Program & Abstract Book

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Abstracts

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POSTER SESSIONS

*Presenter

P001 CLEANING PRACTICES AND THE CLEANLINESS OF FOOD SURFACES

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Cross contamination is increasingly being reported as a contributory factor in outbreaks of food poisoning with a recent UK study implicating it in 39% of general outbreaks. There is data to suggest this could be far higher for food prepared in the home and food service establishments. Prevention depends upon good hygiene practices where cleaning is an important component and effective cleaning has been shown to reduce the potential for cross contamination.

Over 600 food contact and environmental surfaces were assessed in 27 businesses across the food service, retail and manufacturing sectors. Cleanliness was assessed using ATP bioluminescence, microbiological methods and visual methods. Surface condition; cleaning systems, frequency, practices and products; as well as attitudes about cleaning were also assessed. The results were compared to previously published recommendations for clean surfaces.

Visual assessment was a poor indicator of cleanliness with overall 42% of surfaces considered unacceptable compared to 68% for ATP and 66% using microbiological methods. ATP results ranged from <100 to 500 000 RLUs. Microbial counts ranged from <2.5 to >250 CFU/cm^2. After cleaning, surfaces were quickly recontaminated with 71% considered unacceptable within one hour. The nature of the surface was an important determinant of cleanliness with smooth surfaces significantly cleaner ($P < 0.05$). Significantly higher ($P < 0.05$) microbiological counts were obtained from moist surfaces. Sites most likely to fail included a range of hand contact surfaces, including tap, door, and fridge handles which where omitted from most cleaning regimes. 89% of respondents agreed that clean surfaces present a good impression to customers, although 17% did not have any written cleaning instructions.

The results are discussed in relation to cross contamination and cleaning practices; an integrated cleaning monitoring program using ATP bioluminescence in conjunction with visual and microbiological assessments is recommended.

P002 EVALUATION OF HOUSEHOLD CUTTING BOARD CLEAN-UP TECHNIQUES

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Cross contamination is a frequently cited factor in foodborne illness outbreaks. Cutting boards are believed to be a significant source of cross contamination in the home due to the common practice of cutting salad ingredients on a contaminated board. Various surveys show a lack of consumer knowledge concerning cutting board safety. Cutting board cross contamination previously has been verified in consumer and commercial settings using marker bacteria (e.g., *Salmonella* and *Campylobacter*) found during household and commercial surveys. This study was designed to examine the dynamics of household cutting board cleanup. Various types of cutting boards were inoculated with hamburger meat containing approximately 10^8 CFU *Serratia marcescens*, *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes*, or *S. aureus*, and then were cleaned using a variety of methods. Baseline cleaning averages of approximately three log reductions were observed, with no significant differences seen among types of soap (regular versus antibacterial), board condition, cleaning/scrubbing tool, or the rinse water temperature (up to 140°F). Cleanability did vary according to board type, with acrylic being easier to clean than wood ($P < 0.05$). Wiping cutting boards with paper towels after washing resulted in a statistically significant improvement in hygiene when boards were washed imperfectly ($P < 0.05$). Overall, it appears that the maximization of cleaning efficiency comes with the drying of cutting boards and bacterial die-off between uses.

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P003 OZONE: AN ALTERNATIVE DISINFECTANT FOR THE FOOD INDUSTRY

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Within the food industry, disinfection is traditionally achieved by means of heat in the form of hot water or steam or chemicals such as chlorine. However, not only are there growing environmental concerns over the formation of chemical by-products when chlorine is used as a disinfectant but plasmids in some bacteria have been linked with resistance to other important biocides, such as the quaternary ammonium compounds. Such issues have resulted in an increasing interest in the use of alternative disinfectants.

The efficacy of gaseous ozone as a terminal disinfectant has been evaluated. A range of micro-organisms of importance to the food industry were inoculated onto stainless steel squares. Exposure of the contaminated surfaces to ozone resulted in a reduction in microbial viability which ranged, depending on organism type, from 7.56 to 2.41 log values. This represented a significantly greater ($P < 0.05$) reduction in microbial numbers than that observed in the absence of ozone. In the presence of organic material, a reduction in bacterial viability was observed that ranged from 5.64 to 1.65 log values. This reduction was significantly less ($P < 0.05$) than that achieved in the absence of residual food debris although still significantly greater ($P < 0.05$) than that observed in the absence of ozone. The implications of these findings will be discussed in relation to cleaning schedules in food plants.

Although carried out under laboratory conditions, these results suggest that ozone could be used as an effective terminal disinfectant. Its efficacy in reducing the numbers of bacteria present on surfaces coupled with its natural decomposition to oxygen — a non-toxic product — should make this method of disinfection popular within the food industry.

P004 REMOVAL OF MICROORGANISMS FROM INDUSTRIAL SURFACES USING PERACETIC ACID

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Both physical and chemical procedures can be used to remove microorganisms from surfaces. Among chemicals, peracetic acid (PAA) is frequently used in the food industry, but its effectiveness is not well known. We evaluated the efficiency of PAA in destroying *Escherichia coli*, *Staphylococcus aureus* and *Gordona* sp. (an actinomycetes), using two different methodologies: the AOAC suspension method and a method based on the removal of the microorganisms from an artificially contaminated stainless steel template. The surface roughness of the template was similar to that used in the industry. In suspension, D values for *E. coli* and *S. aureus* were 0.5 min and 1.8 min, respectively, using 40mg/L PAA. For *Gordona*, a much higher concentration of PAA was needed (900 mg/L PAA) and the D value was 2.4 min. When the microorganisms were on the surface of the template, we observed that the efficiency of the sanitizer was constant in the first 25 min, but increased afterwards. The contact time between microorganisms and the template also influenced the efficiency of the sanitizer. After 30 min contact between the surface and *S. aureus*, PAA, acting during 35 min, reduced the counts in 8.5 logs. However, when the contact time was increased to 6 h, only 6.4 logs reduction was achieved by the same amount of PAA for the same time of action. Therefore, the earlier PAA is applied to a contaminated surface, the more effective will be the sanitizing procedure.

