30 min of cold shock, as indicated by similar mRNA transcript levels in wild-type and *DsigB* mutant, (iii) expression of the putative cold response genes opuCA, fri, oppA, and ltrC is predominantly σ^B-independent while *bsh* is σ^B-dependent during growth at 4°C, as shown by mRNA transcript levels, and (iv) *opu*CA is transcribed from a σ^B-dependent promoter during growth at 4°C and in the absence of σ^B-expression, while at 37°C, *opu*CA is predominantly transcribed by a σ^B-dependent promoter. Our results indicate that σ^B-contribution to *L. monocytogenes* gene expression during growth at 4°C is limited and that σ^B-independent mechanisms are responsible for growth at 4°C.

P4-52 DSC Exposure of Nutrient Deprived *Listeria monocytogenes* Cells to Food Preservative Stress in the Presence or Absence of Oxygen

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For *Listeria monocytogenes*, heat-stressed cells produce higher counts if enumerated under anaerobic conditions and the alternative sigma factor sB is reported to contribute to survival of in vitro environmental-stressed Gram-positive bacteria. In this study a wild type strain, 10403S, and a D*sigB* mutant strain, FSLA1-254, were stressed by starving in phosphate buffer saline (PBS), coupled with exposure to preservative salts with or without oxygen. Anaerobiosis was created by addition of sodium thioglycolate and boiling with argon gas flush in Hungate tubes before autoclaving. The preservatives used were sodium propionate (2%) (SP), sodium laureate (0.3%) (SLau), sodium diacetate (2%) (SD) and sodium lactate (2%) (SL). Each strain was grown separately in BHI before inoculating into PBS with or without preservatives, and plated on days 0, 2, 4, 7, 14, 21 and 28 on *Listeria* selective agar. Initial control counts were about 9 log CFU/ml. Both aerobic and anaerobic stressed cells starved in the presence of SLau yielded 1 log lower counts than the controls, and by day 7 counts were 2 logs lower for both strains. By day 7, counts of cells exposed to SD were about 3 logs lower than the control. By days 14 or 21, cells of both strains exposed to SD or SLau yielded no detectable CFUs. By day 28, cells exposed to SL and held anaerobically yielded counts that were 3 logs lower than the aerobically starved counterparts. Over 28 days, the mutant strain survived starvation almost as well as the parent strain, suggesting *sigB* alone may not be responsible for *Listeria*'s strong starvation survival response in the presence or absence of preservatives. Anaerobiosis did not affect survival of cells starved in the presence of preservatives, except for starved cells exposed to SL, which yielded 3 log lower counts under anaerobic than aerobic starvation conditions.

P4-53 One-year Starvation-stressed Cells of *Listeria monocytogenes* Scott A Serotype 4b Invade Human Cell Line Caco-2

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One-year starvation-stressed cells of *Listeria monocytogenes* Scott A serotype 4b were evaluated for in vitro virulence, using human cell model Caco-2. *L. monocytogenes* was grown in TSBYE for 18 h, washed twice by centrifugation and resuspended in 0.85% NaCl (physiological saline) to induce starvation-stress at 20°C. Almost 99% of *L. monocytogenes* cells turned into small coccoid-shaped cells as a result of starvation-stress for 30 days or longer. Internalization, multiplication and plaque formation in Caco-2 by non-starved and starvation-stressed cells of *Listeria monocytogenes* were determined by challenging 10^5 to 10^7 cells/ml of *L. monocytogenes* cells onto 10^6 cells/ml of Caco-2 (=MOE of 1:10, 1:1 or 10:1). The numbers of *L. monocytogenes* internalized in Caco-2 increased with increase in infection time (1 to 4 h) and infection dose (MOE of *L. monocytogenes*: Caco-2 = 1:10 to 10:1) both for non-starved cells and starvation-stressed cells. All starvation-stressed cells (30 to 365 days) of *L. monocytogenes* were internalized in Caco-2 except that there were about 1–2 log decreases in counts of internalized *L. monocytogenes* versus non-starved cells at different MOE tested after 4 h infection. After internalization, all starvation-stressed cells of *L. monocytogenes* increased to about 6 log CFU/well in Caco-2 versus 7 log CFU/well for the non-starved cells within 24 h. The numbers of plaques formed in Caco-2 were about 2 log per well for starvation-stressed cells compared to 3 log per well for non-starved cells at MOE of 1:1. The plaque sizes were about 1 to 1.5 mm for starvation-stressed cells versus 2 mm for non-starvation-stressed cells. These studies indicate that *L. monocytogenes* cells after encountering conditions of nutrient depletion or starvation still remain highly virulent.

P4-54 Molecular Characterization of "Unusual" *Listeria monocytogenes* from Brazilian Poultry Slaughterhouses

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Listeria monocytogenes can be isolated from many different sources. As a result, policies around the world touch upon acceptable levels of Lm, and range from tolerable limits to zero tolerance. A zero tolerance policy in Ready-to-Eat poultry products requires active farm-to-fork surveillance programs in order to assess the efficacy of the policies that are in place. Monitoring for *Listeria monocytogenes* requires correct identification at the species level. The major tests include production (or not) of acid (without gas) of D-xylose/L-rhamnose/mannitol, and the lysis of sheep/horse red blood cells. Outbreak or epidemiological investigations further rely on serological and molecular characterization data. In this study, 210 strains of *Listeria monocytogenes*, isolated from manual and automated evisceration poultry abattoirs, were subjected to phenotypic and genotypic characterization. A validated PCR based molecular serology assay was compared with classical immunological serology, with unexpected results. Twenty-nine strains had 4 (expect 3) bands, which were sequenced for analyses. Of the 60 strains that were negative for hemolysis on both blood agar media and chromogenic media (Rapid'L.Mono, Bio-Rad), 49 were shown to be *Listeria monocytogenes*. Unexpectedly, some strains shown to be *Listeria* spp. (i.e. non-Lm) were positive by PCR for the presence of the prfA, hlyA and mpl genes. Other genotypic tests included PCR methods targeting the species monocytogenes (BAX® System and multiplex-PCR based on 16S rDNA sequences and the hlyA gene). There has been a previous report of *L. innocua* possessing some of the pathogenicity island 1 genes of *Lm* (AEM 70: 4526-4266). Our discovery of "unusual" Brazilian strains of *Listeria monocytogenes* having similar characteristics underline the importance of not relying on only a few simple tests when investigating the presence or absence of Lm in industry and processing plants, as well as foods. Further work is ongoing to fully characterize the "unusual" set of *Listeria monocytogenes*.

P4-55 Distribution of Epidemic Clonal Genetic Markers among *Listeria monocytogenes* 4b Strains and Correlation with Molecular Subtypes

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Listeria monocytogenes is the etiologic agent of foodborne listeriosis. Of the 13 serotypes distinguishable within the bacterial species, serotype 4b is most frequently involved in the human disease, although not predominant in foods and environment. The recent genome sequencing of *L. monocytogenes* strains representative of different serotypes has allowed for the identification of genetic markers unique to some serotype 4b isolates implicated in major epidemics; these isolates had previously been grouped into two distinct "epidemic clones" - ECI and ECII - based on a number of subtyping techniques. The genetic markers identified in ECI and ECII *L. monocytogenes* 4b strains might confer on them a higher pathogenic potential and/or other selective advantages: thus, estimating their distribution among *Listeria* strains might be of interest, for both epidemiological and risk assessment purposes. In this work we screened by PCR a panel of 91 *L. monocytogenes* 4b strains of different origins for presence of selected ECI- and ECII-specific genetic markers; PFGE patterns of the strains were also compared, in order to establish the relationship between the EC markers and the molecular subtypes. Results showed that 34 of the 91 *L. monocytogenes* 4b strains (37.4%) possessed either ECI (16 strains) or ECII (18 strains) genetic features. Of the 34 strains, 15 were of clinical origin (41.6% of the 36 clinical isolates tested); 17/50 (34%) were from foods; and 2/5 (40%) were from the environment. The predominance of EC genetic markers observed among clinical isolates is consistent with the hypothesis of a higher pathogenic potential. In addition, EC markers were more frequent in strains isolated from processed foods, and were detected in 2 of the total 5 isolates from the environment, in accordance with the environmental persistence reported for EC strains. PFGE analysis showed a certain degree of genetic differentiation between strains harbouring EC-specific genetic markers.

P4-56 The Role of *Listeria monocytogenes* Serotype 4b Antigens in the Pathogenesis of Listeriosis

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Most foodborne outbreaks of listeriosis are associated with serotype 4b strains of *Listeria monocytogenes*. In this study we investigated the role of gtcA, a gene required for glycosylation of teichoic acid, in the pathogenesis of listeriosis. We found that two different serotype 4b mutants of gtcA, which lack glucose and galactose residues on their teichoic acid, were significantly less virulent than their wild type parents when inoculated intragastrically into A/J mice. To gain some knowledge of whether the effect of gtcA was manifested only in the gastrointestinal tract, we also performed experiments in which this environment was circumvented by inoculating mice intravenously. We found that both gtc A mutants exhibited lower virulence than the parent strain in the liver, and one of the strains also caused less severe infection in the spleens of i.v. inoculated A/J mice. In addition, both gtcA mutants of *L. monocytogenes* were less able to invade, and multiplied to a lower final number of intracellular bacilli in a human intestinal epithelial cell line (Caco-2 cells) in vitro, than their respective wild type parent strains. These data suggest a role for gtcA in the pathogenesis of listeriosis. Future experiments will determine the effect of gtcA on the ability of *L. monocytogenes* to grow on ready to eat meat products, and mechanisms by which gtcA affects serotype 4b antigen expression and virulence.

P4-57 Induction of Apoptosis in an In Vitro HEp-2 Cell Model by *Listeria* spp.

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One of the hallmarks of apoptosis is the activation of caspase pathway and loss of plasma membrane asymmetry. Detecting and monitoring cell progression to apoptosis is essential to understanding the underlining mechanisms of cell-mediated cytotoxicity. In this study we compared the molecular events and morphological features associated with apoptosis and *Listeria* cell-mediated cytotoxicity induced by five *Listeria* strains (*L. monocytogenes* Scott A, *L. innocua*, *L. ivanovi*, *L. monocytogenes* 4245 and *L. monocytogenes* 572) to those of hemolysin deficient *L. monocytogenes* (Hly-) on human larynx epithelial (HEp-2) cells in relation to cytotoxicity, using an alkaline phosphatase (AP) and lactate dehydrogenase (LDH) assay. The HEp-2 cells exhibited significantly lower

($P < 0.05$) LDH release of 23.0 and 24.3% for *L. innocua* and *L. monocytogenes* Hly-, respectively, compared to the high control. Both *L. monocytogenes* Scott A and *L. monocytogenes* 4245 were the most cytotoxic, resulting in similar release of LDH (56.3 and 60.0%) and AP (26.7 and 22.5%) from the HEp-2 cells. Four *Listeria* strains triggered both apoptosis, and necroses were positively correlated with their cytotoxicity. Apoptosis was the prevalent form of cell killing by both *L. monocytogenes* Scott A and *L. monocytogenes* 4245, while *L. monocytogenes* 572 and *L. ivanovii* induced mainly the necrotic mode of cell death. The extent and the timing of apoptosis were strongly dependent on the strain and were mediated by caspase-3 activation. Significant ($P < 0.05$) increases in caspase-3 activity and DNA fragmentation were observed predominantly in Hep-2 cells treated with *L. monocytogenes* Scott A, 4245 and strain 572 when observed by both fluorescent and phase contrast microscopy compared to the mutant strain (*L. monocytogenes* Hly-), which showed lesser morphological and cytopathogenic changes and a lack of DNA fragmentation. Results of this study suggest that hemolysin may play an important role in modulating cell death in human epithelial tissue culture cells.

P4-58 Stability of *Escherichia coli* O157:H7 in Sub-optimal Conditions as Monitored by Multilocus Variable Number Tandem Repeat Analysis

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Enteric pathogens, such as *Escherichia coli* O157:H7, have been shown to contaminate fresh produce. These pathogens can survive in low numbers in water, in soil, and on plants. Two hundred seventy *E. coli* O157 isolates were recovered from surface waters of the Salinas watershed and initially analyzed by PCR for the presence of *fli*C and the virulence genes *eae*, *hly*, *stx*1, and *stx*2. Twenty-five percent did not contain *stx*1. Nine percent did not contain *stx*2. All isolates contained the *fli*C, *eae* and *hly* genes. Multilocus variable number tandem repeat analysis (MLVA) has become the method of choice for high-throughput subspecies typing of *E. coli* O157:H7. Seven repeat loci (Vhec1-7) were analyzed by MLVA and 23 different types were found. Comparison of these types to 106 clinical isolates, representing 66 MLVA types, did not show a match, though some types were nearly identical, differing by only a single repeat change at 1 of the 7 loci. Mutation rates at these same loci have been measured by us and other research groups during repeated, serial passaging on complete media. Under these conditions some loci (e.g., Vhec1) mutated readily, while others (Vhec2, 5, 6) did not mutate. Since conditions in the environment are very different from those in the animal gut or the laboratory, we have tested the rate of change of MLVA type under conditions of low temperature and starvation. Additionally, we monitored change of MLVA type after recovery from plants. Repeated passage on complete media at 25, 15 or 10°C did not substantially increase the mutation rate compared to incubation at 37°C. MLVA type did not change in 28 strains re-isolated after survival for 3 months on plants. Likewise, under starvation conditions in creek water for 7 days, the MLVA type also failed to change appreciably. Therefore, under conditions where the bacteria did not divide or divided minimally, MLVA type appeared stable, indicating that this method may be effective as a trace-back tool to locate sources of environmental contamination.

P4-59 Oxygen Consumption Rate of *Campylobacter jejuni* during Growth and Survival under Various Oxygen Levels

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Campylobacter jejuni is an important foodborne pathogen. The microaerophilic nature of this organism has made its isolation and culture in the laboratory problematic. To gain a better understanding of its physiology, we measured the oxygen consumption of *C. jejuni* strain 81–176, using a Clark-type oxygen electrode at various microaerobic (2–10% oxygen, 10% carbon dioxide, balanced with nitrogen) conditions and at atmospheric oxygen levels (21% oxygen). *C. jejuni* microaerobic cultures that were grown (up to 72 h) to different cell densities in Mueller-Hinton (MH) broth were sampled in the microaerobic glove box, sealed with parafilm and placed in an air-tight container, and then removed from the glove box. Samples (2.5 ml) were then withdrawn through the parafilm membranes, using a syringe fitted with a long blunt needle, and injected into the oxygen electrode chamber to determine the oxygen consumption along with culturable cell counts (colony forming units, CFU) on MH agar. Our results showed that the rate of oxygen consumption was very low (10^{-21}–10^{-19} moles/s/CFU) when *C. jejuni* was grown in MH broth at 42°C under microaerobic conditions. The oxygen consumption rate increased almost instantaneously (within 1 minute) after the microaerobic culture was exposed to atmospheric levels of oxygen, and this activity remained relatively constant (ca. 0.6-2.0 × 10^{-18} /moles/s/CFU) for at least 2 h, or until culturable cell counts started to decline. After prolonged exposure in air, the gross oxygen consumption (moles/s) remained steady longer than the culturable cell counts, resulting in an increase in the CFU-normalized oxygen consumption rate (moles/s/CFU). This report provides a direct and real time means of measuring *Campylobacter* physiological states during growth and survival with minimal manipulation of the test samples, which is pivotal for monitoring such a fast-changing phenomenon.

P4-60 Quorum Sensing and Stress Resistance Relationship in *Salmonella*

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The objective of this study was to evaluate the potential relationship between autoinducer-2 (AI-2)-like activity and resistance of *Salmonella* to heat and acid. Filter (0.22 μm) sterilized supernatants (pH 3.0 and 3.5; adjusted with HCl) of *Salmonella* Thompson RM1987N (AI-2-positive) and RM1987NLUX (AI-2-negative; LuxS mutant of *Salmonella* RM1987N), or *Escherichia coli* O157:H7 86-24 (AI-2-positive) and VS-94 (AI-2-negative; LuxS mutant of *E. coli* 86-24) in Luria-Bertani (LB) + 0.5% glucose broth, were prepared as preconditioned culture (PC) media. *Salmonella* RM1987N and RM1987NLUX were exposed to 55°C (6 h) in LB, and *Salmonella* RM1987NLUX and *Salmonella* Typhimurium DT104 (AI-2-postive) to AI-2-positive and -negative PC media, derived from *Salmonella* or *E. coli* O157:H7. In addition, *Salmonella* RM1987NLUX was acid-challenged in PC (pH 3.5; 35°C), and acid-adapted (tryptic soy broth [TSB] with 1% glucose) and nonacid-adapted (TSB without glucose) cultures of *Salmonella* RM1987N and RM1987NLUX were challenged in LB (pH 3.0; 35°C). At hourly intervals, samples were plated on tryptic soy agar. The Baranyi model (heat) and linear regression (acid) were used to obtain death rates of the pathogen. During heat challenge, survival of *Salmonella* RM1987N and RM1987NLUX was not different (0.4 log CFU/ml; $P \geq 0.05$) in LB, and survival of *Salmonella* RM1987NLUX and DT104 was also not different (< 0.2 log CFU/ml; $P \geq 0.05$) in various PC media preparations. In addition to survival, death rates (-3.17 to -3.90 h^{-1}) were similar in the presence or absence of AI-2-like activity. During acid challenge, survival of *Salmonella*

RM1987NLUX was similar (< 0.5 log CFU/ml; $P \geq 0.05$) in AI-2-positive and -negative PC media, and survival of acid-adapted *Salmonella* RM1987N (4.8 log CFU/ml) and RM1987NLUX (5.1 log CFU/ml) was not different ($P \geq 0.05$) in LB. In general, death rates of *Salmonella* showed no differences (< 0.08 log CFU/h) in media with or without AI-2-like activity. These results suggested that AI-2-like activity may not play a major role in heat and acid resistance of *Salmonella*.