P005 EFFICACY OF TWO SANITIZERS AGAINST FOOD SPOILAGE *BACILLUS* ISOLATES

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The efficacies of a quaternary ammonium compound containing (QACC) and a chlorine dioxide-containing (CDC) sanitizer against a *Bacillus* (B.) *cereus*-like (*Bacillus*5) isolate from an alkaline dairy wash solution and *B. subtilis* EL39, isolated from ropey bread were investigated in vitro. Cells of each isolate attached to stainless steel and planktonic cells sampled from flasks not containing stainless steel surfaces were tested. Cells were grown for 8 or 24 h in tryptone soya broth at 30°C and exposed to 500 ppm (recommended) and 1000 ppm of the CDC sanitizer, and 850 (recommended) and 1700 ppm of the QACC sanitizer for 1 or 10 min. Control cells were exposed to sterile distilled water instead of sanitizer. After neutralization, cells were dislodged where appropriate and enumerated by the droplet plate technique. Generally, attached cells of both isolates were more resistant to the two sanitizers than corresponding planktonic cells. Furthermore, attached cells of both isolates were more susceptible to both sanitizers after 8 h com-

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pared to 24 h, and attached *Bacillus* 5 cells were more resistant to the QACC sanitizer than *B. subtilis* EL39 cells. Both sanitizers were effective (5 log reduction in planktonic cells and 3 log reduction in attached cells) against *Bacillus* 5 and *B. subtilis* EL39 when double the recommended concentrations coupled with 10 min exposure times were used.

P006 EFFECTS OF CLEANERS OF BIOFOULED STAINLESS-STEEL SURFACES IN YOGURT MANUFACTURING EQUIPMENT

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Long processing times cause fouling on equipment surfaces, especially in plate heat exchangers, e.g., in yogurt manufacturing. The growth of harmful thermophilic bacteria in the fouled layers is referred to as biofouling. Bacteria may pass from the surfaces of the heat exchanger to the fermentation tank. These bacteria may prolong yogurt fermentation. Therefore, the correct processing time and cleaning methods should be specified to meet the quality claims of the final product and to keep processing costs as low as possible.

Harmful thermophilic bacteria were isolated from a process line from both hot- and cold-mixing equipment in yogurt manufacturing. Effects of different isolated thermophilic bacteria in yogurt fermentation were tested, and contaminated yogurt milk was burned onto stainless-steel coupons. The tests showed that the contaminants prolonged fermentation. Cleaning efficiency was tested using various cleaning agents in single- and two-phase CIP procedures. The testing equipment developed for burning milk onto steel surfaces and the testing procedure, including determination methods used, can be applied for choosing suitable cleaning agents, for example, for yogurt manufacturing equipment. The results showed that the two-phase cleaning procedure with an alkaline mixture containing chelating agents as well as nitric acid was the most efficient combination for cleaning of burned milk from stainless steel. The acidic treatment enhanced the cleaning result.

P007 INFLUENCE OF PROCESSING FLOW VELOCITY ON ATTACHMENT RATES OF *PSEUDOMONAS FLUORESCENS* ISOLATED FROM THE EGG INDUSTRY

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Pasteurized liquid eggs are sensitive products. Better control of shelf life is needed for extensive development of this market. This can be obtained by control of the pasteurization scales, but also by limiting fouling of the process lines, which causes over-contamination of circulating raw products. The aim of this work was to study the effect of flow velocity of liquid eggs during processing on the fouling of equipment surfaces.

Fouling was performed by circulating liquid whole egg in an industrial scale loop and in a laboratory loop. Three flow velocities were studied at 11°C : 0.13 m.s-1, 0.34 m.s-1 and 0.70 m.s-1, corresponding to industrial practices. Two types of surface materials were examined: stainless steel and Teflon™. Liquid whole egg was artificially contaminated with a strain of *Pseudomonas fluorescens* (10^7 CFU/ml) which was isolated from several pieces of equipment in an industrial processing line.

Results obtained show that flow velocity influences level of bacterial attachment despite the fact that the 3 flow velocities tested were given a laminar flow. Higher flow velocities gave lower numbers of attached bacteria. After 16 h, there were about 10^6 CFU per cm^2 for a 0.13 m.s-1 flow velocity and 10^5 CFU per cm^2 for a 0.70 m.s-1 flow velocity. Differences tend to become smaller after one week of fouling/rinsing/draining cycles. Teflon™ was 2 to 5 times more contaminated than stainless steel.

These results indicate that correct choice of processing parameters such as product flow velocity is of concern for control of bacterial attachment on equipment surfaces.

P008 COMPARATIVE BIOCIDAL CAPACITIES OF OXIDATIVE AND NON-OXIDATIVE SANITIZERS VS. *LISTERIA MONOCYTOGENES, ESCHERICHIA COLI* O157:H7, AND *SALMONELLA* TYPHIMURIUM USING A MODIFIED SURFACE-DRIED FILM ASSAY METHOD

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The increasing focus on contamination of food products with bacterial pathogens like *Listeria monocytogenes, Escherichia coli* O157: H7, and *Salmonella* Typhimurium have put increased emphasis on equipment sanitation by food processing markets. Consequently, the use and efficacy of sanitizers vs. surface dried food pathogens have been under increased scrutiny. USEPA regulations for a sanitizer dictate bactericidal efficacy vs. planktonic cells, but a more practical test model using surface dried bacterial pathogens provides the sanitarian with a more accurate assessment of the biocidal capacities of various EPA approved sanitizers.

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A modified version of the ISO-GRID HGMF Disinfectant Test compared oxidant and non-oxidant biocides using a 60-s contact vs. the above pathogens after they had been surface dried on the filters at a challenge of 6 $\log_{10}$ cycles. Biocides were tested at approved-use concentration levels as sanitizers and compared using $\log_{10}$ reduction vs. positive control filter titers. Against *Salmonella* Typhimurium, the following $\log_{10}$ reductions were determined: 5.4 for 50 ppm Iodophor, a 4.9 reduction for 200 ppm Quat, a 4.7 reduction for 200 ppm hypochlorite, while peracetic acid [VigorOx™] at 100 ppm had a 5.9 reduction. A competitor's specialty PAA product achieved a 6.0 log reduction. The oxidants also outperformed the non-oxidants vs. *Listeria*. Peracetic VigorOx™ at 100 ppm achieved a 6.0 log reduction, while the 50 ppm of Iodophor had a 4.1 log reduction, and the Quat at 200 ppm had a 4.9 log reduction. This screening tool can evaluate sanitation systems vs. dried cell or biofilm challenge models.

P009 ULTRASOUND CLEANING IN CHEESE MOLD HYGIENE

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The hygiene of cheese molds used in forming the cheese into the final product is important because any spoilage microbes in the molds are transferred from the surfaces into the product, causing shelf-life problems in the cheese. The structure of the parts used in cheese molds is often complex, with long, narrow conical channels. These channels are hard to clean with conventional cleaning procedures. Ultrasound cleaning procedures are more efficient than the conventional methods for cleaning these channels. The cleanliness of the cheese mold surfaces during processing was assessed using various methods. The dipslide technique proved to be the best method in detecting contaminants on these surfaces. The organic load in the cleaning water from the cheese mold cleaning equipment was measured using COD (chemical oxygen demand) values and EDTA (ethylenediaminetetraacetic acid) titration.

The results of these two methods showed similar patterns, in which the organic load in the cleaning water attained COD values of approx. 5000-6000 mg O_2/L in 5-6d. This organic load level was used in the pilot studies for ultrasound cleaning. The results showed that the cleaning agent containing lactic acid was the most effective agent for cleaning of soiled surfaces. An increase in the ultrasound intensity from 460W to 740W enhanced cleaning, especially in experiments using artificially aged cheese mold surfaces in water containing an organic load. Experiments with cleaning agent concentrations of 0.5%, 1.0% and 1.5% showed that the concentration should be maintained at least at 1.0% levels.

P010 EVALUATION OF CETYLPYRIDINIUM CHLORIDE IMMERSION AS A METHOD TO REDUCE PATHOGENIC BACTERIA IN FRESH VEGETABLES

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Fresh ready-to-eat vegetables require no further processing prior to consumption. However, several foodborne illnesses have been traced to the consumption of raw vegetables contaminated with *Listeria monocytogenes*, *Salmonella* spp. and/or *E. coli*. Methods to reduce or eliminate pathogenic bacteria from ready-to-eat vegetables need to be found. Cetylpyridinium chloride (CPC) is a quaternary ammonium compound which has been used in mouthwashes due to its high bactericidal potency. The efficacy of CPC immersion to reduce three pathogenic bacteria (*L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7) was studied in our research laboratory. It was found that the numbers of *L. monocytogenes* on inoculated vegetables (broccoli, cauliflower, and radishes) treated with 0.1% and 0.5% CPC for 1 min were reduced at least 2.2 logs and 3.5 logs, respectively, while *S.* Typhimurium was reduced 1.3 logs and 2.0 logs for 0.1% and 0.5% CPC treatments, respectively. A 0.5% CPC treatment reduced the numbers of *E. coli* O157:H7 1.5 to 2.0 logs.

P011 ATTACHMENT AND SURVIVAL OF *SALMONELLA STANLEY* ON CANTALOUPE SURFACE: EFFICACY OF WASHING TREATMENTS AND POSSIBILITY OF TRANSFER TO FRESH-CUT TISSUES

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Salmonella is the most frequently reported cause of foodborne outbreaks of gastroenteritis in the United States. Salmonellosis associated with cantaloupe has been reported. Contamination may have been introduced into the fruit from the rind by cutting or contact of cut pieces with contaminated rinds. Our objectives were to investigate attachment and survival of *Salmonella stanley* on cantaloupe surface, survival of attached bacteria following chlorine or hydrogen peroxide treatments, and transfer of bacteria to the interior tissue during cutting.

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Cantaloupes were immersed in a *S. stanley* inoculum containing 10^8 CFU/ml for 5 min. A population of 3.5 to 4 logs per cm^2 *S. stanley* was recovered from the rinds. Washing with water did not reduce the attached *Salmonella* population. Washing with chlorine or hydrogen peroxide solutions within 24 h of inoculation caused 3.29 log_{10} CFU/cm^2 or 2.1 log_{10}CFU/cm^2 reduction of attached *Salmonella* population, respectively. In samples stored at 4° or 20°C for 72 h, the population was reduced 2.3 log_{10} CFU/cm^2 after washing with chlorine and 1.4 log_{10}CFU/cm^2 for hydrogen peroxide wash. Fresh-cut pieces prepared from inoculated cantaloupes prior to washing treatments showed presence of *S. stanley*. Recovery occurred in samples stored at >8°C for 5 days. However, a chlorine or hydrogen peroxide wash before cutting significantly ($P < 0.05$) reduced the population to below a detectable limit after storage at 4°C for 6 days.

Results of this study suggest that washing cantaloupes with chlorine or hydrogen peroxide and proper refrigeration at ≤ 7°C will reduce growth of *Salmonella* on fresh-cut cantaloupe tissues.

P012 COMBINATION OF CHEMICAL TREATMENTS WITH GAMMA IRRADIATION FOR ELIMINATION OF FOODBORNE PATHOGENS FROM FRESH PRODUCE

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Combining low doses of gamma radiation with water-based chemical treatments was investigated as a potential means of inactivating foodborne pathogens on fresh produce. Fresh products (carrots, strawberries, tomatoes, cantaloupes, cucumbers, lettuce, and apples) were inoculated with *E. coli* O157:H7, *Salmonella* serotypes, or *Listeria monocytogenes*, and treated. Treatments consisted of two irradiation doses, 0.25 or 0.38 kGy, that were followed by one of 12 water-based chemical treatments: water only, 0.5% lactic acid + 100 ppm sorbitan monolaurate (LASPAN), or 1200 ppm acidified sodium chlorite (ASC) each applied at 23°C for 5 or 10 min; water only, LASPAN, or ASC each applied at 54°C for 1 or 2 min. Following treatment, surviving target bacteria were enumerated to determine reductions in viable populations. In general, reductions were 1-2 log_{10} CFU/ml greater when produce was irradiated prior to application of water treatments vs reductions from water treatments alone. The magnitude of this increased kill was approximately that obtained by irradiation alone. Therefore, combining irradiation and chemical treatments produced an apparent additive rather than synergistic effect. All of the water-based treatments when combined with 0.38 kGy irradiation resulted in reduction of *E. coli* O157:H7 and *Salmonella* (10^3-10^5 CFU/ml) to non-detectable levels (<10 CFU/ml). Using low irradiation doses in combination with water treatments may provide a means of eliminating foodborne pathogens from fresh produce without adversely affecting product quality.