P4-61 Invasiveness and Intracellular Growth of Multidrug-Resistant *Salmonella* and Other Pathogens in Caco-2 Cells

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The wide use of antibiotics in medical and agricultural practices has enhanced the dissemination and persistence of multidrug-resistant pathogens in hospitals, food chains, and ecosystems. This increase in multidrug-resistant *Salmonella* strains of human and animal origin has become a major public health concern. For a better understanding of the health consequences of multidrug-resistant bacteria transmitted from animal products to humans, host interactions of *Salmonella* isolates along with other pathogenic and commensal bacteria were evaluated, using a human intestinal Caco-2 cell system. PCR assay was carried out to determine the presence of class 1 integrons and specific antibiotic-resistant genes in the tested multidrug-resistant *Salmonella* isolates. Multidrug-resistant *Salmonella* Agona, *Salmonella* Heidelberg, and *Salmonella* Typhimurium possessed plasmid-mediated class 1 integrons. *Salmonella* Typhimurium phage type DT104 (*S.* Typhimurium DT104) isolated from ground beef showed the well-known genotype and phenotype resistance characteristics of the species, and class 1 integron in the isolate was chromosomally located. Multidrug-resistant *Salmonella* isolates were better able to invade Caco-2 cells than antibiotic-susceptible isolates. Among them, the invasiveness of *S.* Heidelberg 129 and *S.* Typhimurium DT104 isolates into the cells was distinctive. Another intracellular pathogen, *Listeria monocytogenes*, performed the best in invading Caco-2 cells among the tested species. Multidrug-resistant opportunistic pathogens, such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, were also able to invade the cells. The invasion of *S.* Heidelberg 129, *S.* Typhimurium DT104, *L. monocytogenes*, *K. pneumoniae*, and *P. aeruginosa* into Caco-2 cells was not affected even in the presence of commensal *E. coli*. During the intracellular growth of *S.* Heidelberg 129, *S.* Typhimurium DT104, and *L. monocytogenes*, the bacterial counts increased 2-log cycles in 9 h of incubation in Caco-2 cells. Therefore, our findings indicate that these strains could rapidly proliferate after their invasion into the cells.

P4-62 Generation of Accessory Gene Regulator Variants in *Staphylococcus aureus* Biofilms

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Several of the food poisoning-associated enterotoxins produced by *Staphylococcus aureus* are regulated by the accessory gene regulator (Agr) system. Agr mutants are frequently found among clinical isolates, and Agr-negative strains are able to form more robust biofilms under certain conditions both in vitro and in vivo. We hypothesized that Agr "variants", isolates whose Agr phenotype differs significantly from the parent strain, would be generated in *S. aureus* biofilms, and that those with an Agr-negative phenotype would become the numerically predominant subpopulation within the biofilm. We assessed variant generation by growth of *S. aureus* FRI1169 (a strain that is both hemolytic and Agr-positive) in biofilm drip reactors, using a serum-based growth medium. Biofilm samples were harvested at various times and approximately 200 isolates were assayed for hemolytic activity and expression of the Agr autoinducing peptide (AIP). After 14 days of growth, nearly two-thirds of isolates from the biofilm were found to be non-hemolytic and expressed little or no detectable AIP. The remaining biofilm isolates either expressed AIP at levels similar to the parental strain, or overexpressed AIP. When grown as a chemostat culture with use of the same growth medium, variants remained a small portion of the overall population (< 2%). However, when grown in enriched medium at high cell densities in the chemostat, variants were found to form a large portion of the overall population (60%) after two weeks. One explanation may be that higher cell density, whether in biofilms or chemostat culture, contributes to the formation of variant populations. The structure of the biofilm population, with regard to Agr activity may have clinical implications. Agr-positive subpopulations presumably can express many Agr-regulated secreted virulence factors associated with severe staphylococcal disease as well as food poisoning. In contrast, Agr-negative subpopulations tend to produce immuno-evasive factors and surface adhesions that contribute to biofilm formation.

P4-63 DSC Quantitative Analysis of the Growth and Attachment of *Salmonella* Typhimurium Mutants during the Alfalfa Seed Sprouting Process

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Salmonella present on alfalfa seed have been shown to be responsible for foodborne outbreaks. Research has shown that *Salmonella* multiply rapidly during the sprouting process, and can be found in water used to irrigate sprouting seeds. Fundamental research has also shown that bacterial attachment is correlated with various factors, such as motility, lipo-polysaccharide (LPS), and exo-polysacchride (EPS). Wild type *S.* Typhimurium and strains with flagellar mutation (physical absence or loss of motility), major components of EPS (cellulose or colonic acid), LPS (O-antigen), and EPS/LPS synthesis were used to identify cell characteristics responsible for *Salmonella* association with sprouts. Alfalfa seeds were inoculated with wild type and mutant strains of *S.* Typhimurium at (30 CFU/g), and transferred into a bench top-scale sprouting chamber. Microbial populations on seeds, on sprouts or in irrigation water were determined during sprouting, and growth rates and maximum concentrations were compared. Attachment assays on three day old sprouts were also performed according to published methods. Mutants defective in flagellar motility, colonic acid synthesis, or LPS O-antigen showed behavior not statistically significantly different from that of the wild type during the sprouting process. EPS/LPS synthesis mutant showed a reduction in both growth rate and maximum cell concentration. Mutants with no flagella or those that could not synthesize cellulose showed no statistically significant difference in growth rate, but did show a ten-fold reduction in the ability to colonize sprout surfaces. No significant differences were observed for any mutant strains when tested using attachment assays with three day old sprouts. This research provides evidence that the presence of flagella and the ability to synthesize cellulose appear to play an important role in *Salmonella* attachment to sprouts during the sprouting process. Our results also indicate that *Salmonella* attachment assays on sprouts that do not closely mimic the actual sprouting process may not provide reliable results.

P4-64 Microbiological and Toxicological Safety of Dried Spices and Herbs at Import, Production, Retail, and Catering Establishments in the UK

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Spices and herbs are valued for their distinctive flavors, colors and aromas. However, spices and herbs can contain high levels of microbial contamination, including pathogenic bacteria, depending on whether they have received a form of treatment or not. The European Commission Recommendation 2004/24/EC required member states to undertake a program of sampling dried spices and herbs from import, production, retail and catering establishments, for the presence of *Salmonella* spp. and levels of *Bacillus cereus, Clostridium perfringens* and *Enterobacteriaceae,* using the most recent version of EN/ISO standards, and to determine levels of aflatoxins, using the method in Commission Directive 98/53/EC. Microbiological examination of 2963 spice and herb samples in the UK and comparison with criteria stipulated in 2004/24/EC revealed that most (98%) were of satisfactory or acceptable quality. However, 2% of samples were of unsatisfactory quality due to high levels of *B. cereus* ($\geq 10^5$ CFU/g), *C. perfringens* ($\geq 10^3$ CFU/g) and/or presence of *Salmonella* spp. Most (90%) samples examined were recorded as ready-to-use, while another 4% were destined for further treatment or packing. One percent of ready-to-use samples contained salmonellas, 0.5% contained high levels of *B. cereus* and 0.5% high levels of *C. perfringens*. Analysis of 34 samples for aflatoxin revealed that 24% of samples contained unsatisfactory levels of aflatoxins (> 5µg/kg for aflatoxin B1 and/or 10 µg/kg for total aflatoxins). The potential public health risk of using spices and herbs as an addition to Ready-to-Eat foods that undergo no further processing is highlighted in this study. The presence of *Salmonella* spp. and high levels of *B. cereus* and *C. perfringens* in some dried spices and herbs suggests a need for better control in the usage of these ingredients. Ensuring effective ways to reduce microbial loading and destroy pathogens in dried spices and herbs could help to improve their microbiological status.

P4-65 The Importance of Strain Validation Prior to Experimental Use of Nalidixic Acid-resistant *Salmonella* Typhimurium: Alterations in Serotype and Multi-Drug Resistance

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The use of antimicrobial drug-resistant foodborne pathogens to inoculate food products and examine the effects of food processing or interventions on pathogen reduction is a common practice in food microbiology research; inherent in the use of the drug resistant, marker strains is the requirement that they are similar to the wild-type strain. The objective of this study was to develop nalidixic acid resistant mutants of *Salmonella* Typhimurium from a single wild-type strain and examine alterations that would limit the ability of the resistant mutants to reflect the wild-type strain. Thirty nalidixic acid resistant mutants were selected in a separate, step-wise process from a single wild-type strain, *Salmonella* Typhimurium ATCC 14028. Mutants were confirmed as *Salmonella,* using a *Salmonella* latex test, and tested for cross-resistance to other antimicrobial drugs by examining minimum inhibitory concentration for 15 antimicrobial drugs, using 96-well microdilution plates. Twenty-seven strains tested positive with a *Salmonella* latex test; 3 strains did not agglutinate and were sent for determination of serotype and 16S rDNA sequence along with the wild-type strain. Two strains had altered serotypes compared to the typical *Salmonella* Typhimurium serotype displayed by the wild-type strain (4,5, 12: i: 1,2); these strains would be classified as *Salmonella* Typhimurium variant Copenhagen (4,12: i: 1,2) and a monophasic variant of *Salmonella* Typhimurium (4,12: i: -). Twelve multi-drug resistant mutants were also observed. Mutants that have altered their serotype or have acquired multi-drug resistance should not be used as marker strains to reflect the growth and survival of the original, wild-type strain because they will likely not reflect the behavior of the wild-type strain. Performing strain validation prior to using nalidixic acid resistant marker pathogens is vital to ensure that resistant mutants are similar to the original wild-type strain.

P5-01 Molecular Characterization of Toxigenic *Staphylococcus aureus* in Ready-to-Eat Foods in Korea

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Ready-to-Eat foods (ca. 3,000 samples) were selected from nationwide wholesale marts to monitor food contamination by *Staphylococcus aureus* in Korea. Two hundred eighty-five samples (8.6%) were contaminated by *S. aureus*, and major contaminated foods were cream-cake, raw fish, and rice cake with filling. Seventy-three strains (47.4%) were enterotoxigenic (had the toxin gene and could produce toxin). As a result of phenotyping by RPLA, staphylococcal enterotoxin A was detected at a high level (44%). Other toxins such as SEB, SEC, SED, SEA+SEC, SEC+SED were detected in each strain. Toxic shock syndrome toxin-1 (TSST-1) was also detected in thirteen strains. The toxigenic *S. aureus* was grouped into eleven toxin gene types by use of multiplex PCR. The most prevalent genotype was *sea+seh, sei,* and *seg+sei*. The tst gene encoding TSST-1 was also found in thirteen strains. The genes (eta, etb) encoding exfoliative toxin A and B were not detected at all. One hundred twenty-five strains (81%) were resistant to penicillin G and 37% of penicillin resistant strains had multi-drug resistance. Of them, some were identified as methicillin resistant *Staphylococcus aureus* (MRSA), the causative organism of nosocomial infection. For further epidemiological study, we used the pulsed-field gel electrophoresis (PFGE) method on ten strains, including three MRSA isolates which were selected by antibiotic susceptibility and enterotoxin type. Results from PFGE profiles showed that one methicillin-resistant food sample with enterotoxin A and H was closely related to NRS123 (SCC*mec* IVa).

P5-02 *Enterobacter sakazakii* in Milk Kitchens of Maternities in São Paulo State, Brazil

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Enterobacter sakazakii (*Esak*), an opportunistic pathogen, has been gaining much attention from food authorities all over the world. While morbidity associated with this bacterium is low, mortality rates can range from 40–80%. The pathogen affects mainly low-birth-weight neonates (first 28 days), but babies less than 6 months old are also at risk. *Esak* infections have been reported as both sporadic cases and small outbreaks of sepsis, meningitis, cerebritis and necrotizing enterocolitis. In the latter, powdered infant formula

has been incriminated as the possible source of the microorganism. In Brazil, as in several other countries, there is no information regarding the incidence of *Enterobacter sakazakii* in powdered infant formula, in reconstituted formula, and in the preparation areas in hospitals. Also, little is known about the time / temperature conditions that rehydrated formulas are exposed to prior to being fed to children. The objective of this study was to evaluate the presence of *Enterobacter sakazakii* in the environment, utensils, and handlers of powdered infant formula and rehydrated infant formula from milk kitchens from different maternity wards in São Paulo, Brazil. Samples were collected from 2 hospitals (A–school; B-public) and analyzed for *Enterobacter sakazakii* by use of the ISO method. For formula (powdered or rehydrated), the MPN technique was used. *Enterobacteriaceae* populations were determined using Petrifilm. *Enterobacter sakazakii* was found in one unopened formula can collected from Hospital A (0.003MPN/g), although the pathogen could not be detected in other cans from the same lot. *Esak* was also found in leftovers from one nursing bottle from the same hospital and from one cleaning sponge from Hospital B. The *Enterobacteriaceae* population ranged from 10^2 to 10^5 CFU/g in cleaning aids (sponges and/or brushes) from both hospitals and was < 6.7 CFU/g in all the formulas. Cleaning aids seem to be an important harborage site for *Esak* in those hospitals.

P5-03 Monitoring and Risk Assessment of Foodborne Pathogens in Foods in Korea

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Foodborne pathogenic bacteria in various food samples in Korea were monitored and a risk assessment was carried out. A total of 1,240 food samples, including 280 Sassimi (raw fish fillet), 244 processed frozen products, 258 Kimbab (cooked rice wrapped with sea weed), and 337 soybean paste were obtained from 8 cities, including Seoul in Korea. Microorganisms tested were *Bacillus cereus*, *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium perfringens* and *E. coli*. The contaminated microorganisms in food samples were comprised of 10.55% *B. cereus*, 2.7% *S. aureus*, 2.0% *V. parahaemolyticus*, 0.8% *Cl. perfringens*, 0.2% *Y. enterocolitica*, and 0.1% of *L. monocytogenes*,. *Salmonella* spp., *C. jejuni,* and *E. coli* O157:H7 were not detected in any of the food samples. Particularly, *B. cereus* that harbors the enterotoxin gene was detected in various foods and regions in Korea; therefore, it should be given special consideration to prevent a hazardous level of contamination. Consequently, quantitative as well as qualitative control of foodborne pathogenic bacteria is needed and will need to be revised to allow reasonable distribution and production in the fields.

P5-04 Estimation of the Burden of Gastroenteric Diseases in Miyagi Prefecture, Japan

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In Japan, under the Food Sanitation Law, the numbers of food poisoning cases must be reported; however, this passive surveillance does not exactly reflect the real burden of foodborne illness. Underreporting can be attributed to the following factors: Reporting rate: (number of laboratory–confirmed cases reported to the authorities), stool sampling rate (the rate of physicians who take stool specimen from patients for laboratory testing), consultation rate: (rate of patients seeking medical care). There are several studies to estimate the burden of gastroenteritis, such as FoodNet in the US. To estimate the burden of illness associated with *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus*, a small scale pilot study was conducted in Miyagi Prefecture, in the northern part of Japan (population of 2.36 million). Data on laboratory-confirmed infections of the three pathogens were collected from clinical laboratories in the prefecture from April to September, 2005. The stool sampling rate was cited from the previous study. The physician-consultation rates were estimated by analyzing foodborne outbreak investigation data for each pathogen. Each factor was multiplied by the laboratory-confirmed cases. The estimated number of illnesses per 100 thousand in this region was 251 for *Campylobacter*, 26 for *Salmonella* and 11 for *Vibrio parahaemolyticus*. Our preliminary data suggested that there was a significant difference between our estimate of burden of illness and the reported cases. A need for establishment of complementary system for the present passive surveillance of foodborne illnesses in Japan was strongly suggested in order to identify and prioritize food safety issues more precisely and to monitor the effectiveness of risk management options.

P5-05 Detection of *Brucella* spp. in Cheese Samples by Nested-PCR at Hidalgo State, Mexico

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Brucellosis is still an important public pealth problem in many states of Mexico, with constant outbreaks in states like Hidalgo. As a direct consequence of this problem, it is necessary to maintain monitoring of the milk produced by animals in different regions in order to identify possible risks to human health in Hidalgo State, Mexico. Sixteen samples of cheese were obtained at Huichapan, Hidalgo, and sent to the Public Health Laboratory of Guanajuato for analysis. The samples were obtained after an outbreak involving 89 persons in different localities of Huichapan caused by the consumption of cheese made with raw milk. Sera samples were analyzed by an agglutination test for detection of antibodies against *Brucella*. The samples were processed by nested PCR, analyzed for the presence of *Brucella* spp., and investigated for phosphatase activity as a measure of pasteurization. The DNA from a *Brucella melitensis* strain and from *B. abortus* antigen was used as a positive control in the PCR technique. From the 16 samples, 62.5% were positive by PCR, none were positive by culture, and phosphatase was detected only in two. According to the results, we are actually improving the nested PCR technique at Hidalgo State, to use it as a routing screening of milk products. It is important to keep on with this work in Mexico in order to acquire greater and more accurate knowledge about our brucellosis situation and for the development of properly planned strategies coordinated by our State Public Health authorities aimed at the control of this disease.

P5-06 Probabilistic Risk Assessment for Viral Foodborne Disease

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Recent estimates indicate that human enteric viruses, particularly the noroviruses, are responsible for a large proportion of the world's overall foodborne disease burden. These viruses are transmitted by human fecal contamination, often associated with poor personal hygiene practices of infected food handlers. We developed a probabilistic risk assessment model to describe the risk of foodborne viral gastroenteritis associated with poor personal hygiene practices of infected food handlers. Inputs considered in the model include, but are not limited to, norovirus contamination levels in the environment and feces, rate of transfer of norovirus particles between alternative surfaces (e.g., hands, foods, food contact surfaces), probability and efficiency of sanitation practices such as washing hands and food contact surfaces, and behavioral factors such as frequency of bathroom use and frequency of contact. These inputs were incorporated into a mathematical model that simulates norovirus contamination among food products, food contact surfaces, employees' hands, and the environment with which hands and surfaces come into contact. A key feature of the model is that it has a temporal dimension, and hence contamination levels are estimated with respect to time during the simulation period. Over time, the contamination levels on hands decrease due to virus inactivation and hand washing/disinfection, while contamination levels on food contact surfaces decrease only as a function of surface washing/disinfection. What-if scenario and sensitivity analyses were applied to evaluate potential mitigation strategies. Results showed that, based on an average number of food servings, the probability of wearing gloves, the number of bathroom visits, and the degree of contamination in the bathroom area substantially affected the contamination level and hence the risk of norovirus infection. This work provides a mathematical approach to modeling the transmission of gastrointestinal viruses and a means by which to compare proposed mitigations aimed at reducing the transmission of foodborne viruses.