P013 INACTIVATION OF BACTERIAL FOOD-BORNE PATHOGENS ON FRESH PRODUCE USING WATER-BASED CHEMICAL TREATMENTS

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The objective of this study was to evaluate potential antimicrobial treatments for fresh produce. Because water is used in chilling, washing, and transportation of fresh produce, water-based chemical treatments were tested. Fresh products (carrots, strawberries, tomatoes, cantaloupes, cucumbers, lettuce, and apples) were inoculated with *Salmonella* serotypes, *E. coli* O157:H7, or *Listeria monocytogenes*, and treated. The 12 tested treatments were: water only, 0.5% lactic acid + 100 ppm sorbitan monolaurate (LASPAN), or 1200 ppm acidified sodium chlorite (ASC), each applied at 23°C for 5 or 10 min; and water only, LASPAN, or ASC each applied at 54°C for 1 or 2 min. All treatments were applied by immersion of intact produce into solutions with constant agitation. Following treatment, target bacteria were enumerated to determine reduction in viable populations. Water treatments had little effect on bacteria, although reductions of > 2.0 log_{10} CFU/ml were noted in limited cases. Treatment with LASPAN resulted in a reduction of 0 - 3.4 log_{10}CFU/ml. Reductions of > 4.0 log_{10} CFU/ml resulted from ASC treatment in most but not all cases. Reductions in bacterial populations varied greatly, and were affected by treatment, temperature of application, bacterial type, and product type. No one treatment tested seemed to be effective against all bacteria on all products.

P014 GROWTH OF *ESCHERICHIA COLI* O157:H7 AND NATURALLY PRESENT MICROORGANISMS IN HEATED FRESH-CUT LETTUCE

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Lettuce has been implicated in outbreaks of *E. coli* O157:H7. Blanching (50°C for 90 s) inhibits PAL (phenylalanine ammonia-lyase) activity and

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reduces subsequent browning in fresh-cut lettuce. However, little is known about the growth characteristics of microflora after blanching. The objective was to determine if blanching affected survival and subsequent growth of naturally present microorganisms and *E. coli* O157:H7 in fresh-cut lettuce. Iceberg lettuce leaves were cut into 30 cm^2 segments. After dipping samples in water containing 20 mg/L chlorine for 90 s at 50°C, leaves were inoculated with 10^6 CFU/g *E. coli* O157:H7. Samples were then packaged and stored at 4°C for 21 days. Populations of total aerobic mesophilic and psychrotrophic bacteria, *Enterobacteriaceae*, lactic acid bacteria and *E. coli* O157: H7 were enumerated at 0, 7, 10, 14 and 21 days throughout storage. Treatment of lettuce by warm (50°C) chlorinated water delayed browning by at least 3 days. Blanching of lettuce had no appreciable effect on growth of *E. coli* O157: H7. In both blanched and unblanched samples, populations of *E. coli* O157:H7 increased by about 3 logs after 21 days of storage. Similarly, survival and growth of lactic acid bacteria and *Enterobacteriaceae* were unaffected by blanching. Populations of aerobic mesophilic and psychrotrophic bacteria increased to about 8-10 log CFU/g in both blanched and unblanched lettuce. The results suggest that blanching retards the brown discoloration of lettuce without affecting survival and growth of resident microorganisms.

P015 BACTERICIDAL EFFECT OF CHLORINE DIOXIDE AGAINST *SALMONELLA* SPP., *ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* INOCULATED ON APPLES AND LETTUCE

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Chlorine dioxide (ClO_2) has been explored for its bactericidal effect in poultry chilling water and seafood wash water. It is also explored as an alternative to aqueous chlorine. The objective of this study was to investigate the concentration and time-related killing effectiveness of aqueous ClO_2 in eliminating *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* inoculated on the surface of lettuce and the blossom end of apples. ClO_2 working solutions at 10, 20, 40, 100, and 200 ppm were freshly prepared from a stock solution following the instructed procedures. After the inoculated test samples were dipped in these solutions for 1, 3, 6 or 10 min, they were blended with 9 volumes of Butterfield's Buffer for 2 min and the suspensions subjected to bacterial enumeration on various selective media applying spiral plating and pour plate methods. The treatment of apples with 200 ppm ClO_2 for 10 min caused a 3-log reduction of *Salmonella* spp. and 1- to 2-log reduction of *E. coli* O157:H7 and *L. monocytogenes*. For *Salmonella* spp. on the lettuce, a 1-log reduction occurred following a 10-min treatment with 200 ppm ClO_2. However, only a 0.7- to 1.0-log reduction occurred with *E. coli* O157:H7 and *L. monocytogenes* on the lettuce. Thus, ClO_2 treatment is more effective against *Salmonella* spp. than against *E. coli* O157:H7 and *L. monocytogenes*. The treatment was found to be more effective in removing these pathogens on apples than on lettuce.

P016 MODELING UV INACTIVATION OF *ESCHERICHIA COLI* IN APPLE CIDER FOR QUANTITATIVE RISK ASSESSMENT

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Ultraviolet (UV) tubes used for non-thermal pathogen reduction in apple cider were tested to quantify the bactericidal effect of UV radiation on *Escherichia coli* ATCC 25922, a non-pathogenic surrogate of *E. coli* O157:H7. Experimental data from each tube that produced at least a 5-log reduction in triplicate trials was subjected to comprehensive statistical analysis. The variability, both in each tube and between tubes, was determined for 70 tubes. A beta distribution approximated the mean log reductions of all of the tubes (Kolmogorov-Smirnov test (KS), 0.0246), while the between-replicate variability was fit to a logistic distribution (KS, 0.0305). Taken together, these two distributions can model UV treatment as part of a quantitative risk assessment for *E. coli* O157:H7 in apple cider. The distributions were coded into @RISK and Analytica, two software programs commonly used in risk assessment. Simulations run in both programs show similar trends in their results, despite differences in the graphing routines of the two programs.

P017 EFFICACY OF SURFACE HEAT TREATMENT ON APPLES IN THE PRODUCTION OF APPLE CIDER

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Surface heat treatment of apples was evaluated in a cider mill as a means of reducing the microbial load in the final juice. Apples were treated in-line

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for approximately 90 s in a heatable water tank with a submerging belt at 4 different temperature ranges, 60 to 70°C, 70 to 80°C, 80 to 90°C, and 90 to 100°C. The level of naturally occurring aerobic microorganisms as measured using standard plate count on incoming fruit averaged approximately 3.04 +/- 0.53 log (CFU/gm). At the highest temperature range, reductions up to 1.5 logs were achieved. Reductions in juice were less pronounced, averaging less than 1 log. These results supported results of previous studies performed under laboratory conditions, which found similar reductions in naturally occurring microflora. Additional studies using inoculated apples indicate that surface heat treatment can effectively destroy all vegetative cells on the surface of fruit. Remaining populations consisted of internalized vegetative cells and heat resistant spores.

P018 SURVIVAL AND SPATIAL LOCATION OF *SALMONELLA STANLEY* IN ALFALFA SEED AND SPROUTS

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Consumption of *Salmonella* contaminated alfalfa sprouts has resulted in several outbreaks of foodborne illness. A putative source of the pathogen is contaminated seed. During sprout germination and growth, the pathogen likely increases significantly in number prior to harvest of the mature sprout. Treatment of mature sprouts to kill pathogens is not always efficacious and may be related to spatial location of the pathogen on the sprout. We have constructed green fluorescent protein (GFP)-expressing *E. coli* JM109 and *Salmonella stanley* to investigate spatial location. Fluorescence microscopy and laser scanning confocal microscopy were coupled with microbiological analysis of samples providing data on spatial location and relative number of marker bacteria. Seeds were contaminated by immersion in a suspension of GFP-*S. stanley* or GFP-*E. coli* JM109 (10^8 CFU/ml), removed, and allowed to air-dry overnight. Inoculated seeds were germinated and grown for 7d at room temperature. Samples (25 g) were taken at 24 h intervals for microbiological analysis and for microscopy. *S. stanley* counts from a representative experiment were 3.7, 7.0, 7.0, 6.9, 6.0, 5.4, 5.3 and 5.0 $\log_{10}$ CFU/g for inoculated seed, 1, 2, 3, 4, 5, 6, and 7 d sprouts, respectively. Similar increase and decline in bacterial number occurred with GFP-*E. coli* JM109. Microscopy revealed marker bacteria at subsurface locations in association with root, hypocotyl, and cotyledon tissue samples. Results of this study indicate that bacteria contaminating the outer surface of the seed can localize at a subsurface level during sprout growth. Studies are in progress to determine optimal chlorine concentration and stage of growth for efficacious treatment of sprouts.

P019 ASSESSMENT OF THE MICROBIAL EFFICACY OF A PROTOTYPE GRAS PRODUCE WASH ON APPLES

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The US EPA is currently seeking a standard method for evaluating the antimicrobial efficacy of produce washes for home use. We developed a standard method, which was consistent with the recommendations of an EPA Scientific Advisory Panel. The method provided consistent results across three laboratories when used to evaluate the efficacy of a prototype Fit Produce Rinse*, comprised of GRAS ingredients, against *Salmonella* strains inoculated onto tomatoes. The objective of the current research was to extend the standard method to apples. Golden Delicious apples were inoculated with a five-strain cocktail of *Salmonella* serotypes that had been isolated from patients and fecal material associated with produce-related foodborne illness. Inoculated apples were held at room temperature for 1 or 24 h prior to washing with the produce rinse, sterile USP water, or neutralizer control. The efficacy of the produce wash after a 30-s exposure period resulted in an average 2 to 4 log reduction of *Salmonella* on the apples over that achieved with sterile USP water or neutralizer control regardless of hold time ($P < 0.01$). A mixed linear model was used to assess the $\log_{10}$ CFU with respect to efficacy and associated components of variance.

P020 INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* IN APPLE CIDER AND ORANGE JUICE BY OZONE

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Inactivation of *E. coli* O157:H7 and *Salmonella* in apple cider and orange juice treated with ozone was evaluated. A five-strain mixture of *E. coli* O157:H7 or *Salmonella* was inoculated (7 log CFU/ml) into apple cider and orange juice. Ozone (0.9 g ozone/h) was pumped into juices at 4°C for up to 180 (apple cider) or 240 min (orange juice). Samples were withdrawn at 30-min intervals, diluted in 0.1% peptone water, neutralized with 1.0 N NaOH, and surface plated onto recovery media.

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Recovery of *E. coli* O157:H7 was compared on tryptic soy (TSA), sorbitol MacConkey (SMAC), hemorrhagic colitis (HC), and modified eosin methylene blue (MEMB) agars; recovery of *Salmonella* was compared on TSA, bismuth sulfite (BSA), and XLT4 agars. *Salmonella* survived better in orange juice than *E. coli* O157:H7 ($P < 0.05$), although their survival in apple cider did not differ ($P > 0.05$). After treatment, *E. coli* O157:H7 in apple cider was reduced by 4.81, 4.64, 6.80, and 6.85 log CFU/ml as determined on TSA, HC, MEMB, and SMAC, respectively. *Salmonella* was reduced in apple cider by 4.46, 6.06, and 6.54 log CFU/ml as determined on TSA, BSA, and XLT4, respectively. *E. coli* O157:H7 populations in orange juice declined 5.39, 5.69, 5.52, and 5.33 log CFU/ml as determined on TSA, HC, MEMB, and SMAC, respectively. *Salmonella* populations in orange juice declined 4.18, 4.31, and 4.88 log CFU/ml as determined on TSA, BSA, and XLT4, respectively. Ozone treatment of apple cider and orange juice may provide an alternative to pasteurization for acceptable reduction of *E. coli* O157:H7 and *Salmonella*.