P5-07 Pre-harvest Control Factors Affecting Prevalence of Shiga Toxin-producing *Escherichia coli* in Feedlot Cattle

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Since 1982, many human illness outbreaks have been traced to beef contaminated with Shiga toxin-producing *Escherichia coli* (STEC). The illnesses includes the bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). The increased number of outbreaks have raised concerns about beef safety. To effectively address these concerns, it is critical to evaluate the role of beef cattle in STEC infection and to identify on-farm factors that eliminate or decrease STEC carriage by cattle entering the food chain. This study was designed to determine the effects of pre-harvest factors on STEC prevalence in California feedlot cattle. From four feedlots (ranging from 13,000 to 46,000 Holstein steers), 642 fecal samples were collected over one year. Approximately 40 samples were collected from each feedlot in each season, with 321 steers in the growing phase and 321 in the finishing phase. One of the feedlots tested negative for STEC at all times whereas the remaining feedlots had prevalence rates ranging from 1.9 to 4.3%. Prevalence rate of STEC was not affected by season (averaging 2.5%) but tended to decrease more ($P = 0.13$) during finishing than during growing (1.9 vs 3.1%). The STEC isolates belonged to 10 serotypes (O86:H19, O125:H19, O127:H19, O136:HUT [untypeable H antigen], O153:H- [nonmotile isolate], O165:HUT, OUT [untypeable O antigen]:H2, OUT:H20, OUT:H-, and OUT:HUT). Of these, two (OUT:H2 and OUT:H-) caused HUS and one (OUT:HUT) caused other human illnesses. Five of these STEC (O86:H19, O125:H19, O127:H19, O165:HUT, and OUT:H20) have not been reported previously in cattle and *E. coli* O157 isolates were not found in the cattle tested. Of the pre-harvest factors tested, low ($P < 0.05$) STEC prevalence rates were associated with heavier cattle, clean feed bunks, and increasing dietary forage from 10 to 15%. Identification and implementation of pre-harvest control measures appears to have the potential to decrease beef contamination with STEC.

P5-08 Tracking *Salmonella* Typhimurium ST1 from Contaminated Poultry Feed to a Cluster of Human Salmonellosis

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Microbiological risk assessments generally rely on cross-sectional surveys at individual steps in the food chain to provide data for modeling exposure which, combined with dose-response information, is used to estimate risks to consumers. Where possible, epidemiological data should be accumulated to validate such risk estimates. On this occasion, a contamination event involving *Salmonella* Typhimurium ST1 was specifically tracked through the food chain. The hazard was first detected in broiler chickens and trace back identified raw wheat as the source. The prevalence of *Salmonella* in broiler flocks increased from around 12% to 50% but ST1 cleared completely within 4 weeks. During this period, the prevalence of all salmonellae on broiler carcasses immediately after immersion chilling increased from the usual 5.5% to 23%, although this peak was limited to the two weeks during which flock contamination was greatest. ST1 was also detected on associated chilled poultry product from retail food outlets in the same region (50% whole birds, 25% portions; 78% < 9 MPN/rinse, all < log 1.7 MPN/rinse), peaking within three weeks of the onset of slaughter of the affected birds but not being detected after four weeks. Concurrently, notifications of human salmonellosis from which ST1 was isolated rose in the associated health district but then immediately decreased when the contaminated poultry product cleared from the market. This real-time study demonstrated that transient low level contamination of animal feeds can result in tightly time-bound clusters of human salmonellosis.

P5-09 Prevalence and Characterization of *Bacillus cereus* Isolated from Cereal Grains in Korea

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A total of 293 retail samples of cereal grains were examined for the presence of *Bacillus cereus*. Only 25% (73) of the samples were found to be contaminated with *B. cereus* by biochemical tests and 16S r-RNA PCR amplification. Among the 73 samples, 15 samples (20.5%) from brown rice, 23 (31.5%) from glutinous rice, 16 (22%) from barley, and 19 (26%) from job's tears were contaminated

with *B. cereus*. *Bacillus cereus* isolates obtained from a variety of cereal grains were screened for the presence of four pathogenic target genes (*nhe*A, *bce*T, *hbl*D-C, and *cyt*K) by PCR. Results showed that the *nhe*A gene had the highest detection ratio, (98.6%), followed by the *bce*T gene (93.1%), *hbl*D-C gene (83.6%) and *cyt*K gene (54.8%). Seventy-three select isolates were tested for enterotoxin production by the reverse passive latex agglutination (RPLA) test. Positive results were obtained for 83.6% of the isolates. Additionally, we found that 37 strains from the total of 76 *B. cereus* isolates contained 4 different types of toxins. The isolates were further examined for drug resistance patterns. All of isolates were very susceptible to most of the antibiotics tested, but they were highly resistant to ampicillin, cefepine, oxacillin and penicillin. The genetic relationships of *B. cereus* isolates were analyzed using the BioGen program, which grouped them into 38 clusters and showed less than 0.5 similarity among strains. From these results, *B. cereus* isolates did not show significant genetic homology.

P5-10 Predictive Modeling on the Growth of *Bacillus cereus* in Various Cereal Grains

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We present a predictive model for the growth of *Bacillus cereus* on various cereal grains (brown rice, barley, glutinous rice, and job's tear) at various temperatures (5°–40°C). A cocktail of *B. cereus* strain (ATCC 12480, 13061, 14579) did not grow at below 10°C, grew slowly at 15°C, and rapidly above 20°C. The experimental growth data was fitted with the Gompertz equation. Specific growth rate (SGR) and lag time (LT) was obtained using GraphPad prism program. To assess the effect of temperature on the growth of *Bacillus cereus*, a square root model was chosen and was constructed using SAS program. Result showed that LT was less effective in predicting the growth kinetics of *B. cereus*, thus modeling was performed only for SGR. The model was validated using statistical indicators, such as mean square error (MSE), bias factor (BF) and accuracy factor (AF) between the experimental values and predicted values. MSE obtained for various cereals was in the range of 0.0007 to 0.00477, while BF for brown rice, barley, glutinous rice and job's tear was 0.972, 0.9436, 1.37, and 0.906, respectively, and AF was 1.058, 1.060, 1.37, and 1.132, respectively. Results showed that the relationship between the induced data and the experimental observed data had high fitness. The correlation coefficient ($r^2 \geq 0.9564$) for the developed secondary models indicated that the model had good predictive ability. Thus, these results indicate that the developed model could be used to predict the growth of the *B. cereus* in various cereal grains for simulated temperature conditions.

P5-11 Fate of *Listeria monocytogenes* in Minas Frescal Cheese

DSC

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Soft cheeses are a well known cause of listeriosis outbreaks. The so called "queso blanco" or "Latin-style fresh cheese" represents a heterogeneous group of white, unripened soft cheese produced and consumed in different Latin America countries. Minas frescal cheese is the Brazilian representative of this group and it can be produced by different technologies. Several studies have shown that *Listeria monocytogenes* (Lm) occurrence in Minas cheese is highly variable, while a high population of fecal coliforms (>10^4/g) is very common. The objective of this study was to evaluate the fate of Lm in Minas frescal cheese produced by direct acidification of the milk with lactic acid. Pasteurized milk was spiked with *Listeria monocytogenes* (10^6 CFU/g) and coliforms (10^7 CFU/g) and cheeses were prepared in the laboratory following regular commercial procedures. The cheeses packed in polyethylene bags were divided in 3 groups and each group was stored under one of the following conditions: up to 20 days at 5°C; up to 20 days at 12°C (abusive temperature); up to 8 days at 5°C/16 h followed by 25°C/8 h (to simulate open market conditions). At intervals of 5 days for cheeses stored at 5°C and 12°C, and every 2 days for the other group, triplicate samples were taken and *Listeria monocytogenes*, coliforms and lactic acid bacterial populations were determined using standard procedures. Sample pH and water activity were also measured. Cheeses prepared with milk spiked only with *Listeria monocytogenes* (10^6 CFU/g) or coliforms (10^7 CFU/g) were used as controls. Studies were duplicated four times. A trend in the inhibition of *Listeria monocytogenes* was observed in the cheeses when in the presence of coliforms (at least 1 log difference at any temperature). The values of pH and a_w had not been sufficiently low to cause this inhibition.

P5-12 Enterotoxin Production by *Bacillus cereus* Strains and Prevalence and Characterization of *Listeria* Species Isolated from Ricotta Cheese

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The objective of this work was to identify the presence of mesophilic and psycrotrophic *Bacillus cereus* using the recommended APHA method, and identify capacity for enterotoxin production as well as the contamination profile of *Listeria* species, using the Canadian Health Product and Food Branch recommended method, API *Listeria*-kit (bioMérieux) and PCR-RFLP based allelic analysis of virulence genes *act*A and *hly* in 45 samples of 15 different commercial ricotta cheeses. Mesophilic *B. cereus* counts varied in the range of 10^2–10^6 CFU/g in 53.3% (24/45), of samples while psycrotrophic *B. cereus* was presented in 2.2% (1/45) of the samples at 1.3×10^6 CFU/g. The potencial of enterotoxin production by 42 strains (39 mesophilic and 3 psycrotrophic) was investigated using BDE-VIA kit (Tecra) and PCR-based methods for *hbl*A, *hbl*D e *hbl*C genes and for *nheA*, *nheB* e *nheC* genes. Eleven different strain profiles using the molecular methods; 26.2% (11/42) of the isolates were positive for the three HBL encoding genes, 45.2% were positive for one or two genes and 28.6% were found; 80.9% were positive for the three NHE enconding genes and 19.1% for one or two genes. The results obtained with BDE-VIA kit revealed that 85.7% (36/42) of the isolates were NHE producers. *Listeria* sp. were detected in 20% (9/45) of the samples as a cause of *L. monocytogenes* in 6.7% (3/45), *L. grayi* in 2.2% (1/45), *L. innocua* in 13.3% (6/45), *L. seeligeri* in 2.2% (1/45) and *L. welshimeri* in 6.7% (3/45) was identified. The virulence genes *act*A type 4 and *hly* were identified in all of *L. monocytogenes* isolates, classified as lineage I. These results show the high enterotoxigenic potential of the *B. cereus* isolates and the risk of this product as a cause of food poisoning.

P5-13 *Enterobacter sakazakii* Infection in CD-1 Neonatal Mice

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Enterobacter sakazakii has been associated with nosocomial infections in premature and very low birth weight human infants. The affected infants were exposed to *E. sakazakii* when fed contaminated reconstituted powdered infant formula. To develop an animal model for *E. sakazakii*, experimental CD-1 suckling mice were challenged orally with 0.1 ml reconstituted powdered infant formula inoculated with 9 log or 11 log CFU *E. sakazakii* strain MNW2 on postnatal day 3. Deaths occurring immediately after or less than 15 h post-treatment were suspected to result from gavaging technique and were not included in the analysis. Twenty deaths occurred at least 15 h post-treatment and were assumed to result from *E. sakazakii* infection. The surviving mice were euthanized and weighed on postnatal day 10. Brains, ceca, and livers were excised and pooled into groups within each litter for culturing. *E. sakazakii* was isolated from brain, liver, and cecum tissues in animals treated with 11 log CFU as compared to only brain and liver tissues in neonates administered 9 log CFU. *E. sakazakii* was not found in control tissues. One out of three litters at 9 log CFU had neonatal deaths associated with *E. sakazakii* treatment whereas all litters (4/4) treated with 11 log CFU had at least three neonatal deaths. There was 17.8% (5/28) lethality among pups administered 9 log CFU and 34.8% (15/43) lethality among pups given 11 log CFU as compared to no deaths among control pups. *E. sakazakii* infection in neonatal mice may be similar to that in premature human neonates because of their underdeveloped CNS at full-term birth. Thus neonatal mice may potentially serve as a model for *E. sakazakii* infection in premature and very low birth weight human infants.

P5-14 Association of Autoinducer-2-like Activity with Heat and Acid Resistance of *Escherichia coli* O157:H7

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This study evaluated whether autoinducer-2 (AI-2)-like activity (quorum sensing molecule) may be involved in the heat and acid resistance of *Escherichia coli* O157:H7. AI-2-positive or -negative preconditioned media (PC-media; pH 7.0) were prepared from cell-free supernatants of *E. coli* O157:H7 86-24 (AI-2-positive) and VS-94 (AI-2-negative; LuxS mutant of *E. coli* 86-24) in Luria-Bertani (LB) plus 0.5% glucose broth. Heat challenge of the pathogens was conducted as follows; (i) *E. coli* O157:H7 ATCC43895 (AI-2-positive; 8.5 log CFU/ml) was exposed to AI-2-positive and negative PC-media (55°C, 4 h); and (ii) *E. coli* 86-24 and VS-94 were exposed to LB (52°C, 6 h). Acid resistance of *E. coli* O157:H7 ATCC43895 or VS-94 was determined in AI-2-positive and -negative PC, adjusted to pH 3.0 (35°C, 6 h). Survivors were determined by plating on tryptic soy agar and incubating at 35°C for 48 h. Death rates and fitted lines were obtained with the Baranyi model (heat) or linear regression (acid). Survival of *E. coli* ATCC43895 during heating was higher ($P < 0.05$) in AI-2-positive than AI-2-negative PC, and survival of *E. coli* 86-24 was higher ($P < 0.05$) than that of *E. coli* VS-94 in LB. In contrast, average death rates (-1.51 to -1.65 h-1) were not different in the presence or absence of AI-2-like activity, but fitted curves of *E. coli* O157:H7 showed a more obvious tailing effect in the presence of AI-2-like activity. *E. coli* ATCC43895 had higher ($P < 0.05$) survival in AI-2-positive than in AI-2-negative PC, and *E. coli* VS-94 had more ($P < 0.05$) survival in AI-2-positive than in AI-2-negative PC during acid challenge. Death rates of *E. coli* O157:H7 strains that were acid challenged were also slightly higher (0.2 log CFU/h) in the presence of AI-2-like activity. These results indicate that AI-2-like activity may be partially associated with heat and acid resistance in *E. coli* O157:H7.

P5-15 Challenge of Cooked and Packed Rice with *Clostridium botulinum* Spores Using Post-process and In-process Methods

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More than 87,000 tons/year of cooked and packed rice is consumed in Japan (2004 data). These products are often packaged with oxygen-absorbing agents and are distributed without refrigeration. No incident of botulinal poisoning has occurred due to these products. Some post-process challenge studies with *C. botulinum* spores have demonstrated growth and toxin production in these products. Post-process challenge, however, does not represent the actual contamination route for these products with *C. botulinum* spores; hence, we examined if toxin production would occur using a more realistic in-process challenge. Our post-process challenge was done using commercially packaged cooked rice, with spores of *C. botulinum* types A and B at inoculum levels of approximately 1,000 to 0.1 spore/gram of cooked rice. The in-process challenge was done at approximately 1,000 spores added per gram of rice-water mixture before cooking. The cooking was done at 100°C for 15 min followed by 105°C for an additional 15 min, simulating a commercial method. The post-process challenges produced toxic samples after 40 days to 9 months of 30°C incubation, with the rate of toxin production depending on inoculum sizes. The heaviest inoculation resulted in bacterial growth and toxin production within 40 days, but with the lowest inoculum, 0.1 spores/gram, no toxic samples were produced even after incubation for 9 months. With the in-process challenge, inoculated spores at 1,000 per gram became totally undetectable after cooking even with an enrichment, as a plate count method had failed to detect the presence of the organism. A three-month incubation failed to produce a single toxic sample, and *C. botulinum* remained undetectable. The results suggest that the packaged cooked rice product has a good degrees of safety, though incubation for longer periods is warranted to assure the ultimate safety.

P5-16 DSC Development and Validation of a Tertiary Model for Predicting Growth Kinetics of *Listeria monocytogenes* Scott A

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Tertiary models are used in the food industry to predict kinetics of pathogen growth and to help assess food safety. Therefore, we developed and validated a tertiary model for *L. monocytogenes* in broth. In this study, we used only the major parameters affecting the growth kinetics of *L. monocytogenes* in Ready-to-Eat meat products to develop this model, including temperature (4, 10, 17, 24, 30, 37°C), pH (5.5, 6.0, 6.5, 7.0) and lactate/diacetate mixtures (0, 0.3, 1.0, 1.8, 3.0). Kinetic data obtained were fit to the Baranyi model to determine initial density, lag time, specific growth rate, and maximum population density. Secondary models for lag time and specific

growth rate as a function of temperature, pH and lactate/diacetate mixtures were developed and combined with the primary model to create a tertiary model that predicted pathogen density at any combination of the described cultural conditions. Models were evaluated for performance using the prediction bias (Bf) and accuracy factors (Af), and the acceptable prediction zone method (%RE) for data used (dependent) and not used (independent) in model development. Percent RE in the acceptable prediction zone for the tertiary model was 82.1 for dependent data and 84.6 for independent data. The Bf of 1.06 and Af of 1.15 for dependent data and Bf of 1.04 and Af of 1.17 for independent data were obtained for the tertiary model, indicating fail-safe predictions for pathogen density. Overall, this study showed that the tertiary model provided acceptable predictions of *L. monocytogenes* growth in the matrix of conditions described, and can be used as a tool to estimate the impact of food formulation and storage conditions on the growth of *L. monocytogenes* in the retail market.

P5-17 A Dynamic Approach to Predicting Bacterial Inactivation during High Hydrostatic Pressure Treatment

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Recently, a number of studies have been published on the microbial inactivation kinetics of high hydrostatic pressure (HHP) treatment. The patterns of inactivation kinetics of microorganisms by HHP-treatment do not follow first-order kinetics as thermal inactivation does; they are generally nonlinear curves with tailing. In order to describe the non-linear kinetics of HHP-induced microbial inactivation, appropriate functions such as log-Logistic, Gompertz, or Weibull have been applied to the inactivation curve. In the present study, inactivation kinetics of *Escherichia coli* by high hydrostatic pressure treatment was examined from 200 MPa to 400 MPa in 50 MPa increments at 15°C. Although the time course of HHP-induced *E. coli* inactivation successfully fitted the Weibull function, this procedure involved fitting, and not prediction. The objective of this study was to develop a novel HHP-induced bacterial inactivation model in order to simulate the inactivation kinetics under unknown conditions. The maximum inactivation rate during HHP-treatment was calculated from the inactivation curves at different pressure conditions on a semi-log plot. The relationship between the square root of the absolute value of the inactivation rate and treatment pressure showed high linearity with $R^2 = 0.98$. The newly developed differential equation model that substituted the square root function of the inactivation rate was overall capable of simulating the inactivation kinetics during HHP-treatment at constant pressure. Furthermore, the new model could successfully simulate the inactivation kinetics during dynamic pressure conditions, which included come-up time, changes in holding pressure during treatment, and pressure-release time. Repeated pressure treatment was incapable of successfully simulating by use of the developed model. However, the modeling procedure presented in this study will contribute to the advancement of predictive modeling for HHP-induced microbial inactivation.