P021 EFFICACY OF ALLYL ISOTHIOCYANATE IN KILLING ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157:H7 ON ALFALFA SEEDS

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Volatile compounds occurring in the essential oil of plants were tested for their efficacy in killing *Escherichia coli* O157:H7. Experiments using an agar disk assay revealed that exposure of the pathogen to 50 µl of eugenol, carvacrol, linalool, or methyl jasmonate in a 950-cc jar at 20, 37, or 47°C for up to 48 h failed to inhibit colony formation. However, exposure to 8 µl of allyl isothiocyanate (AIT) (equivalent to 8.4 ppm in the air within the jar, if completely volatilized) resulted in more than a 7-$\log_{10}$ reduction in population of *E. coli* O157:H7 at 37°C within 48 h; a significant ($P < 0.05$) reduction in population also occurred in the presence of 4 µl of AIT compared with 2 µl, which had no lethal effect.

At 20°C, the lethality of AIT was substantially less. Treatment with 10 µl of AIT for 5 h at 47°C resulted in death of 6 $\log_{10}$ CFU/ml. At an initial population of 2.7 $\log_{10}$ CFU/g of wet alfalfa seed, *E. coli* O157: H7 was not recovered by direct plating or enrichment after exposure to 53 ppm AIT for 24 h at 37 or 47°C. Exposure of dry seeds containing 2.9 $\log_{10}$ CFU of *E. coli* O157:H7 per g to 105 ppm AIT for 24 h at 47°C did not eliminate all viable cells. The use of AIT as an alternative to chlorine for the purpose of killing *E. coli* O157:H7 and perhaps other pathogens on alfalfa seeds holds promise.

P022 EVALUATION OF CHEMICALS FOR THEIR EFFECTIVENESS IN KILLING *SALMONELLA* ON ALFALFA SEEDS

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Nine outbreaks of salmonellosis associated with alfalfa sprouts have been documented in the US since 1995. This project was undertaken to evaluate various chemical treatments for their effectiveness in killing *Salmonella* on alfalfa seeds. The effect of presoaking on the efficacy of treatments was also evaluated. Submerging inoculated seeds in solutions containing 20,000 ppm free chlorine [$Ca(OCl)_2$], 5% Na_3PO_4, 8% H_2O_2, 1% $Ca(OH)_2$, 1% calcinated calcium, 5% lactic acid, or 5% citric acid for 10 min resulted in reductions of 2.0-3.2 $\log_{10}$ CFU/g. Treatment with 1,060 ppm Tsunami™ or Vortex™, 1,200 ppm acidified $NaClO_2$, or 5% acetic acid were less effective in reducing *Salmonella* populations. With the exceptions of acetic, lactic, and citric acids, treatments did not adversely affect the ability of seeds to germinate. Presoaking seeds in water, 0.1% EDTA, 1% Tween 80 (a surfactant), or 1% Tween 80 plus 0.1% EDTA for 30 min before treatment with water, 2000 ppm $Na(OCl)_2$, or 2% lactic acid had a minimal effect on reducing populations of *Salmonella*. Treatment with 1% $Ca(OH)_2$ in combination with the 1% Tween 80 enhanced inactivation by 1.3 $\log_{10}$ CFU/g. Results indicate that, although several chemical treatments reduce *Salmonella* populations of up to 3.2 $\log_{10}$ CFU/g, none of the treatments eliminated the pathogen without reducing the ability of seed to germinate.

P023 FACTORS AFFECTING THE THERMAL INACTIVATION OF BACTERIA IN POULTRY PRODUCTS DURING AIR CONVECTION COOKING

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Air convection cooking is widely used for commercial processing of poultry products. Variations in process conditions, such as temperature, humidity, and air velocity, during cooking can affect the thermal inactivation of bacteria. The objective of this project was to evaluate the effect of these factors on thermal inactivation of *Salmonella* and *Listeria* in poultry products during air convection cooking. Ground and formed chicken breast meat patties were inoculated with *S. senftenberg*, *S.* Typhimurium, *S. heidelberg*, *S. mission*, *S. montevideo*, *S. california*, and *L. innocua* M1, and processed in a pilot-scale, air-impingement oven. Process

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conditions included dry bulb temperature of 173-218°C, air velocity of 7.1-12.7 m^3/min, and steam flow of 0-114 kg/h. The inoculated meat (10^7 CFU/g) was processed to internal temperatures of 55-75°C, and bacterial survivors were enumerated. Microbial lethality was significantly affected by the process humidity, and the effect varied with the product final internal temperature. Lethality differences as large as 5 log were observed between treatments. Also, depending on the final internal temperature, the bacterial survivors were not uniformly distributed among individual patties. In an internal temperature range of 60-70°C, standard deviations of 2 and 3 logs were obtained, respectively, for survivors of *Salmonella* and *Listeria*. The results indicate that the thermal inactivation of bacteria in chicken patties processed via air convection cooking is significantly influenced by the process conditions. Existing inactivation models should be applied to commercial processes with caution, giving careful consideration to the appropriateness of the model to the process conditions.

P024 FATE OF *SALMONELLA* SPP. DURING HEATING AT DIFFERENT RATES IN SOUS-VIDE COOKED BEEF

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Inactivation rates of a cocktail of *Salmonella* spp. in sous-vide cooked beef exposed to varied heating rates of one to three h from 10°C to the processing temperature of 58°C were examined. The primary thermotolerance response of the organism, as a result of increasing cooking times to the target temperature, was a switch to nonlinear inactivation kinetics, resulting in the presence of shoulders (lag time) in the survival curves. From the survival curves obtained in this study, values for two parameters (the asymptotic D-value and the "lag" time) were estimated. The computations for the nonlinear curves suggested that the treatment affects the initial cell resistance (lag time) but that once this initial period is past, the heat resistance, as measured by the asymptotic D-value, is not affected by the rate of cooking. At 58°C, the asymptotic D-value was estimated to be between 5.2 and 7.4 min. To compute the time to achieve a target lethality (TL), at 58°C, of more than a 4 $\log_{10}$ relative decline, the lag time must be added to the product of TL and the asymptotic D-value. These findings should have substantial practical importance to food processors of sous-vide cooked beef that is processed by slow heating rate/long come-up times and low heating temperatures.