P5-18 A Novel Mathematical Modeling Approach to Determine Inactivation of *Listeria monocytogenes* F4244 and *Escherichia coli* O157:H7 C7927 Exposed to Gaseous Chlorine Dioxide

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The food industry is constantly looking for new techniques to rapidly detect pathogens and to evaluate efficacy of antimicrobial treatments. Our objective was to develop a rapid technique for enumerating surviving cells of *Listeria monocytogenes* and *E. coli* O157:H7 after exposure to chlorine dioxide (ClO_2) gas. Twenty microliters of 10^0-10^7 CFU/ml of LM and EC was inoculated into wells of a 96 well micro-titer plate. Plates were exposed to > 90% RH for 10 minutes prior to treatment with gaseous ClO_2 (100, 200, and 300 ppm for *Listeria monocytogenes*; 200, 300, and 400 ppm for *E. coli*). During treatment, plates were removed at pre-set time points (0, 2, 4, 6, 8, or 10 min) and neutralized immediately with 30 l neutralizing buffer followed by 50 l of a non-selective growth media. Plates were incubated at 37°C for 4 h to recover injured cells followed by addition of 100 l of 2X selective media. The plates were then incubated at 37°C and OD_{490nm} was measured hourly for 24 h. Non-linear mathematical models were used to model microbial growth and to correlate with surviving cells exposed to ClO_2 gas. An additional mathematical model was used to determine inactivation kinetics. Mathematically derived D-values for 100, 200, and 300 ppm ClO_2 for *Listeria monocytogenes* were 3.68 ± 0.04 min, 3.52 ± 0.11 min, and 1.87 ± 0.03 min, while *E. coli* D-values for 200, 300, and 400 ppm ClO_2 were 1.78 ± 0.09 min, 1.09 ± 0.004 min, and 0.91 ± 0.001 min, respectively. Inactivation kinetics for *Listeria monocytogenes* were higher ($P < 0.05$) than inactivation kinetics for *E. coli*, however, there was no difference ($P > 0.05$) for z-values, with *Listeria monocytogenes* giving a z-value of 678 ± 28 ppm ClO_2 and *E. coli* giving a z-value of 671 ± 44 ppm ClO_2. This approach of rapid enumeration, along with the use of mathematical modeling to describe microbial growth and inactivation, provides a useful tool to rapidly screen for antimicrobial effectiveness.

P5-19 DSC Comparison of Primary Predictive Models to Study the Growth Variability of *Listeria monocytogenes* Ribotypes at Low Temperature

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Previous studies have compared growth variability of *Listeria monocytogenes* strains using only a single primary model. This study evaluated the performance of four primary models to define the growth kinetics of *L. monocytogenes* ribotypes grown at low temperature to determine the best predictive model. Nineteen *L. monocytogenes* food or animal isolates with distinct ribotype or serotype were grown in tryptic soy broth at 4, 8, and 12°C with initial inoculation levels ≤ 10 CFU/ml. Separate regressions were performed on semi-logarithm growth curves to fit linear (Monod) and non-linear (Gompertz, Baranyi-Roberts, and Logistic) equations and determine model parameters. The lag times in the linear model were determined by the intercept of the regression line of the exponential phase with the line originating from initial concentration. Models were selected analyzing the residual scatter plot and residual sum of squares. The parameters of the best fitting model were used to select the fastest growing strains. On 57 growth curves (19 strains at 3 temperatures), the linear equation was a good fitting model for 93% of the growth curves, followed by Gompertz (70.1%), Baranyi-Roberts (52.6%) and Logistic (42.1%) models. Statistical comparison through F-test also is underway. The parameters from the linear model showed significant variation ($P < 0.05$) in maximum growth rates and lag times among the strains. Since strains with greatest maximum growth rate did not necessarily have the shortest lag time, the selection of the fastest strains was based on calculating the time to detect (TTD) 100 CFU/ml from 1 CFU/ml. The average TTD of the 19 strains ranged from 223 to 359 h, 72 to 99 h, and 48 to 56 h at 4°C, 8°C, and 12°C, respectively. These results indicated that a simple linear model could adequately describe bacterial growth and be used for selection of fastest growing organisms.

P5-20 Transfer Coefficients and Predictive Models for *Listeria monocytogenes* Transfer during Slicing of Deli Meats

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Contamination of deli meats with *Listeria monocytogenes* during retail handling has been identified as a serious public health concern, with delicatessen slicers and knife blades cited as major vectors for *Listeria* transfer. Based on previous work, a predictive model was developed for *Listeria* of transfer during slicing of deli meats with a mechanical delicatessen slicer and kitchen knives. Transfer coefficients were calculated for two different *Listeria* transfer scenarios – (a) inoculated blade to uninoculated product and (b) inoculated product to uninoculated product via an uninoculated blade. A mathematical model was then developed to predict numbers of *Listeria* transferred during slicing of roast turkey breast, salami, and bologna. The model and subsequent computer program in GWBasic are based on the following three assumptions: (1) the expected number of *Listeria* cells transferred during slicing is the fraction "f1" that describes the number of *Listeria* cells on the blade just before each sequential slice, (2) the expected number of *Listeria* cells transferred to surrounding areas is the fraction "f2" that describes how many cells are on the blade just before slicing, and (3) the number of *Listeria* cells on the blade available for transfer before any slicing begins is N0. High inoculation levels (10^8 and 10^5 CFU/blade) were similar for both observed values and values predicted using the model ($R^2 > 0.90$). At low-level inoculations (10^3 CFU/blade), variance between observed and predicted values was much greater (R^2 ~0.50), with the model approaching random distribution. Use of a single model for all transfer scenarios can be advantageous when discreet variables are unknown or are likely to significantly affect the outcome. After further improvements at the low inoculation level, this approach to modeling various scenarios of bacterial transfer should be beneficial in refining current risk assessments for deli meats sliced at retail.

P5-21 Modeling Hand Hygiene: The Influence of Biological and Psychological Factors on Illness Rate

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The objective of this study was to create a quantitative microbial risk model to compare the reduction in foodborne illness associated with various hand hygiene regimens employed in a food service setting. Data on the effectiveness of hand washing with an antimicrobial soap (MICRELL, GOJO Industries Inc., Akron, OH), sanitizing with an alcohol-based hand sanitizer (PURELL, GOJO), and a regimen of both interventions together were obtained under controlled laboratory conditions. Transfer of contamination from hands to food was based on previously published data. Dose response functions were obtained from the literature for *Salmonella*, *Escherichia coli* O157:H7 and Hepatitis A. A computer simulation model in Analytica (Lumina Decision Systems, Los Gatos, CA) was created to study the relationships between the factors listed above and illness rate. Average log reductions were 2, 2.5 and 3 from antimicrobial soap, hand sanitizer and combined regimens respectively. The illness rates associated with each regimen vary with compliance, but at the highest compliance rates, a 0.5 log increase in effectiveness can reduce illness rates by 20%. Hypothetically increased efficacy of 1 or 2 log reductions reduced illness more than 20 and 40 percent, respectively (assuming 100 percent compliance). The effect of the dose-response curve was quite profound: in some simulations illness rates were doubled or tripled, depending upon the agent and percent compliance. The model predicts that higher efficacy and higher compliance both serve to reduce risk. The results also show the importance of considering the dose-response curve together with the product efficacy, and the expected degree of compliance of any intervention when managing the risk of contaminated hands. Risk modeling appears to be a good method to assess the impact of hand hygiene products on illness in situations where traditional epidemiology approaches are not practical.

P5-22 Molecular Typing of *Staphylococcus aureus* Isolated from Food Handlers of Food Processing Plants

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Staphylococcus aureus is the principal cause of foodborne diseases in Colombia; there is no information available about the molecular types of the circulating strains present in food handlers, who are the most common cause of cross contamination. In this study we analyzed 26 *S. aureus* isolates obtained from hands, nose and throat swabs of food handlers from two processing plants, using RAPDs (Random Amplification of Polymorphic DNA) with the primer HLW-74 to determine the molecular types among these strains. The technique was standardized by use of a reference strain evaluating different concentrations of DNA, dNTPs, $MgCl_2$, Taq polymerase and amplification cycles. From the banding pattern, a binary matrix was constructed and a dendogram was obtained, using SYNTAX 2000, a hierarchical Clustering program. Two major lineages (I and II) were identified and the 26 isolates formed a total of 12 minor clusters. These isolates were grouped into four major clusters (Type A to type D) or 6 sub-clusters (Type 1 to type 6) based on the similarities among strong band patterns. All the nose isolates were contained by Lineage I; 5 isolates were Type A and 8 were Type B, while Lineage II was constituted exclusively by hand isolates. 100% of throat isolates were grouped within Lineage I, Type B, and isolates belonging to hands, throat and nose of the same person were classified within different types, whereas different strains obtained from the throat of the same food handler displayed an exact fingerprint. There was no correlation among types within a specific processing plant. The genetic diversity among the hand isolates was relatively low compared to that of nose and throat isolates. These results show that there exists heterogeneity among strains from the same person, suggesting that each food handler can hold different *S. aureus* strains in the nose, throat and hands.

P5-23 An In-home Investigation of the Conditions under Which Refrigerated Foods are Stored

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The consumer's role in food safety is critical because many foodborne illnesses are believed to occur from improperly home prepared or stored foods. Recommendations have been made for proper storage of refrigerated foods and leftovers. However, little research has been done in the home to see the actual storage conditions of cold foods. The objective of this project was to evaluate the temperatures of refrigerators, the length of time foods were stored, and the conditions of leftovers found in the refrigerator. In-home refrigerator inspections were conducted in 200 homes in 4 states by trained observers. A checklist was used that allowed the observer to record the condition of the refrigerator, presence of targeted foods, dates on containers, packaging, and other related information. Thermo loggers were placed in the refrigerator to record temperatures over time. Most foods in the refrigerators were

within a few days of the dates on the containers. However, several instances occurred where dates could not be located on the package, or products were present for more than a month after the date on the package. Most products were stored in their original package. Raw meat was most frequently found on the top or middle shelf. Spoiled or old foods were found in 27% of the refrigerators. Other foods were stored in the meat compartment with raw meats in 26% of the homes, and unsealed and opened containers and bags were found in 33% of the refrigerators, both of which could cause cross contamination. Leftovers were rarely dated (1.1% of 175 homes), and non-food items were found in 34.3% of the homes. Doors were warmer than other compartments. Mean temperatures for the bottom shelf, top shelf, and door were 38°, 35.5°, and 41.3°F, respectively. Thus, unsafe conditions for storage of cold foods did exist in many homes.

P5-24 Temperature Control of Meat during Transport and Retail Display

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Quantitative risk assessments addressing pathogens on meat depend on identifying stages in the farm-to-fork continuum where bacterial pathogens might be introduced and survive or multiply. In response to data gaps identified in New Zealand, a survey was undertaken to monitor temperatures of red meat and poultry during transport from slaughter facility to retail outlet and during subsequent storage and processing. The survey was conducted in two South Island and two North Island locations during winter and summer. Temperature data were gathered using a combination of data loggers and infrared thermometers. Results from the transport temperature study were examined in relation to the New Zealand Industry Standard for transport and storage (IS9). On the majority of occasions meat was transported in such a manner that the preservation temperature (7°C) of products was maintained throughout transport, even in the warmer summer conditions. All delivery vehicles surveyed were refrigerated with delivery times less than 3 h. Results from the survey of meats on retail display also indicated that temperatures were being well controlled, with only 1% of readings exceeding 13°C, which is the maximum display temperature allowed by the New Zealand Food Hygiene Regulations. Where both window display cases and non-window display were used to store meat, it was found that in most cases the temperatures were higher in window displays. Retail premises with food safety programs based on a hazard analysis critical control point (HACCP) system exhibited better temperature control than those that have not yet implemented such a control system. The temperature data provided will allow assessment of potential growth of pathogens if present on meat surfaces.

P5-25 Effect of Nisin-EDTA on Kinetics of Growth and Inhibition of *Listeria monocytogenes* and Mesophilic Aerobic Bacteria in Apple Cider

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Listeria monocytogenes is a significant food safety concern because this organism is widely distributed in the environment, can grow under refrigeration conditions, and has been frequently found in certain food processing establishments. Therefore *L. monocytogenes* can potentially contaminate liquid foods. In this study, we investigated the effect of pH (4–7) and nisin (200 to 300 IU) in 2 mM EDTA or 0.3% citric acid on growth parameters, including lag period (LP), generation time (GT) and growth rate (GR) of *L. monocytogenes* in tryptose soy broth (TSB) and apple cider. The growth data were analyzed and fitted to the modified Gompez model. LP, GT and GR in broth at pH 4 was 4.47 h, 1.05 h and 0.287 log (CFU/ml)/h, respectively. At pH 7 LP, GT, and GR was 0.587 h, 0.513 h and 1.933 log (CFU/ml)/h, respectively. A similar pattern of growth behavior was also observed in apple cider (pH 3.67). The addition of nisin in combination with EDTA and citric acid led to inhibition of *L. monocytogenes* in apple cider. Maximum growth for aerobic mesophilic bacteria in apple cider treated with nisin alone was slightly inhibited but was bactericidal when combined with EDTA and citric acid. A significant ($P < 0.05$) increase in the LP of *L. monocytogenes* in samples containing nisin-EDTA and nisin-citric acid combination was observed, and at > 6 h of exposure both treatments were bactericidal to the pathogen and aerobic mesophilic bacteria in apple cider. The results of this study suggest that a concentration of 250 IU nisin in combination with ETDA or citric acid can be used to inactivate *Listeria monocytogenes* in apple cider.

P5-26 The Survival of *Listeria* spp. on Poultry Skin in the Presence of Lactic Acid Bacteria

DSC

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There are specific sites on poultry skin that have physical structures suitable for the irreversible attachment of bacteria. A study was conducted to determine if two lactic acid bacteria, *Lactobacillus acidophilus* and *Lactobacillus plantarum*, are capable of lowering the recovery of *Listeria* spp. on poultry skin. Samples of poultry skin were inoculated with *Listeria monocytogenes* Scott A, *Listeria monocytogenes* Scott A +LAB, *L. innocua* , or *L. innocua* + LAB and stored at 4°C for 1, 3, 5, and 7 days. Poultry skin samples were rinsed in sterile deionized water to remove unattached bacteria, and the skin was stomached in peptone water for 60 s. The samples were spiral plated onto TSAYE and MOX agar and incubated for 18–24 h at 37°C. A reduction was seen with *L. plantarum* + *L. monocytogenes* on days 1, 3, and 7 which resulted in a 0.43, 0.24, and 0.79 log reduction, respectively. The addition of LAB to *L. innocua* showed that *L. plantarum* on days 1, 3, 5, and 7 caused a 0.92, 0.49, 0.51, 1.75 log-reduction, respectively. *L. acidophilus* was not as effective as *L. plantarum* in lowering the recovery of both *L. monocytogenes* and *L. innocua*. Results indicate that the addition of select LAB may be an effective way of reducing the recovery of *Listeria* spp. on poultry skin during storage at 4°C for up to 7 d. The addition of *L. plantarum* as a competitive inhibitor may be an effective way of controlling *Listeria* spp.

P5-27 Efficacy of Lactic Acid Alone or Combined with Sodium Lauryl Sulfate for Control of *Listeria monocytogenes* in Vacuum-packaged Frankfurters Made with or without Sodium Lactate

DSC

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The inhibition of *Listeria monocytogenes* by lactic acid (LA) and sodium lauryl sulfate (SLS) was evaluated in frankfurters containing 64% beef and 36% pork and made with or without 2% (w/w) sodium lactate (SL). The frankfurters were dipped for one or three min in solutions made from all combinations of 0 and 5% (v/v) LA and 0, 0.5 and 1% (w/v) SLS. The treated product was

surface-inoculated with 10^7 CFU of a five-strain mixture of *L. monocytogenes* per link, vacuum packaged and stored at 4.0°C for 90 days. Dipping in 5% LA or 1% SLS alone reduced initial numbers of the pathogen by 0.4 to 0.7 log. In contrast, the combination of 5% LA and 1% SLS reduced the bacterial population by 3.8 log CFU/link. Extending the dipping time from one to three min had no immediate inhibitory effect on the initial population of the pathogen, but some residual bacteriostatic effect occurred when the samples without SL were dipped in LA plus SLS (0.5 or 1.0%). No increase in inhibition of the pathogen occurred on frankfurters that contained SL. The Hunter values (L-, a- and b-), were not affected by the dipping solutions or SL and did not change during storage. The pH of the frankfurters was not changed by SL, but dipping in solutions that contained LA decreased pH of the product. The pH of the product prepared without SL decreased during storage but it was stable in frankfurters made with SL. These results show that using LA (5%) plus SLS (1%) on frankfurters formulated with SL controls *L. monocytogenes* in these products. Sensory evaluation is needed to investigate the effects, if any, of these treatments on quality.

P5-28 Effect of Antimicrobials, Point of Inoculation and Home Storage Conditions on *Listeria monocytogenes* Growth on Commercial Uncured Turkey Breast

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Contamination of Ready-to-Eat (RTE) meat and poultry products with *Listeria monocytogenes* is an ongoing public health concern and may occur at all segments of the food chain. Growth of *L. monocytogenes* on uncured Ready-to-Eat turkey breast was evaluated under conditions simulating two contamination scenarios. Product with potassium lactate-sodium diacetate and control product was sliced and inoculated (to simulate contamination during manufacturing) with a 10-strain mixture of *L. monocytogenes* (1–2 log CFU/cm^2), vacuum-packaged, and stored at 4°C (First-Shelf-Life; FSL). At 5, 15, 25 and 50 days, packages were opened, slices were tested, and packages with remaining slices were folded using rubber bands. In addition, at these time intervals, original vacuum-stored (4°C) product was sliced, inoculated as above, and packaged in delicatessen bags simulating contamination at retail or home (Second-Shelf-Life; SSL). Both sets of bags were maintained at ambient temperature (25°C) for 90 min (simulating temperature abuse), and then stored at 7°C for 12 days ("home storage"). Microbiological analyses indicated that antimicrobials inhibited ($P < 0.05$) growth of *L. monocytogenes* during FSL (4°C) compared to the control, with populations after 50 days reaching 5.3 and 7.2 log CFU/cm^2, respectively. During SSL, average growth rates (log CFU/cm^2/d) varied from 0.14 to 0.16 and 0.25 to 0.51 for products with and without antimicrobials, respectively; growth rates in product without antimicrobials decreased ($P < 0.05$) with age of the product. FSL inoculation of product without antimicrobials and SSL inoculation of product with antimicrobials were generally associated with the highest and lowest pathogen levels during "home storage", respectively. Irrespective of contamination scenario, however, 12-day "home storage" could result in pathogen levels of 7.3–7.7 log CFU/cm^2 in product without antimicrobials. These findings emphasize the importance of controlling *L. monocytogenes* throughout the food chain, and can be useful in establishing risk-based consume-by date labels for refrigerated RTE meat and poultry products.