P025 SURVIVAL OF INOCULATED *ESCHERICHIA COLI* O157:H7 ON BEEF JERKY DRIED AT 62.5°C FOLLOWING FOUR PREPARATION TREATMENTS

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The fate of *Escherichia coli* O157:H7 was determined during preparation and drying of whole muscle beef jerky using four pre-drying treatments designed to promote the recommended 5-log reduction for processed fermented and semi-dry foods. Pre-drying preparation treatments of inoculated meat slices included: (1) dipping in water (96°C, 15 s), followed by marinating (4°C, 24 h); (2) seasoning (4°C, 24 h), followed by dipping in a pickling brine (78°C, 90 s); (3) dipping in acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h); (4) marinating (4°C, 24 h), followed by dipping in acetic acid solution (57.5°C, 2.5%, 20 s). Following pre-treatments, slices were dried in dehydrators at 62.5°C air temperature. Samples were analyzed (bacterial enumeration with selective and nonselective agar media, pH, and a_w) prior to and following each preparation step and at 0, 4, 6, 8 and 10 h of drying. The study was replicated twice with three samples analyzed per replication. Depending on initial inoculum level (5.3 to 7.6 CFU/cm^2) and agar medium (tryptic soy agar — TSA, Sorbitol MacConkey agar — SMAC, and modified eosin methylene blue agar — MEMB), count reductions after 10 h of drying were 4.3-5.1, 5.7-6.3, 4.8-5.2 and 4.7-4.8 log CFU/cm^2 for the four treatments, respectively. Pre-drying Treatment 2 (seasoned, then dipped in pickling brine) resulted in the greatest pre-drying log reduction (3.1-4.1 log CFU/cm^2) and had the highest total reduction after 10 h of drying (5.7-6.3 log CFU/cm^2). These results should be useful in developing guidelines for jerky preparation by consumers.

P026 PHYSICAL VARIABLES AND YEAST INACTIVATION DURING THERMO-ULTRASONICATION

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Application of low frequency ultrasound (20 kHz) promotes microbial inactivation during heat treatments. Cavitation effect has been associated with the lethality of ultrasound. Physical variables that may enhance cavitation during ultrasonication need to be evaluated. The aim of this work was to estimate, using a two-level factorial design, the effect

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of selected categorical (presence or absence) variables: air bubbles (1 ml every 3 min), cavitation nuclei (5 boiling chips), and pulsed ultrasound (5 s on, 5 s off), as well as the effect of selected numerical variables: wave amplitude (75 or 115 microns), sample volume (50 or 100 ml), and process temperature (35 or 55°C) on the inactivation of *Saccharomyces cerevisiae.* Treatments were carried out in a double wall vessel with circulating water to maintain constant temperature. Ultrasound was applied with a 13-mm horn. For each experiment, the yeast was inoculated (10^6 CFU/ml) into Sabouraud broth; after 5, 10, or 15 min, samples were taken out, and plated into potato-dextrose agar. Longer treatments increased yeast inactivation. An analysis of variance demonstrated that temperature, wave amplitude, application of ultrasound pulses and presence of boiling chips significantly ($P < 0.05$) affected yeast response after 5, 10, or 15 min of thermoultrasonication. Increasing wave amplitude and the application of ultrasound pulses in combination with elevated temperature significantly ($P < 0.05$) increased yeast decimal reductions. The presence of cavitation nuclei only enhanced ultrasound effects at 35°C, while at 55°C the thermal effect predominated. The increased yeast lethality observed on selected thermoultrasonication treatments suggests a raised cavitation effect during the application of ultrasound.

P027 EFFECTS OF PULSED ELECTRIC FIELD PROCESSING USING A STATIC CHAMBER ON THE SURVIVAL OF *LISTERIA MONOCYTOGENES*

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The survival of *Listeria monocytogenes* during pulsed electric field processing was measured as a function of field strength (20-48 kV/cm), temperature (0-60°C), and pulse number (5-50 pulses) in a static chamber. PEF treatment in a static chamber simplifies the measurement of lethality by eliminating complexities of continuous treatment. A gellan gum gel matrix was used to hold bacterial cells in place during treatment. The overnight culture (10^9 CFU/ml) was suspended in gellan gum solution (0.25%) and $CaCl_2$ (0.03%) solution was added to aid gelling. The solution was allowed to gel in the static chamber, and the appropriate PEF treatment (with a pulse width of 3.25 microsec and a pulse interval of 1 min) was given under isothermal conditions. After the treatment, samples were taken from the treatment and control areas. They were dissolved using a calcium sequestrant, diluted, and plated for enumeration of both injured and noninjured survivors. There was no inactivation of the organism at lower field strengths and slightly less than a log reduction at 48 kV/cm. With pulse number, there was a 0.5 (5 pulses) to 1 log reduction (10 and 50 pulses). An increase in inactivation was seen with increasing temperature, with a maximum of 4.7 logs at 55°C, showing a synergy between PEF and temperature. There was no significant injury of the bacterial cells due to PEF. PEF alone appears to be limited in its effectiveness towards inactivating *L. monocytogenes* using a static chamber.