P5-29 Antimicrobial Activity of Lauric Arginate and Benzoic Acid against *Listeria monocytogenes* and *Escherichia coli* O157:H7

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Lauric arginate (LA), a GRAS cationic surfactant, inhibits both Gram negative and Gram-positive bacteria. Benzoic acid is a lipophilic weak acid used in many acidified beverage systems to inhibit growth of pathogenic and spoilage organisms. Growth inhibition of five strains of *Listeria monocytogenes* and *Escherichia coli* O157:H7 by a combination of lauric arginate (0–64 ppm) and benzoic acid (0–9000 ppm for *L. monocytogenes* and 0–1000 ppm for *E. coli*) at pH 5, 6 and 7 was investigated. Microorganisms were cultured at 32°C in TSB and growth in the presence of antimicrobials monitored over 48 h using a microbroth dilution assay. Growth in the absence of antimicrobials was generally lower at reduced pH regardless of organism. Growth inhibition of *L. monocytogenes* and *E. coli* O157:H7 in the presence of combinations of LA and benzoic acid was strongly pH dependent. Compounds inhibited growth at pH 5, while no inhibition was observed at pH 7. At pH 5, benzoic acid accounted for most of the growth inhibition. LA inhibited *L. monocytogenes* at concentrations > 64 ppm but had no effect against *E. coli* at this concentration. Results showed that combining benzoic acid and LA did not lead to a synergistic effect. We suggest that lack of inhibition at pH 7 is due to pH-induced charge neutralization and may be aggravated by complexation due to interaction between compounds. The pH of aqueous solutions of 64 ppm was 4.84; upon addition of benzoic acid, the pH gradually increased to reach 7 at a benzoic acid concentration of 9000 ppm. Isothermal titration calorimetry experiments confirmed direct interaction between the two compounds. Our results suggest that compounds may be used in combination at low pH to inhibit growth of microorganisms but that combination of compounds at higher pH may lead to charge neutralization and complete loss of the antimicrobial activity of both compounds.

P5-30 Formation of Mixed Micelles Improves Antimicrobial Activity of Lauric Arginate against *Listeria monocytogenes* and *Escherichia coli* O157:H7 at Elevated pH

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Lauric arginate (LA) is a GRAS cationic surfactant with broad spectrum antimicrobial activity. However, LA has been shown to rapidly lose solubility and antimicrobial activity at elevated pH (> 5). The objective of this study was to determine whether a combination of LA with nonionic surfactants to form mixed micelles can overcome pH-related stability and antimicrobial activity problems. Growth inhibition of two strains of *Listeria monocytogenes* (101, Scott A) and *Escherichia coli* O157:H7 (932, 1730) by a mixture of LA (0–64ppm) and the nonionic surfactant Tween 20 (0-5wt%) at different pHs (5, 6 and 7) was investigated. Microorganisms were cultured at 32°C in TSB and growth monitored periodically over 48 h, using a microbroth dilution assay. Solubility was assessed by UV-spectroscopy. Addition of 64 ppm LA at pH 5 led to inhibition of all tested pathogens over a 24-h period but no inhibition of any

organism was observed at pH 6 and 7. Solubility decreased with increasing pH and LA precipitated from solution at pH 7. Addition of Tween 20 to LA resulted in significant improvements in both antimicrobial activity and physical stability. Combination of 5 wt% Tween 20 with 64 ppm LA inhibited all tested cultures at all tested pHs (5–7) and did not precipitate from solution at pH 7. Addition of Tween 20 lowered the minimum inhibitory concentration at a given pH, e.g., at pH 5, 16 ppm and 32 ppm LA with 5 wt% Tween 20 inhibited growth of both strains of *E. coli* O157:H7 and *L. monocytogenes*, respectively. Results were attributed to formation of a mixed micellar system composed of both LA and Tween 20 monomers less susceptible to charge neutralization that may occur at elevated pH. Our results show that combinations of lauric arginate with a nonionic surfactant to form a mixed micellar system can help maintain stability and antimicrobial activity at pH > 5.

P5-31 DSC Antimicrobial Efficacy of Cranberry or Grape Seed Extract Alone or Combined with Sodium Lauryl Sulfate against *Listeria monocytogenes* in Vacuum-packaged Frankfurters at 4°C

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A study was conducted to evaluate the antimicrobial efficacy of cranberry extract (CB) and grape seed extract (GS) against *Listeria monocytogenes* in refrigerated vacuum packaged frankfurters. Frankfurters formulated with or without 2% (w/w) sodium lactate (SL) were immersed (2.0 min) in CB (2% or 3% v/v) or GS (2% or 3% w/v) alone or combined with 1% (w/v) sodium lauryl sulfate (SLS). Frankfurters dipped in sterile distilled water served as control. After dipping, frankfurters were drained (15 s), placed in vacuum packaging bags, and inoculated with a 5-strain mixture of *L. monocytogenes* to give ~5 × 10^7 CFU/frankfurter. All samples were vacuumed packaged and stored at 4°C for 90 days. At set time intervals *L. monocytogenes* survivors were enumerated by washing the surface of frankfurters with sterile 0.1% peptone, plating aliquots (0.1-ml) of diluted wash solution on Modified Oxford Medium (MOX), and counting bacterial colonies on the agar after incubation (35°C, 48 h). CB (3%) and GS (3%) reduced initial numbers of *L. monocytogenes* by 0.7 and 4.0 log, respectively, on frankfurters without SL. The addition of 1% SLS enhanced their antimicrobial activity. CB (2 or 3%) + 1% SLS and GS (2 or 3%) + 1% SLS decreased initial numbers by ~ 4 and 6 log, respectively. Greater reductions in initial numbers of the pathogen were observed in frankfurters with SL. CB (2 or 3%) + 1% SLS and GS (2 or 3%) + 1% SLS produced reductions of ~ 5.0 and 7.0 log, respectively. While survivors grew in frankfurters without SL, no growth occurred in frankfurters that contained SL. Based on these results the combined application of CB or GS with SLS on the surface of frankfurters formulated with 2% SL) has good potential for destroying *L. monocytogenes* and inhibiting growth of survivors in these popular Ready-to-Eat meat products.

P5-32 Antimicrobial Effectiveness of Sodium Phytate against *Listeria monocytogenes* in Laboratory Media

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Phytic acid, which occurs naturally in most plant seeds, may be inhibitory to bacteria due to its ability to chelate polyvalent cations. This study evaluated the antimicrobial effectiveness of sodium phytate (SP) against 4 strains of *Listeria monocytogenes* at 10^3 CFU/ml in nutrient broth (NB) at pH 7.5 to 9.0. Minimum inhibitory concentrations (MICs) were determined at 72 h for stationary-phase cultures in NB (35°C) using the tube dilution method. Additionally, the effect of cations (Fe_3+, Mn_2+, Ca_2+, and Mg_2+) on growth inhibition of *L. monocytogenes* by 4.0% SP in tryptic soy agar (TSA) was evaluated using an agar diffusion method. Sterilized solutions (0.1 M) of $FeCl_3$, $MnCl_2$, $CaCl_2$ and $MgCl_2$ were added to wells in TSA + SP (pH 8.5) inoculated with *L. monocytogenes* to give ~ 10^8 CFU per plate. The diameter of the zone of growth around the well after incubation (35°C, 72 h) was measured to evaluate the extent to which each cation relieved growth inhibition by SP. The inhibitory effect of SP against *L. monocytogenes* increased with increasing pH. For three of the *L. monocytogenes* strains the MICs at pH 8.0, 8.5, and 9.0 ranged from 2.0 to 4.0%, 0.125 to 0.25%, and 0.06 to 0.50%, respectively. One *L. monocytogenes* strain (TS 218) grew in NB + SP at pH 7.5, 8.0 and 8.5; however, the MIC for this strain decreased dramatically at pH 9.0 (MIC = 0.50%). Fe3+ produced the largest zone of growth on TSA + SP. The decreasing order of effectiveness of cations in relieving inhibition of the pathogen was: Fe_3+ > Mg_2+ > Mn_2+ >Ca_2+. Based on these results SP seems to have good potential for use in controlling growth of *L. monocytogenes*. Further research is needed to evaluate the antilisterial effects of phytate in appropriate food systems.

P5-33 The Antilisterial Effects of Decanol in Ready-to-Eat Meat Products, Bologna and Country Ham

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The antilisterial effectiveness of decanol was determined for low fat (10%), high water activity (a_w 0.92) base bologna prepared in-house with the addition of only 2% sodium salts, compared to sliced commercial bologna (containing curing agents) and country cured ham (containing 4% sodium salts). Sliced samples were inoculated with a mixture of six *Listeria monocytogenes* strains for a final inoculum concentration of 10^7 CFU/cm^2. Decanol concentrations ranged from 0.1% to 10%. Samples were vacuum packed and stored at 4°C or 10°C for up to eighty days prior to microbial analysis. A decanol concentration of 5% in samples stored at 4°C was effective at reducing the *Listeria monocytogenes* population by three log units ($P < 0.05$). However, other treatments did not show a significant effect on *Listeria monocytogenes* population ($P > 0.05$), whether samplese were stored at 4°C or 10°C . On the other hand, the synergistic effect of decanol (0.5% and 5%) and curing agents (sodium phosphate, sodium acetate, sodium chloride, sodium nitrite, sodium diacetate, and sodium erythorbate) in commercial bologna had a significant effect on the *Listeria monocytogenes* population compared to the control, resulting in a five-log reduction ($P < 0.05$). The combination of decanol (5% and 10%), low water activity and sodium salts in country ham inhibited the *Listeria monocytogenes* population and resulting in a two-log reduction of *Listeria monocytogenes*. These results indicated that decanol, a natural compound, can be used in combination with other treatments in reducing and controlling *Listeria monocytogenes* in Ready-to-Eat meat products.

P5-34 Bactericidal Activity of Methanobactin Combined with Various Surfactants against *Listeria monocytogenes* Scott A

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Methanobactin is a novel extracellular chromopeptide produced by the methanotroph *Methylosinus trichosporium* OB3b, a nonfastidious bacterium important in global carbon cycling and single-cell protein production. This study investigated the effect of pH (5.75 to 6.25) and surfactants (Tween 20, Tween 80, and sodium lauryl sulfate) at 0.25 and 0.50% on the antimicrobial efficacy of copper-bound methanobactin against *Listeria monocytogenes* Scott A in brain heart infusion (BHI) broth. Minimum inhibitory concentrations (MICs) were determined at 24 h for stationary-phase *L. monocytogenes* cultures in BHI (32°C) using a broth microdilution method. Growth was monitored spectrophotometrically (595 nm) and viability after 24-h exposure was determined by surface plating samples onto BHI agar and enumerating colonies (72 h, 32°C). The MIC of methanobactin alone was 2.06 mM, regardless of pH. Tween surfactants, especially Tween 80, were antagonistic to methanobactin activity. This antagonistic effect was more pronounced with increasing pH and surfactant concentration. In contrast, growth did not occur in samples containing sodium lauryl sulfate (SLS) with or without methanobactin. Synergistic combinations of SLS (0.25%) and methanobactin (1.03 mM) at pH 5.75 reduced *L. monocytogenes* populations by 5.33-log cycles, whereas 2-fold higher concentrations of methanobactin (2.06 mM) without SLS were required to achieve this same reduction. Increasing the pH only slightly reduced the effectiveness of the SLS/methanobactin treatments. These results clearly demonstrate that surfactants, depending on type, can have an antagonistic or synergistic effect on methanobactin activity. A combination of SLS with methanobactin showed improved listericidal activity, demonstrating that this system has good potential for use in food applications.

P5-35 Combined Effectiveness of Lactic Acid and Sodium Lauryl Sulfate in Destroying *Salmonella* Enteritidis, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Raw Whole Almonds

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A study was conducted to determine the combined effectiveness of lactic acid (LA) and sodium lauryl sulfate (SLS) for inactivating enteric pathogens on raw whole almonds. Almonds (5 kernels per sample) were inoculated with *Salmonella* Enteritidis, *Escherichia coli* O157:H7 or *Listeria monocytogenes* to obtain 8.0 log CFU/sample. Samples were air-dried at 23°C for 24 h then dipped (2 min) in distilled water (H_2O), 5% (v/v) LA, 2% (w/v) SLS, or 5% LA + 2% SLS at 22°C. Inoculated almonds that were not immersed in H_2O or chemical solution served as control. Bacterial survivors were determined by washing almonds in buffered peptone water and plating the diluted wash solution on tryptic soy agar containing 0.6% yeast extract. Inoculated agar plates were incubated (35°C) and bacterial colonies were counted at 48 h. Numbers of viable pathogens on almonds ranged from 6.29 to 6.81 log CFU/sample after drying and before treatment. Reductions in numbers after dipping of almonds in H_2O ranged from 0.58 to 0.69 log. LA (5%) reduced numbers of *S.* Enteritidis, *E. coli* O157:H7 and *L. monocytogenes* by 1.18, 1.15, and 1.47 log, respectively. Log reductions after dipping in SLS were 1.09 (*S.* Enteritidis), 1.20 (*E. coli* O157:H7) and 1.77 (*L. monocytogenes*). In contrast, 5% LA + 2% SLS reduced *S.* Enteritidis, *E. coli* O157:H7 and *L. monocytogenes* by 3.54, 3.44, and 4.61 log, respectively. Based on these results the combined application of 5% LA and 2% SLS has good potential for destroying pathogens on raw almonds.

P5-36 Reduction of *Bacillus cereus* in Cooked Rice Treated with Sanitizers and Disinfectants

DSC

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This study aimed to identify effective washing and sanitation programs to minimize the contamination of cooked rice by *B. cereus*. The effectiveness of five sanitizers, including QAC, alcohol, chlorine, CaO, and H_2O_2, was evaluated in relation to the survivability of *B. cereus* spores in cooked rice and resulting sensory properties of the rice. The water-treated cooked rice contained remaining *B. cereus* spores at 1.09 log CFU/g. In contrast, treatment with the minimum inhibitory concentrations of the sanitizers, such as 200 ppm of QAC, 50% of alcohol, 100 ppm of chlorine, 650 ppm of CaO, and 500 ppm of H_2O_2, decreased the spores in the cooked rice to a level below the detection limit (< 0.15 CFU/g). The sensory properties of the sanitizer-treated (1,000 ppm of H_2O_2, 100 ppm of chlorine, and 800 ppm of CaO) cooked rice did not differ significantly from those of the water-treated cooked rice. As a result, 500 ppm of H_2O_2, 650 ppm of CaO, and 100 ppm of chlorine were found to effectively eliminate *B. cereus* spores during the cooking of rice.

P5-37 Effects and Interactions of pH and Water Activity (a_w) on the Thermal Resistance of *Listeria monocytogenes* F4258: Examining the Impact of Acid Adaptation

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Listeria monocytogenes F4258 was initially grown in acidogenic TSB + 1% glutamine + 1% glucose (TSBG+G) and non-acidogenic TSB + 1% glutamine + 0% glucose (TSBG-G) to produce cells that were and were not pre-adapted to a moderately acidic environment, respectively. The thermal resistance of the cultures was determined by transferring the cells to BHI adjusted to different combinations of pH (3.0–8.5) and a_w (0.960 – 0.987), then heating at 58°C for specified time periods, using a submerged coil heating apparatus. Survivor curves were determined by plating on BHIA (injured and non-injured cells) and VJA (non-injured cells only). The survivor data were fitted to a 2-phase log-linear inactivation model, and TL, D, and T4D values were calculated. Comparison of T4D values demonstrated that the microorganism was most thermally resistant over the range pH 5.0 to 7.0, with thermal resistance reduced substantially at pH values ≤ 4.5. Water activity had less impact. The acid adapted cells were more thermally resistant in the lower pH heating menstrua, while the non-adapted cells were more heat resistant in the pH 4.5–7.5 range. T4D values based on the BHIA data were typically 1.5 to 2.5 greater than T4D values based on the VJA data, indicating that the cells go through an injury phase before becoming non-viable.

P5-38 Fate of *Bacillus anthracis* (Sterne) in Pasteurized Whole Liquid Egg Stored at Different Temperatures and Cooked Using a Commercial Grill

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Commercial pasteurized whole liquid egg (WLE) was inoculated with heat-shocked (80°C, 10 min) or non-heat shocked spores of *Bacillus anthracis* (Sterne) (ca. 4.0 log spores/ml), stored at 4°, 15° or 25°C for up to 14 days, and cooked using a commercial "clam-shell" type grill set at 149°C. For each sampling interval, two 52-ml portions of WLE inoculated with heat-shocked or non-heat shocked spores were placed in an aluminum mold to form a WLE "patty" with standardized size (21.6 × 7.7 cm) and thickness (2.5 mm) and cooked for either 2, 5 or 8 min. The average internal temperature of the WLE during cooking was 92°C +/- 6°C. When inoculated WLE was stored at 4°C, pathogen numbers decreased by 0.5 log spores/ml (heat shocked) and 2.0 log spores/ml (non-heat shocked). In contrast, when inoculated WLE was stored at 15°C for 3 days or 25°C for 1 day, pathogen numbers increased by 3.0 log spores/ml (heat shocked) and 2.0 log spores/ml (non-heat shocked) and 0.2 log spores/ml (heat shocked) and 1.9 log spores/ml (non-heat shocked), respectively. When inoculated WLE was stored at 4°C and cooked for 2, 5 or 8 min, pathogen numbers decreased ca. 1.0 log spores/g for both heat shocked and non-heat shocked spore preparations. However, after storage at 15 or 25°C and cooking for up to 8 min, pathogen numbers decreased by ≥ 6.0 log spores/g for both heat-shocked and non-heat shocked spores. Thus, storage at abusive temperatures of 15 or 25°C allowed for germination and growth of *B. anthracis* in WLE and consequently much greater susceptibility to killing by a subsequent heat treatment. Storage of WLE at 4°C did not allow for spore germination. These data reinforce the need for additional research to lessen the potential risk due to contamination of WLE with a threat agent.

P5-39 The Thermal Resistance of *Yersinia pseudotuberculosis* in Apple and Orange Juice and Its Relationship to pH

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The effect of pH on the thermal resistance of *Yersinia pseudotuberculosis* was determined for five different pH values at five different temperatures, each measured using a submerged coil device. Thermal resistance was greatest at neutral pH and decreased as the pH decreased. The estimated D-values for phosphate buffer pH 7.0 at 61.8, 59.8, 57.8, 55.8 and 53.9°C were 0.25 ± 0.04, 0.54 ± 0.19, 2.57 ± 1.37, 9.22 ± 2.54, and 22.99 ± 4.54 min, respectively. The corresponding z-value at pH 7.0 was 3.75 ± 0.23. The thermal resistance at pH 6.0 and pH 8.0 was not significantly different from that found at pH 7.0. However, D- and z-values calculated for pH values < 6.0 were significantly lower. The D-value measured in buffer pH 4.0 at 57.8°C was only 0.16 ± 0.02 min. The z-value for the same conditions was 6.69 ± 1.13. In addition to the effect of pH, the thermal resistance of *Y. pseudotuberculosis* in single strength apple and orange juice was established using the same methods and found to coincide with thermal resistance in buffer at similar pH. However, *Y. pseudotuberculosis* in concentrated apple or orange juice was much less resistant to heat, indicating additional inactivation, most likely due to increased acid concentration.

P5-40 Effects and Interactions of Temperature, Sodium Lactate, Sodium Diacetate and Pediocin on Starved Cells of *Listeria monocytogenes*

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The effects and interactions of temperature (55–60°C), sodium lactate (SL; 0.0–4.8%), sodium diacetate (SDA; 0.0–0.4%) and pediocin (P; 0.0–10000 AU) on starved cells of *L. monocytogenes* inoculated on the surface of frankfurters were investigated and a predictive model was developed. A central composite design was chosen, with five levels of each parameter; twenty-five combinations were obtained. The organism was starved in Butterfield's phosphate buffer at 37°C for 24 h. The D-values, calculated using linear regression, ranged from 1.4 to 21.8 min. Among the parameters, temperature had the most prominent effect on inactivation of *L. monocytogenes*. Both SL and SDA showed protective effects at high temperatures. *L. monocytogenes* was inactivated more rapidly in the presence of SDA at lower temperatures; D-values decreased from 26.8 to 14.8 min. An increase in the D-value (26.8 to 33 min) with an increase of the pediocin concentration was observed at all heating temperatures. The interaction of pediocin with SL was significant. With the increase in pediocin concentration, the predicted D-value increased from 14.8 to 20.4 min. Similarly, with the increase in SL concentration, the predicted D-value increased from 14.8 to 23.8 min. The combined effect of SL and SDA on the predicted D-values was statistically significant ($P < 0.05$). Changes in the protein expression pattern of starved cells of *L. monocytogenes* inoculated on frankfurters having different concentrations of SL, SDA and pediocin at different temperatures were analyzed using 2-D PAGE. The synthesis of a number of proteins by starved cells of *L. monocytogenes* exposed to various temperatures was reduced compared to the control. Repressed proteins (26) and novel proteins (23) were more abundant than the amplified (9) proteins. Starvation rendered *L. monocytogenes* more susceptible to heat, and a number of genes were down regulated, as seen from the higher number of repressed proteins.

P5-41 Efficacy of Ultraviolet Light and Citric Acid to Reduce *Listeria monocytogenes* in Chill Brine

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Chill brines used during Ready-to-Eat meat processing may be an important source of post-processing contamination by *Listeria monocytogenes*. The purpose of this study was to determine the efficacy of ultraviolet light (UV) in combination with citric acid to reduce *L. monocytogenes* in chill brines. Fresh brine (60 L; 8.0% w/w NaCl) was inoculated with a *L. monocytogenes* cocktail (~6 log CFU/ml). Citric acid (0.1% w/w) was added to the inoculated brine and the solution was exposed to UV in an UltraViolet Water Treatment Unit (Model: AMD 150B/1/2T D; Aquionics Inc., Erlanger, KY; Peak output: 254 nm) fitted with an in-line chiller unit to maintain a brine temperature of -1°C during treatment. Samples were withdrawn at 0, 1, 5, 15, 30, 45, 60, 75, 90, 105 and 120 min during treatment, serially diluted in 0.1% peptone, and surface plated onto Modified Oxford Agar (MOX). When *L. monocytogenes* was no longer detectable via direct plating, enrichment was performed in Brain Heart Infusion Broth. Suspect colonies on MOX were confirmed by use of API *Listeria*. When treated with UV alone, *L. monocytogenes* populations decreased from ~6 log CFU/ml to below the

limit of detection (1 log CFU/ml) within 5 min. However, *L. monocytogenes* was detectable by enrichment through 120 min. When treated with citric acid alone (i.e., no UV), populations decreased to below the detection limit in 30 min, but were detectable by enrichment through 120 min. When treated with UV and citric acid, *L. monocytogenes* decreased to the detection limit in 5 min and was not detected by enrichment after 15 min. The results of this work indicates that combinations of UV and citric acid may be more effective than either treatment alone for the reduction of *L. monocytogenes* in chill brines.

P5-42 Effect of Modified Atmosphere Packaging on Irradiated Ground Beef

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The combination of antimicrobial treatments with gamma irradiation has been proposed as a way to increase the antimicrobial efficiency of antimicrobials while reducing required irradiation doses. This study used 3-strain mixtures of *Escherichia coli* and *Salmonella* spp. separately to evaluate the microbiological and quality changes in inoculated ground beef after 17 days storage at 4°C following gamma irradiation and combined with modified atmosphere packaging (MAP). Microbial reductions for *E. coli* inoculated to 7 log CFU/g in ground beef irradiated to 1.2 kGy, 1.6 kGy, and 2.0 kGy were 2.0, 2.7, and 3.9 log CFU/g, respectively. Microbial reductions for *Salmonella* inoculated to 7 log CFU/g in ground beef irradiated to 1.2 kGy, 1.6 kGy, and 2.0 kGy were 3.1, 4.4, and 5.8 log CFU/g, respectively. The observed electron beam irradiation-induced microbial reductions for *Salmonella* and *E. coli* could be maintained without growth of surviving cells on ground beef when combined with modified atmospheres of 100% N_2, 30% CO_2:70% N_2, 30% CO_2:70% Ar, and 20% He:80% Ar followed by refrigerated storage (4°C) for up to 17 days. Declines in pathogen viabilities were apparent when irradiation doses exceeded 1.6 kGy. With higher dosage (above 1.6 kGy) greater lipid oxidation occurred for the Ar-packaged ground beef samples. It is not known to what extent 15–25 μg/g increases in oxidized lipid could influence ground beef sensory qualities. Digitally-recorded photographs of ground beef color during the refrigerated storage of irradiated and MAP beef did not show noticeable differences in color, except after the packaging film had been compromised. Microbial reductions were significant and ground beef quality during storage could be increased using MAP combinations.

P5-43 Differentiation of *Escherichia coli* O157:H7 Processing-resistant Isogenic Mutants Recovered from High-pressure Processed Apple Juice by Fourier-transform Infrared Spectroscopy

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Escherichia coli O157:H7 pressure-resistant mutants can develop by repeated exposure to high pressure processing (HPP). Emergence of such mutants may compromise the safety of food (e.g., fruit juices) treated with this technology. Methods to identify isogenic mutants are scarce. However, Fourier-transform infrared (FT-IR) spectroscopy could be used to identify microorganisms on the basis of unique, reproducible, biochemical fingerprints from major cellular components. The objective of this study was to identify and differentiate *E. coli* O157:H7 EDL-933 wild type and three pressure-resistant isogenic mutant, recovered from HPP-treated apple juice by FT-IR and multivariate analysis. The wild type (EDL-933) and mutant stains were streaked onto tryptose agar (TA) and incubated (37°C, 24 h). Single colonies (~10^8 CFU) resuspended in distilled water (10 μl) were placed onto multiple-bounce ZnSe crystals for attenuated total reflectance (ATR) analysis. Soft independent modeling of class analogy (SIMCA) models from derivatized infrared spectra, were created for each microorganism. For models validation, sterile apple juice samples (1 ml each) were individually spiked with each strain (~ 1.0×10^7 CFU/ml) and treated with high pressure (500 MPa for 5 min at 23 ± 2°C). Mutant strains were detectable (~ 4.0×10^1 CFU/ml) only in HPP-treated juice. Control samples were kept at atmospheric pressure (0.1 MPa at 23 ± 2°C). Treated and control samples were plated onto TA and incubated (37°C, 24 h), and isolated colonies were analyzed by FTIR for microbial identification, using the SIMCA models. SIMCA models, using the 1400-900 cm-1 spectral region, permitted the chemically-based differentiation, mainly in the 900-1100 cm-1 region, of EDL-933 and isogenic mutant strains, presumably corresponding to the cell envelope's lipid and carbohydrate signals. FTIR analysis of recovered mutants and analysis in SIMCA models resulted in 100% correct microbial identification. In conclusion, FT-IR and multivariate analysis can be used for rapid differentiation and identification of *E. coli* O157:H7 HPP-resistant mutants recovered from apple juice.

P5-44 Inactivation of Barotolerant *Listeria monocytogenes* in Fat Emulsions by Tert-Butylhydroquinone and High-pressure Processing

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Persistence of *Listeria monocytogenes* in the food-processing environment may cause periodic contamination of Ready-to-Eat (RTE) meats during post-processing steps, in spite of conscientious sanitation programs. Thus, post-packaging pasteurization techniques such as high-pressure processing (HPP) are receiving greater attention by the food industry and are beginning to be employed commercially. Despite its benefits, HPP also has limitations due to the resistance of some pathogenic strains to this process. In our previous studies, combining a food-grade additive, tert-butylhydroquinone (TBHQ), with HPP was successful in eradicating *L. monocytogenes* in buffers and some foods. In this study, the efficacy of TBHQ in enhancing the lethality of HPP was further investigated in two fat emulsions that mimic low-fat (e.g., poultry deli meats) and high-fat (e.g., meat sausage) products. Two oil-in-water emulsions, containing soybean oil, buffer and some emulsifiers, were prepared with two levels of soybean oil (5% and 35%) to create low-fat and high-fat emulsions, respectively. Barotolerant *L. monocytogenes* OSY-328 was inoculated (at 10^7 CFU *Listeria*/ml) into the emulsions, and TBHQ (dissolved in propylene glycol) was added to achieve a final concentration of 200 ppm in the fat phase. Samples were pressurized at 400 MPa for 5 min at 25°C or 45°C. USDA enrichment procedures were used to detect the viability of the pathogen after the treatments. The TBHQ-HPP combination was clearly superior to HPP alone in eliminating *L. monocytogenes* in the emulsions. While percent *Listeria*-positive samples after HPP alone were as high as 93% at 25°C and 33% at 45°C, TBHQ-HPP treatment at both temperatures completely eliminated *Listeria* populations in the low-fat emulsion and in > 93% of the high-fat emulsion samples tested. These results suggest that addition of TBHQ to meat products, followed by pressurization, may significantly reduce *Listeria* risk in RTE meats.

P5-45 Use of Bromine Chemistry during Poultry Immersion Chilling (Post-chill Tank, Supplemental Chiller, and Combination of the Two)

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A critical step in a poultry HACCP food safety plan is reduction of bacteria following carcass chilling. The poultry industry continues to need improved and sustained reduction in post-chill *Salmonella* incidence (SAL), with multiple interventions required. Management of *Escherichia coli* (EC), Aerobic Plate Count (APC) and SAL incidence is essential in meeting future USDA post-chill carcass bacteria criteria. Five trials (n = 210 per treatment) were conducted to measure the effectiveness of bromine (Br_2) broad-spectrum antimicrobial either applied in final chiller or combined with post-chill, in controlling poultry carcass *Escherichia coli*, APC, and SAL bacteria. In both trials, poultry carcasses were spotted with approximately 3 logs each of EC and SAL bacteria. Following a 1-h drying period during which bacteria will adhere to skin, whole carcasses were treated with Br_2 solution either applied in the final chiller for 15 min or combined with post-chill for various contact times. Carcass rinses were plated and counted using standard microbiological techniques. Final chiller procedures were applied on each carcass at either 0, 4, 8, or 12 ppm "free" or active Br_2 addition during 15-min immersion chilling; in Trial 1, they were washed for either 0, 5, 25, or 50 s and, in Trial 2, for either 0, 15, 25, or 50 s (both trials used 80–82 ppm total Br_2), and dripped 10 s before rinsing. Results required to achieve < 10% SAL standard and acceptable bacteria counts (significance $P < 0.05$): (1) At least 12 ppm final chiller free Br_2 levels with at least 15-s contact time and (2) A combination of both final chiller Br_2, with < 12 ppm free Br_2, and post-chill wash in a multiple intervention approach. The conclusion is that either final immersion chiller Br_2 addition or a combination with post-chill wash will achieve the desired SAL goal. EC, APC, and shelf life (at least 4-day improvement over control) followed a similar pattern.

P5-46 Inactivation of Coccidian Parasites by Water Purification Chemicals and Treatment Device for Campers and Hikers

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Cryptosporidium parvum is a coccidian parasite that has been occasionally identified in surface waters. If campers or hikers ingest water directly from streams, reservoirs, or rivers, they might become ill from this parasite. Some purification tablets or devices sold in camping equipment stores make label claims that their products will inactivate *Cryptosporidium*. This study was undertaken to test such claims. Because *Cryptosporidium* is a human parasite and is hazardous to handle, we chose to work with a related coccidian parasite, *Eimeria acervulina*, a parasite of chicken, as a surrogate. Five different purification chemicals (two iodine, two chlorine dioxide, one mixed oxidants) and a battery operated ultraviolet light treatment device were tested. The test was based on the ability of *Eimeria acervulina* oocysts to sporulate. Treated oocysts will not sporulated if inactivated. Under the microscope, unsporulated oocysts appear as an undifferentiated mass while sporulated oocysts contain four sporocysts. Between 1×10^5 to 2×10^6 unsporulated oocysts in 250 ml of water were exposed to treatments, sporulated in 2.5% potassium dichromate at 29.5°C for a minimum of 20 h, and then counted microscopically as sporulated or unsporulated. Heated unsporulated oocysts at 80°C for 30 min served as negative controls, and positive controls (no treatment), were also used in every test. With exposures of up to 4 h, none of the chemicals completely inactivated oocysts. In contrast, exposure to ultraviolet light for one min appeared to be effective. We conclude that ultraviolet light, like heat, is an effective treatment against coccidian parasites in water for campers and hikers.

P5-47 Reduction of Foodborne Bacterial Pathogens by Silver/Zinc Antimicrobial Coatings on Stainless Steel

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Cross-contamination of food by contaminated food processing and domestic household surfaces is common. The foodborne bacterial pathogens *Salmonella*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* O157:H7 can readily be spread from contaminated meats to contact surfaces and may survive for periods ranging from days to weeks. These food processing and preparation surfaces may remain contaminated even after routine cleaning and disinfecting. Zeolite (sodium aluminosilicate) powder is comprised of a three-dimensional grid-like structure resulting in a network of pores running throughout the crystals. It has a strong affinity for metal ions that are able to reside within these structures. Zeolites act as ion exchangers, releasing metal ions into the environment in exchange for other cations. Bacterial test solutions were added to coated stainless steel pans [2.5% (wt/wt) silver, 14% (wt/wt) zinc ions] and uncoated stainless steel pans, to a depth of 1.3 cm. The experiments were conducted in triplicate. The pans were then covered and incubated with agitation at 37°C. Significant reductions ($P < 0.05$) were observed for *Salmonella* Typhimurium (> 4.7-log decrease), *E. coli* O157:H7 (3.9-log), *C. jejuni* (> 6.0-log) and *L. monocytogenes* (> 5.3 log) within 24 h in comparison to uncoated steel pans. In a separate set of triplicate experiments, significant reductions were also found with *L. monocytogenes* in the presence of beef extract on coated stainless steel panels within 4 h at 24°C (> 3.78-log) and within 24 h at 4°C (2.17-log). In contrast, the numbers of *L. monocytogenes* on uncoated stainless steel panels increased under these conditions. This report demonstrates that zeolite coatings containing silver and zinc ions impart antimicrobial properties to stainless steel surfaces. Such coatings might aid in the prevention of cross contamination of foods in food processing plants, restaurants and household kitchens, potentially reducing the incidence of foodborne diseases caused by these organisms.

P5-48 Bacteriocidal Effects of CaO (Scallop-shell Powder) on Foodborne Pathogenic Bacteria

DSC

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Bacteriocidal effects of calcium oxide (CaO) on microorganisms (*Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Typhimurium) in the conditions of broth and sesame leaf were evaluated. First, each bacterial reduction was determined in CaO solution (0.01, 0.03, 0.05, 0.1, 0.15, and 0.2% w/v) for either 15 s, 30 s, 1 min, 2 min, 3 min, 5 min, 10 min, or 30 min. The reduction of *E. coli* (99%; 2.78 log CFU/mL), *L. monocytogens* (45%; 1.44 log CFU/mL), and *S.* Typhimurium (70%; 2.08 log CFU/mL) was greatest in 0.05% CaO solution for 10 min compared to the other CaO concentrations and exposure times. Exposure to CaO provided 0.55–1.49,

0.85–2.56, 0.16–1.08, 0.30–1.14, and 0.19–1.07 log CFU/g reduction of total aerobic bacteria, total coliforms, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* Typhimurium, respectively, in sesame leaf. The results of this study provide evidence that CaO is potentially useful as a substitute for chemical synthetic substances in disinfection and sanitization.

P5-49 Antimicrobial Effects of Concrete Coated with Polyurethane Containing Different Concentrations of Copper Oxide against *Listeria monocytogenes* at Different Temperatures

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Our study evaluated the effectiveness of polyurethane containing copper oxide (PCO) coated on the surface of concrete in reducing *L. monocytogenes* counts. Copper oxide (CO) at concentrations of 20, 40, 60, 80 and 100% were added to polyurethane, and 1 ml of each mixture was spread over the surface of Petri dishes containing concrete. A decimally diluted overnight culture of *L. monocytogenes* (10 µl) was inoculated into Modified Welshimer Broth (10 ml), and poured over the surface of concrete samples or Petri dishes (control). The bacterial counts were determined at 0, 2, 4, 6, and 8 days at 4°C and for 0, 1, 2, and 3 days at 25 and 37°C. The broth was collected, decimally diluted and plated onto Oxford agar. Plates were incubated at 37°C for 48 h and CFU/ml determined. Our results showed that at 4°C *L. monocytogenes* counts on the surface of concrete coated with PCO were significantly reduced during the first 2 h by 1 to 2 log for all concentrations of CO. By day 8 the *L. monocytogenes* counts at 4°C were further reduced by 4.5 log with 80 and 100% CO compared to the control. At 25°C all PCO concentrations reduced *L. monocytogenes* counts to non-detectable levels by day 4. However, at 37°C *L. monocytogenes* counts were reduced to non-detectable levels for all concentrations of PCO on the surface of concrete by day 3. Our results indicated that concrete coated with PCO could possibly be used to control Lm in hard-to-clean areas of food processing plants.

P5-50 Survival of Stationary Phase and Acid-adapted *Escherichia coli* O157:H7 in Single Strength Lemon and Lime Juice

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Survival of *Escherichia coli* O157:H7 at room temperature (22°C) in single strength lemon and lime juices was evaluated. The juices contained no preservatives and had intrinsic pH values of 2.5–2.6 and titratable acidities of 4.51–4.53% (w/v citric acid). A higher than 5-log reduction of stationary phase cells was achieved in both lemon and lime juices after 72 h of incubation. Similar results were obtained when pH was adjusted to 2.7, a value above the highest value measured (pH 2.6) in plant, during the reconstitution of single strength lemon and lime juice from juice concentrates. Lemon juice seems to have a higher inhibitory activity than lime juice on *E. coli* O157:H7. Stationary phase *E. coli* O157:H7 survived better than acid adapted cells in the same experimental conditions. Storage of lemon and lime juice at room temperature (22°C) for three days may be an alternative option to heat treatment to ensure the required 5-log reduction of the vegetative pathogens in these products. Validation studies confirmed that more than 5-log reduction occurred in shelf-stable lemon and lime juice after 72 h of incubation at 22°C.

P5-51 DSC Antimicrobial Effects of Dehydrated Powder and Essential Oil of Clove and Cinnamon against *Salmonella* Enteritidis in Eggnog

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The antimicrobial effects of dehydrated powder and essential oil of clove and cinnamon were studied in two types of eggnogs (one with rum and wine and the other with milk and cream as principal constituents) stored at 4 and 8°C for 96 h. In eggnog with rum and wine, clove powder was added at 0.0025, 0.005, and 0.0075% concentrations and cinnamon powder was added at 0.0075 and 0.01% concentrations. In the case of milk and cream eggnog, clove and cinnamon powder were added at 0.01 and 0.015% concentrations. For essential oils, concentrations of clove and cinnamon used were 0.044, 0.055, and 0.033, 0.044%, respectively, in both eggnogs. Also, a combination of essential oils of clove and cinnamon was tried (0.02% clove plus 0.01% cinnamon, and vice versa). The lowest concentration used for both powder and essential oils was based on the minimum inhibitory concentration determined in earlier experiments. All eggnogs were inoculated with 10^4 CFU/ml *Salmonella* Enteritidis (SE) and sampling was done at 24 h for 4 days. There was a one log reduction in counts of SE in rum and wine eggnog samples, with or without spices, at both 4 and 8°C. In the case of milk and cream eggnog with powdered clove and cinnamon, there was a one log reduction each in counts of SE at 4°C, whereas in the case of essential oils, a one log reduction was seen only with cinnamon. However, at 8°C, a one log increase in counts of SE in control and in eggnog with powdered clove was observed. With powder and essential oil of cinnamon, a one log reduction was observed at both 4 and 8°C. Combination treatments were not effective. The results from this study show that these spices are limited in their ability to inactivate SE in eggnog, and different forms of spices may have differing effects on SE inactivation.

P5-52 Withdrawn

P5-53 Thymol, Carvacrol and Potassium Sorbate Combinations as Antimicrobial Agents

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The possibilities of formulating effective antimicrobial agent mixtures can be expanded by combining natural and traditional agents. Individual and combined effects of thymol (Th), carvacrol (Cr) and potassium sorbate (KS) concentrations on the growth of *Escherichia coli*, *Listeria innocua*, *Salmonella* Typhimurium, and *Staphylococcus aureus* were evaluated. Tripticase soy broth was prepared with NaCl and hydrochloric acid to reach a_w 0.99 and pH 5.5. The necessary amount of Th, Cr and/or KS (0, 15, 30, 45,

up to 240 ppm) was added, the broths were inoculated, (10^6 cells/mL) and incubated at 35°C. Optical density (OD) was measured every day for 7 days. Also, for those combinations in which no change in OD was detected after 7 days, 0.1 mL was spread plated over tripticase soy agar in order to determine bactericidal or bacteriostatic effects. Minimal inhibitory concentrations (MIC) for phenolic compounds (Th and Cr) and KS were determined, as well as the inhibitory concentrations for every antimicrobial combination. Fractional inhibitory concentrations (FIC) and FIC Index were then calculated. *S. aureus* and *L. innocua* exhibited higher resistance than *E. coli* and *S.* Typhimurium to the evaluated antimicrobials. MICs varied from 600 (KS) to 150 ppm (Th). Combinations of 50 ppm Th, 50 ppm Cr and 50 ppm KS inhibited the growth of the four tested bacteria, being synergistic, since calculated FIC indexes were lower than 0.7. Combinations of carvacrol, thymol and potassium sorbate drastically reduced individual inhibitory concentrations, and in several cases the observed effect was bactericidal. Combining agents from different sources may well increase the efficacy of natural antimicrobials in foods.

P5-54 Cinnamon, Orange and Grapefruit Essential Oil Vapors as Antimycotic Agents in Bread

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Vapors of essential oils are being evaluated as sources of natural antimicrobials. The effect of cinnamon (C), grapefruit peel (G) and orange peel (O) essential oil vapors (0.25, 0.50 or 1.00 mL/L of air) on *Aspergillus niger*, *Aspergillus flavus* and *Penicillium corylophilum* growth response were evaluated. Cinnamon sticks, dried grapefruit and orange peels were milled and water vapor distilled to obtain the essential oils. For every mold, PDA plates and a bread analogue (prepared under aseptic conditions) were inoculated (2 µL of a 10^6 spore/mL), incubated at 25°C in 5 liter hermetic chambers, and observed every third day for 14 days, with the colony diameter being measured. Increase in C, G or O extract concentration in the air significantly ($P < 0.05$) affected mold colony diameter. Minimal inhibitory concentration for each mold depended on the extract concentration, being higher for O, followed by G and C vapors. In PDA and bread, an atmosphere with 1.00 ml essential oil/L air had an important effect on mold growth; colony diameters were significantly ($P < 0.05$) lower than those obtained in the controls (air). In general, the antimycotic effect of the oils was higher in the bread analogue than in PDA. *A. flavus* was more resistant than the other molds. In PDA, *A. flavus* colony diameter was 90% lower than in the control when 0.50 mL C or G or O vapor/l air was used, whereas the other evaluated molds were completely inhibited. Therefore, C, G and O vapors are promising antifungal agents for foods, such as in some types of bread, where the essential oils have compatible flavor and odor.

P5-55 Evaluation of the Listericidal Effect of Oregano Essential Oil and Nisin in Fresh Pork Sausages

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Listeria monocytogenes is an important foodborne pathogen that can cause infections in humans, with mortality up to 30%. In Brazil, 80% of fresh pork sausages were shown to be *L. monocytogenes* positive. Essential oils from herbs, as well as bacteriocins, were shown to be effective antimicrobial agents, with potential applications in food systems. In a previous in vitro study, we demonstrated that the combination of 0.5% oregano essential oil and 200 ppm nisin was able to inhibit the growth of *L. monocytogenes* on agar plates, presenting a synergistic effect, i.e, the oil enhanced the activity of the bacteriocin. In this study, we evaluated the antilisterial effect of 0.5% oregano essential oil (Duas Rodas, BR) and 200 ppm nisin (Nisaplin, US) in fresh pork sausages prepared with deboned minced pork meat, 2% salt and 0.015% nitrite, plus spices, emulsifier and antioxidant, and experimentally contaminated with *L. monocytogenes* Scott A (10^6 CFU/g). The growth of the pathogen was monitored in the refrigerated product (5°C) up to 10 days by means of plate counting. Controls without antimicrobials were included in the experiments. Results indicated that oregano essential oil, used alone, was not effective. Nisin alone caused a 2-log reduction immediately after contact, but during storage the surviving cells showed the same multiplication rate as in the control (a0.5), keeping the counts 2 log lower up to 9 days. When used in combination, the two antimicrobials caused a 4-log reduction of counts immediately after addition and, compared to the control, the multiplication rate of the surviving cells during storage under refrigeration for up to 10 days was significantly lower (a0.5). However, samples containing these antimicrobials in the tested concentrations failed the sensory acceptance tests (a0.5). These results indicate that the combination of these antimicrobials can be an additional hurdle for the control of *L. monocytogenes* in fresh pork sausages, but the final sensory attributes of the product may hamper their application.

P5-56 Antimicrobial Properties of Phenolic Compounds from Sorghum

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Defatted bran fractions (DBF) prepared from a condensed tannin (CT) sorghum variety (red) and a condensed tannin-free (CTF) sorghum variety (white) were analyzed for their content of total phenols (TP) and condensed tannins (CT). TP were determined using the Folin-Ciocalteu method and CT with the vanillin-HCL method. Red sorghum bran contained more TP and CT (33.18 mg tannic acid equivalent (TAE)/g and 117.98 mg catechin equivalent (CE)/g of the bran fractions, respectively) than white sorghum bran (6.81 mg TAE/g and 8.52 mg CE/g of the bran fractions, respectively). Freeze-dried sorghum crude phenolic extracts (CPE) obtained from DBF of CT and CTF sorghum varieties were evaluated for their antimicrobial activities against *Bacillus cereus* ATCC 1178, *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 7644. The extracts were tested at 1, 2, 4 and 20% concentrations (w/v) in methanol, using the paper disc diffusion method. The CTF sorghum CPE at 1, 2 and 4% was effective against Gram-positive bacteria, *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644 at 20%. The CT sorghum CPE was effective against *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644 at 1, 2, 4 and 20%. None of the tested sorghum extracts inhibited the Gram-negative bacterium, *E. coli* ATCC 25922.

P5-57 Application of Allyl Isothiocyanate to Control *Escherichia coli* O157:H7 in Dry Fermented Sausages

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E. coli O157:H7 is able to survive the normal commercial process used for fermented sausage manufacture. The effectiveness against *E. coli* O157:H7 of a natural antimicrobial compound allyl isothiocyanate (AIT), added as a microencapsulated powder to dry fermented sausage was examined. Preliminary experiments tested the minimum inhibitory concentrations of AIT against the main starter cultures commercially used in the manufacture of dry sausages and against five different strains of *E. coli* O157:H7. Results showed that *E. coli* O157:H7 was more susceptible to AIT than the starter cultures. Preliminary work also showed that gum acacia was a suitable wall material for AIT microencapsulation. Pilot plant experiments were conducted to examine the inhibitory action of AIT microencapsulated in gum acacia against a five-strain cocktail of *E. coli* O157:H7 in dry sausages manufactured with two commercial starter cultures (*Pediococcus pentosaceus* and *Staphylococcus carnosus*). Bacteria were inoculated in four sausage batters to yield 6–7 log CFU/g of each type. Microencapsulated AIT was added (or not) to the sausage batter to give 0, 500, 750 and 1000 ppm. Sausages were fermented at < 26°C and 88% RH for 72 h and then dried at 75% RH and 13°C for at least 25 days. Water activity, pH, level of starter cultures and *E. coli* O157:H7 were monitored during fermentation and drying. The pH of all sausages successfully dropped to levels of 4.7–4.8 after 48 h fermentation. Sausages containing microencapsulated AIT showed a slight reduction in starter culture numbers after 28 days of processing. However, *E. coli* O157:H7 was reduced > 5 log CFU/g in sausages containing 500, 750 and 1000 ppm AIT after 40, 21 and 16 days of processing, respectively. Sensory evaluation of sausages without *E. coli* O157:H7 but treated with AIT showed that 500 ppm AIT was the most acceptable treatment and that these sausages might be marketed as a speciality product with a spicy flavor.

P5-58 Absolute Fx, a Natural Peptide-based Antimicrobial with Broad-spectrum Antimicrobial Activity

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A large number of compounds are available for use as disinfectants; however, some are highly toxic or corrosive and produce harmful by-products. Absolute Fx is a peptide-based antimicrobial containing three plant extracts with the combined properties of thionins, amino acid-enriched peptides and a new class of cationic peptides. It exhibits properties similar to those of quaternary ammonium compounds, but without their toxic characteristics. A quantitative suspension test was used to assess the efficacy of Absolute Fx against the foodborne pathogens *Listeria monocytogenes* (Gram-positive), *Salmonella* and *Escherichia coli* (Gram-negatives). Absolute Fx was also tested against the feline calicivirus strain F-9 that is commonly used as a human norovirus surrogate. After 30 s of exposure to a 1:400 dilution of Absolute Fx, a 6.25 log, 5.03 log and 5.87 log reductions were observed against *L. monocytogenes*, *Salmonella* and *E. coli*, respectively. At a concentration of 1:800, reductions of 6.25 log, 5.47 log, and 5.87 log were achieved. A 1 to 2-log reduction was observed with feline calicivirus within 10 min to 1 h of exposure. Absolute Fx therefore shows promise as a natural, non-toxic antimicrobial against a range of microorganisms, including both gram-positive and gram-negative bacteria and viruses.

P5-59 Enhancing Antimicrobial Activity of Lysozyme against *Listeria monocytogenes* Using Immuno-nanoparticles

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Listeria monocytogenes is a gram-positive pathogen frequently involved in outbreaks of foodborne disease. The natural (generally recognized as safe) and inexpensive qualities of lysozyme make it a widely used food preservative for controlling foodborne pathogens. However, its efficacy against pathogens may be reduced by the undesirable interactions with food components and non-target bacteria. Nanoparticles functionalized with pathogen-specific antibodies (immuno-nanoparticles) may serve as a carrier of natural antimicrobials to target the specific pathogen and improve the stability of antimicrobials in foods. The objective of this research was to study the antimicrobial activity of lysozyme adsorbed on immuno-nanoparticles against *L. monocytogenes* Scott A. Polystyrene nanoparticles with active carboxyl groups were conjugated with anti-*L. monocytogenes* through covalent bonding. Immuno-nanoparticles were then coated with lysozyme by direct adsorption. The antimicrobial activity of lysozyme adsorbed on immuno-nanoparticles was compared to that of free lysozyme in phosphate buffered saline. Several factors, such as the amount of anti-*L. monocytogenes* and lysozyme and the adsorption time, were optimized for the most efficient inhibition. A modified Lowry method was used to quantify the lysozyme adsorbed on immuno-nanoparticles. Nanoparticles were conjugated with 0.04 μg anti-*L. monocytogenes* per ml, and at that concentration, immuno-nanoparticles demonstrated enhanced antimicrobial activities of lysozyme. Lysozyme (35 μg/ml) adsorbed on immuno-nanoparticles reduced the *L. monocytogenes* Scott A population from 5.2 log CFU/ml to below the detection limit (< 1 log CFU/ml) in 3 h. However, when free lysozyme (50 μg/ml) was used, ca. 2.2 log CFU/ml of the *L. monocytogenes* Scott A remained culturable after 5-h treatment. Compared to 50 μg/ml of lysozyme, increased free lysozyme concentrations of up to 500 μg/ml had nearly identical inhibitory effects on *L. monocytogenes* Scott A. Our study revealed that the use of lysozyme-adsorbed immuno-nanoparticles is more efficient than direct addition of lysozyme in inhibiting *L. monocytogenes*.

P5-60 IgY as a Natural Food Preservative for Meat Safety

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Consumers demand safe and natural antimicrobial agents to replace those chemicals and antibiotics used in food preservation. The key objective of this paper was to examine natural ovo-antimicrobial components, egg yolk antibodies called IgY, as potential food preservatives that can prevent or inhibit microbial contamination in meat products. Twelve groups of chickens were immunized, using different inactivated bacteria such as *Bacillus cereus*, *Aeromonas hydrophila*, *E. coli* O157:H7, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Listeria monocytogenes*, *Campylobacter jejuni*, *Lactobacillus*, *Clostridium perfringens*, *Saccromyces cerevisae*, *Stapylococcus aureus* and *Staphylococcus epidermidis*. IgY activity in egg yolks were monitored by indirect ELISA during the immunization period.

Results showed that the level of specific IgY activity against each bacterium increased between week 2 and week 4 and thereafter remained relatively high. Eggs from each group were collected to prepare a specific anti-bacteria IgY preservative. Raw fat and lean pork mix patties and sausages were used to study the effect of each specific IgY preservative on the total growth of each bacterium. Seasoning, water, filler and specific IgY preservative (0%, 0.1%, 0.2%, 0.5%) were added. The mix was stuffed into natural hog casings, cooked in a smokehouse (slowly increasing the temperature from 40°C to 70°C) for 2 h and then stored. During storage, a sample from each treatment of pork patties and sausages was prepared, serially diluted and spread on 3M aerocount, coliform, and yeast/mold plates. The results showed that the addition of 0.5% specific anti-bacteria IgY preservative significantly ($P < 0.05$) reduced the aerobic bacteria count in comparison with no addition (control group) at all storage times. Therefore, the IgY preservative has the potential for use as a natural antimicrobial agent to prevent or inhibit the growth of aerobic bacteria in meat products during storage.

P5-61 Comparison of Lactate-diacetate and a Biopreservative for Control of *Listeria monocytogenes* on Vacuum-packaged Wieners

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Three separate batches of wieners were manufactured in a pilot plant, with or without added sodium lactate and sodium diacetate at a concentration that reflects current commercial application in the United States to restrict the growth of *Listeria* monocytogenes during storage at 4°C. Batches of wieners were also prepared with 1/2 the concentration of sodium lactate/diacetate mixture. The wieners were inoculated with a cocktail of approximately 1×10^3 *L. monocytogenes* per gram (including serotypes 1/2a, 1/2b, 3a and 4b) and the biopreservative Micocin™ (*Carnobacterium maltaromaticum* CB1) to give approximately 1×10^4 cells per gram. The *Listeria* were enumerated on PALCAM agar during a 10-week storage period, and if counts decreased below the minimum detection level, enrichment techniques were used to detect any surviving *Listeria*. In the control samples without the chemical or the biopreservative, *Listeria* increased to a population of 10^7 to 10^8 CFU/g. In contrast, in the presence of the chemical preservative, the numbers of *Listeria* remained at the inoculum level throughout storage. In the presence of Micocin,™ an initial decrease below the inoculum level was achieved and counts were maintained below the inoculum level throughout storage. However, when the combined treatment of the commercial or 1/2 concentration of sodium lactate/diacetate and Micocin™ was used, *Listeria* were generally not detected by enrichment methods throughout the 10-week storage life of the product. The combination of chemical and biopreservative antimicrobials could allow meat processors to implement Alternative 1 for control of *L. monocytogenes* in Ready-to-Eat meats.

P5-62 *Carnobacterium maltaromaticum* CB1 Preserves Sensory Quality of Raw Sausage and Prevents Growth of Inoculated *Listeria monocytogenes*

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Fresh pork sausages were manufactured on three occasions in a registered pilot plant facility, on the basis of a commercial formulation. *Carnobacterium maltaromaticum* CB1 was added to the sausage mix to give counts of approximately 1×10^4 CFU per gram. The sausages were aerobically packaged and frozen immediately after production. The product was later thawed and stored at 4°C for up to 20 days. Samples from three experiments were evaluated for appearance, odor and flavor attributes by a consumer panel and on two occasions by a trained sensory panel. Separate product was produced and inoculated with a cocktail of four strains of *Listeria monocytogenes*. The microbiology of the inoculated sausages was analyzed throughout the storage life. The presence of bacteriocins produced by CB1 was demonstrated in the stored sausages. In the initial trial, the sausages were inoculated at 10^3, 10^4, 10^5 and 10^6 CFU of CB1 per gram. At 10^5 and 10^6 CFU/g, rapid spoilage occurred, but at 10^3 and 10^4 CFU/g, the replicated studies showed that the product was still acceptable after 15 days of storage; in fact, both the consumer and the trained taste panels revealed significantly improved color stability (bloom) in the inoculated product. In this study, the adventitious microflora grew at a rate equivalent to that of the inoculated culture, and time to spoilage of the inoculated and uninoculated samples was the same. Separately, it was shown that after 15 days storage the *C. maltaromaticum* CB1 inoculum inhibited the growth (6 to 7 log) of a cocktail of *Listeria monocytogenes*. This research shows that *C. maltaromaticum* CB1 can be added as a biopreservative to a raw pork product without having a detrimental effect on the sensory quality, reducing the risk of *L. monocytogenes* growing to potentially hazardous levels.

P5-63 Isolation of *Bacillus subtilis* from Meju (Fermented Soybean Cake) and Its Effect on the Growth and Aflatoxin Production of *Aspergillus parasiticus*

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Bacillus subtilis is a predominant species of bacteria in Korean soy sauce, soybean paste, and meju (fermented soybean cake). This study was performed to investigate the possibility of an inhibitory effect of *B. subtilis* on the growth of and aflatoxin production by *Aspergillus parasiticus* in meju. All of the preparation methods for meju followed the recommendations of the Korea Food Research Institute. A *B. subtilis* strain was isolated from meju, and its effect on the growth and aflatoxin production of *A. parasiticus* ATCC 15517 was observed. Microorganisms were grown in a modified APT broth and incubated at 30°C for 12 days. Aflatoxins were determined by use of high performance liquid chromatography (HPLC). A remarkable inhibition of the growth of *A. parasiticus* was observed during the incubation period in the presence of *B. subtilis*. Dry mycelial weight was significantly reduced by 75% in comparison to the control at the end of the incubation period ($P < 0.05$). Lower levels of aflatoxin production by *A. parasiticus* were detected in the presence of *B. subtilis*. At the end of the incubation period, total aflatoxin production was significantly inhibited by more than 50% ($P < 0.05$). These results indicate that *B. subtilis* inhibits the growth and aflatoxin production of toxigenic *Aspergillus* in meju. Although its effect on aflatoxin production was less pronounced, we might expect more inhibition by other bacteria related to fermentation in meju.

P5-64 Antifungal Agents from Lactic Acid Bacteria

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Recently, increased public concern over chemical food additives has prompted the search for more natural antifungal agents. One source of these agents is lactic acid bacteria (LAB), which have been used to ferment foods for years and are known to positively influence the gastrointestinal tract of humans. Lactic acid bacteria produce various substances during lactic fermentation that are desirable for food flavor and texture and that may also inhibit undesirable microbial flora. Different mechanisms have been suggested for this antimicrobial activity, including competitive growth, metabolites, altered pH, or a combination of these factors. This research was designed to screen lactic acid bacteria for antifungal properties and elucidate the extent of inhibition of mold growth. The overall goal was to isolate food grade lactic acid bacteria from fermented dairy and plant foods and screen them for antifungal activity against selected spoilage molds. To accomplish this, an initial screening method to detect any antifungal activity of the isolates against *Fusarium graminearum* was developed, and those isolates that showed antifungal activity were further tested against several spoilage molds. Inhibition of mold growth was used as a measure of antifungal activity and was determined by measuring mold colony diameter in the presence and absence of the LAB over seven days. The spoilage molds used were *Alternaria alternata*, *Alternaria* sp., *Penicillium roqueforti*, *Penicillium* sp., *Cladosporium cladosporioides*, *Cladosporium* sp., *Phoma* sp., and *Aspergillus niger*. Among the LAB isolates, those obtained from fermented plant foods were stronger inhibitors of mold growth than those from dairy products, when tested against *Fusarium graimearum*. In subsequent studies using spoilage molds, *Alternaria* spp. and *Cladosporium* spp. were the most sensitive to the inhibitory activity. The most inhibitory LAB isolate, obtained from hot Kimchi, was able to completely prevent mold growth of all species tested, except *Aspergillus niger*, which grew slightly.

P5-65 Chitosan Protects Cooked Ground Beef and Turkey against *Clostridium perfringens* Spores during Chilling

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We investigated the inhibition of *Clostridium perfringens* spore germination and outgrowth by the biopolymer chitosan during abusive chilling of cooked ground beef (25% fat) and turkey (7% fat) obtained from a retail store. Chitosan was mixed into the thawed beef or turkey at concentrations of 0.5, 1.0, 2.0, or 3.0% (w/w) along with a heat-activated three-strain spore cocktail to obtain a final spore concentration of 2 to 3 log CFU/g. Samples (5 g) of the ground beef or turkey mixtures were then vacuum-packaged and cooked to 60°C for one hour in a temperature-controlled water bath. Thereafter, the products were cooled from 54.4°C to 7.2°C in 12, 15, 18 or 21 h, resulting in 4.21, 4.51, 5.03, and 4.70 log CFU/g increases, respectively, in *C. perfringens* populations in the ground beef control samples without chitosan. The corresponding increases for ground turkey were 5.27, 4.52, 5.11, and 5.38 log CFU/g. Addition of chitosan to beef or turkey resulted in concentration- and time-dependent decreases in the spore counts. At 3%, chitosan reduced by 4 to 5 log CFU/g *C. perfringens* spore germination and outgrowth ($P \leq 0.05$) during exponential cooling of the cooked beef or turkey in 12, 15, or 18 h. The reduction was significantly lower ($P < 0.05$) at a chilling time of 21 h, about 2 log CFU/g, i.e., 7.56 log CFU/g (unsupplemented) versus 5.59 log CFU/g (3% chitosan). The results suggest that incorporation of 3% chitosan into ground beef or turkey may reduce the potential risk of *C. perfringens* spore germination and outgrowth during abusive cooling from 54.4°C to 7.2°C in 12, 15 or 18 h.

P5-66 Complex Coacervation May Reduce Antimicrobial Activity of Chitosan

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Chitosan ([1->4]–2-amino-2-deoxy-beta-D-glucan), a positively charged polysaccharide, exhibits high antimicrobial activity against some strains of bacteria, fungi, and yeast when dispersed in acidic aqueous environments. The objective of this study was to investigate whether addition of the oppositely charged polysaccharide to chitosan, i.e., kappa-carrageenan, which yields mutual complexes, may reduce the antimicrobial activity of chitosan. Chitosan (0.1–0.7wt%; Mw=300kDa; degree of acetylation ~ 85%) was dispersed in TSB containing 0.5% acetic acid. Carrageenan (0.1–0.7wt%) was added and the pH adjusted to 4.5. Dispersions were heat sterilized, inoculated with two strains of *Listeria monocytogenes* (Scott A and 310) and *Salmonella* Typhimurium (2486 and 2576) to ~10^7 CFU/ml and incubated at 25°C. Cultures were enumerated after 12, 24, 48, 72, and 96 h by plating on standard methods agar and incubating for 24 h at 37°C and 32°C for *Salmonella* and *Listeria*, respectively. Controls consisted of TSB and TSB + 0.5wt% acetic acid. After 96h, counts of *Listeria* and *Salmonella* had increased by > 1 log in the TSB control and decreased by ~1 log in the presence of 0.5wt% acetic acid. Counts of both *Salmonella* strains and *Listeria monocytogenes* Scott A decreased by 4.5 – 5.5 log at the highest concentration of chitosan (0.7wt%), while counts of *Listeria monocytogenes* 310 declined by 3.5 log. Increasing concentrations of kappa-carrageenan led to reductions in antimicrobial activity of chitosan, and above a critical kappa-carrageenan concentration, chitosan antimicrobial activity was completely lost. For example, addition of 0.1, 0.2, 0.3 and 0.4wt% kappa-carrageenan to TSB plus acetic acid solutions containing 0.5 wt% chitosan reduced the initial number by 4.3, 2.4, 1.5, and 0.8 log, respectively. Results were explained in terms of "complex coacervation", whereby the functional acetyl groups of chitosan bind to carrageenan, rendering chitosan ineffective. Our study highlights the importance of product formulation when using antimicrobial agents such as chitosan to preserve foods.

P5-67 Activity of Bovine Lactoferrin against *Escherichia coli* O157:H7 Strains and Meat Starter Cultures in Broth and During Dry Sausage Manufacture following Its Microencapsulation

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Studies have shown the potential value of using lactoferrin (LF) as a natural antimicrobial in foods, but the presence of divalent cations limits its antimicrobial activity. The objectives of the present study were to assess the antimicrobial activity of LF against five non-pathogenic strains of *E. coli* O157:H7 as well as meat starter cultures in a broth system under conditions similar to those used in the production of dry fermented sausages. A second objective was to determine whether microencapsulation of LF could improve

reduction of *E. coli* O157:H7 viability during dry fermented sausage manufacture. The effect of LF alone or with different chelating agents on the growth of 5 strains of *E. coli* O157:H7 and 7 meat starter cultures was evaluated in LB or APT broth, respectively, containing 2.9% NaCl at 13 or 26°C. LF alone was bacteriostatic against *E. coli* O157:H7 strains 0627 and 0628, but other strains of the organism grew. Antimicrobial effectiveness of LF was enhanced by EDTA, but LF alone did not affect the growth of meat starter cultures in broth. However, when LF plus EDTA and sodium bicarbonate (SB) was used, the growth of all meat starter cultures except *Lactobacillus curvatus* was reduced. The resistant starter culture mixture was used to ferment sausages inoculated with the *E. coli* O157:H7 strains 0627 and 0628 containing microencapsulated LF (a paste-like and a dried powder form) with or without EDTA and SB, as well as unencapsulated LF. The reduction of *E. coli* O157:H7 was significantly higher ($P < 0.05$) in treatments containing LF, and the largest reduction (4.2 log) was obtained when unencapsulated LF was used. These results suggest that non-encapsulated LF can cause significant reductions in the viability of some strains of *E. coli* O157:H7 in fermented sausage and can enhance safety.

P5-68 Susceptibility of CDC Reactor Grown *Listeria monocytogenes* and *Escherichia coli* O157:H7 Biofilms to Eugenol and Carvacrol Encapsulated in Surfactant Micelles

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Listeria monocytogenes and *Escherichia coli* O157:H7, two major foodborne pathogens, are able to form biofilms on many food-contact surfaces, including stainless steel, one of the most common materials used in food processing. Once established, such biofilms may lead to cross contamination and post-processing contamination, thereby causing food spoilage and serious illnesses. A study was carried out to evaluate the effect on biofilms of a new antimicrobial system composed of surfactant essential oil components (0.9% Eugenol or 0.7% Carvacrol) encapsulated in surfactant micelles (5% Surfynol). *Listeria monocytogenes* (strain 101) and *Escherichia coli* O157:H7 (strain 932) were grown in a CDC Biofilm reactor at 32°C for 48 h. Biofilms on stainless steel chips were harvested and immersed in solutions of antimicrobial-containing micelles for 0, 2, 10 and 20 min. Biofilms were removed by scraping, cell clumps were disrupted by shaking (250 rpm) with glass beads (5 mm), and surviving cells were enumerated by plating. Exposure to carvacrol for two min reduced *E. coli* O157:H7 biofilms by 3.9 logs and *L. monocytogenes* biofilms by 2.7 logs. When exposed to eugenol micelles for two min, biofilms of *E. coli* O157:H7 were reduced by 3.6 logs and cells numbers within *L. monocytogenes* biofilms were reduced by 2.0 logs. Upon further exposure (10, 20 min), an additional cellular destruction of less than 1 log was observed for both organisms with both antimicrobial systems. Results showed that both antimicrobial systems were more effective against *E. coli* O157:H7 than *L. monocytogenes*. In addition, the majority of antimicrobial activity of the compounds occurred after a 2 min exposure and further incubation produced only a marginal decrease in cell numbers. We conclude that the delivery of carvacrol and eugenol in surfactant micelles may have applications as a food-grade disinfection agent for food-contact surfaces.

P5-69 Effect of Antimicrobials Eugenol and Carvacrol Encapsulated in Surfactant Micelles on *Listeria monocytogenes* and *Escherichia coli* O157:H7 Colony Biofilm Growth

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Listeria monocytogenes and *Escherichia coli* O157:H7 are important foodborne pathogens able to form biofilms by attaching to the surfaces of food or to food-contact surfaces. In this work, we investigated the antimicrobial effect of the encapsulated essential oil compounds carvacrol and eugenol upon *Listeria monocytogenes* (Scott A, 101, 108, and 310) and *Escherichia coli* O157:H7 (H1730, E0019, F4546, and 932) colony biofilm growth. Carvacrol and eugenol were encapsulated in aqueous micellar nonionic surfactant dispersions (Surfynol 485W) at concentrations ranging from 0.3 to 0.9% (v/v) at a surfactant concentration of 5% (v/v). Colony biofilms were grown on polycarbonate membranes resting on agar plates, and cells were enumerated after 0, 3, 6, 9, 24, 48 and 72 h of incubation. Results showed that *E. coli* O157:H7 strains were more sensitive than *L. monocytogenes* strains to both antimicrobials. *E. coli* O157:H7 cell numbers were reduced below detectable levels after exposure for ≥ 3 h to both antimicrobials at any concentration, except for *E. coli* O157:H7 strain F4546, which grew in the presence of 0.3% and 0.5% eugenol. *L. monocytogenes* Scott A and 101 were more resistant to eugenol, showing growth at 0.3 and 0.5% and cellular destruction at 0.9%, while all carvacrol concentrations tested led to a reduction of cell numbers below detectable levels. While carvacrol was equally effective in destruction of *L. monocytogenes* 108 at any concentration, this strain was able to grow in the presence of 0.3% eugenol. Cells of *L. monocytogenes* 310 were destroying by both essential oils. Results from model colony biofilm studies are a promising indication that surfactant-encapsulated lipophilic antimicrobials are able to not only reduce growth but also destroy bacterial cells.

P5-70 Efficacy of Acidified Sodium Chlorite against *Pseudomonas aeruginosa* and *Burkholderia cepacia* Attached to Conveyor Belt Surfaces

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Food defense concerns about accidental or intentional contamination of food contact surfaces underscore the need to assess the efficacy of promising disinfectants against threat agents or their surrogates, using in situ studies. Antibacterial activity of acidified sodium chlorite (ASC, Sanova™) against *Pseudomonas aeruginosa* (*Pa*) and *Burkholderia cepacia* (*Bc*) was evaluated on smooth ("new") and sanded ("worn") conveyor belt materials (polyethylene, polypropylene, acetal, polyester). Belt coupons were inoculated with 6 to 9 log CFU *Pa* or *Bc* in 1% fish slurry. Inoculum was spotted or spread over the coupon surface and allowed to dry for 1.5 h at room temperature or overnight at 4°C. Inoculated coupons were sprayed with PBS or with freshly prepared 100 to 2000 ppm ASC and allowed to stand for 2 min; they were then dipped in 0.1% sodium thiosulfate for 5 to 10 s. Treated coupons were placed in Whirlpak™ bags containing 25 ml wash buffer (PBS, 0.5% Tween 20, 0.1% glycine) and sonicated for 10 min. Surviving cells were enumerated on TSA-YE after overnight incubation at 37°C. The efficacy of ASC was similar on all belt materials. Treatment with 400 ppm ASC resulted in a 2-log decrease in numbers of *Pa* cells spread on new belt material. Spot-inoculated surfaces were difficult to disinfect; a 1-log decrease in numbers of *Pa* recovered was seen only after treatment with 1000 ppm ASC. Two thousand ppm ASC was required to

P5-24 Temperature Control of Meat during Transport and Retail Display — will be presented by Andrew Hudson, NZFSA, Institute of Environmental Science and Research, Christchurch, New Zealand

P5-52 Time Change (was P3-46) — A Preliminary Study of Environmental *Escherichia coli* Source Tracking by Microarray — WENDY MADUFF and Trevor Suslow, University of California-Davis, Davis, CA, USA

P5-73 Time Change (was P2-33) Thermal Inactivation of Newcastle Disease Virus (Ulster Strain) in Chicken Meat: Determination of Dt and Z Values – will be presented by David E. Swayne, USDA-Southeast Poultry Research Laboratory, Athens, GA, USA

WEDNESDAY AFTERNOON – AUGUST 16

S25 Hot Topics in Food Safety
Macleod D
Organizers: Jeffrey M. Farber and Stan Bailey
Convenors: Frank Yiannas and Gary R. Acuff

3:00 Presenter and Title Change — Food Safety Regulatory Issues: Getting Involved— Robert Buchanan, FDA-CFSAN, College Park, MD, USA