P028 INACTIVATION OF *LISTERIA MONOCYTOGENES* IN BRINE CHILLER WATER FOR THERMALLY PROCESSED MEAT PRODUCTS USING A RECIRCULATING ELECTROCHEMICAL TREATMENT SYSTEM

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A recirculating electrical treatment system consisting of a storage tank and a treatment chamber was designed and evaluated for its efficacy to inactivate approximately 10^6 CFU/ml of *Listeria monocytogenes* in brine chiller water for chilling thermally processed bacons. Samples were taken at 0, 5, 10, 15, 20, 25, 30, 45, and 60 min from the storage tank and the treatment chamber flow. Three current levels (7, 17.5, 35 mA/cm^3) and three temperatures (-8, 0, 4°C) were tested for their effects on bacterial reduction. At -8°C and 35 mA/cm^3, 15 and 30 min treatments eliminated *L. monocytogenes* from the treatment chamber flow and the storage tank. On the other hand, 60 min was needed to eliminate *L. monocytogenes* from the treatment chamber and reduce its population by 5.1 log CFU/ml in the tank at 17.5 mA/cm^3. Only 0.98 and 0.77 log CFU/ml reduction was achieved in the treatment chamber and in the tank at 7 mA/cm^3 for 60 min. At 35 mA/cm^3, 15 min of treatment at all three temperatures eliminated *L. monocytogenes* population from the treatment chamber, but 20, 25, and 30 min was needed for complete inactivation of *Listeria* from the tank at 4, 0, and -8°C, respectively. The pH and conductivity of the treated brine chiller water did not change significantly. Results showed that the recirculating electrical treatment was a convenient and effective antimicrobial method for *L. monocytogenes* and provided the potential as a practical online treatment method for *L. monocytogenes* in a brine chilling system.

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P029 INFLUENCE OF GAMMA IRRADIATION ON *SALMONELLA* SPP. INCORPORATED INTO OYSTERS

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Irradiation is considered one of the most efficient technological processes to reduce the number of microorganisms in food. It can be used to improve the shelf life of food products as well as increase their safety. Oysters are considered one of the most important vehicles of pathogenic bacteria due to their feeding characteristics and because they are often ingested raw. The aim of this study was to evaluate the influence of gamma radiation process on high levels of *Salmonella enteritidis* and *Salmonella infantis* incorporated into oysters (*Crassostrea brasiliana*) as well as on the survival of the oysters. The oysters were submitted to gamma radiation treatment (^{60}Co) with doses ranging from 0.5 kGy to 3.0 kGy. Three trials were conducted for each serotype. The dose of 3.0 kGy was, in general, sufficient to reduce the level of both serotypes by 6 log, and animals were not killed by this radiation dose. Therefore, application of 3.0 kGy is effective on the inactivation of *Salmonella* spp. in oysters.

P030 LOSS OF CRYSTAL VIOLET BINDING ACTIVITY IN *YERSINIA ENTEROCOLITICA* FOLLOWING GAMMA IRRADIATION

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Low dose ionizing radiation or cold pasteurization, effectively eliminates the pathogen *Yersinia enterocolitica* from red meats and poultry. It is possible, although unlikely, that a small number of bacteria could survive the cold pasteurization process. Pathogenicity of *Y. enterocolitica* is dependent upon the presence of a large virulence plasmid. The ability of ionizing radiation to eliminate the large virulence plasmid from *Y. enterocolitica* was investigated. *Y. enterocolitica* strains which contained the large virulence plasmid were inoculated to a density of 10^9 colony forming units per gram in Butterfield's Phosphate Buffer (BPB) or vacuum-packed raw pork. The suspensions were then irradiated in a self-contained ^{137}Cs source, at a sample temperature of 0°C, to a dose of 1.0 kGy. A radiation dose of 1.0 kGy was sufficient to reduce the population of viable cells by five $\log_{10}$ in number regardless of the suspending medium. Following growth on Brain Heart Infusion Agar, the survivors were tested for the ability to bind the dye crystal violet, which is associated with the presence of the large virulence plasmid. The percentage of colonies that did not bind crystal violet increased ten-fold over the value for the untreated controls regardless of the suspending medium. Molecular biological analysis of *Y. enterocolitica* that survived cold pasteurization indicated that loss of the large virulence plasmid, as opposed to mutation of a single gene responsible for crystal violet binding activity, was the major mechanism by which loss of crystal violet binding activity was induced.

P031 EFFICACY OF DISINFECTANTS IN KILLING SPORES OF *ALICYCLOBACILLUS ACIDOTERRESTRIS* AND PERFORMANCE OF MEDIA FOR ENUMERATING SURVIVORS

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The objective of this study was to determine the efficacy of chemical disinfectants in killing spores of *Alicyclobacillus acidoterrestris*, a fruit juice spoilage bacterium. Direct plating media were evaluated for their suitability to support germination and outgrowth of spores surviving exposure to these disinfectants. Significant ($P \leq 0.05$) reductions of 2.2, 0.4, and 0.1 logs in the number of viable *A. acidoterrestris* spores in a five-strain mixture were achieved when spores were suspended in solutions containing 200 ppm free chlorine, 500 ppm acidified sodium chlorite (ClO_2), or 0.2% H_2O_2, respectively, for 10 min at 23°C. When treated with either 1000 ppm chlorine or 4% H_2O_2, the number of spores was reduced by more than 5 logs. Treatment with 8% trisodium phosphate or 80 ppm Tsunami™ did not significantly reduce numbers of viable spores. K agar (pH 3.7) was judged best for recovering chemically treated spores, compared to orange serum agar (pH 5.0) and potato dextrose agar (pH 3.5). Apples, surface inoculated with *A. acidoterrestris* spores, were treated with 500 ppm chlorine or 1200 ppm acidified sodium chlorite for 1 min. Significant ($P \leq 0.05$) decreases in the number of viable spores occurred, but reductions were less than 1 log. Further work is needed to determine the concentration and length of time required for disinfectants, in combination with surfactants or solvents, to kill *A. acidoterrestris* spores on apples and other fruits.

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P032 EFFICIENCY OF SANITATION PROCEDURES AGAINST *LISTERIA MONOCYTOGENES*: APPLICATION TO COLD-SMOKED FISH INDUSTRY IN FRANCE

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Listeria monocytogenes is a foodborne pathogenic bacteria able to contaminate cold-smoked fish. Contamination with *L. monocytogenes* may be linked both to the fresh fish and environmental contamination. The aim of this work was to evaluate efficiency of sanitation procedures used in three smoking industries in France, against *L. monocytogenes.*

Efficiency of cleaning and sanitation agents was tested against two strains of *L. monocytogenes* in suspension or attached to stainless steel and polyurethane.

Efficiency of agents was individually tested on cells in suspension without organic matter at 20°C and with organic matter at 20, 10 and 5°C. For attached cells, agents were tested individually and in combination.

Results showed that all tested agents are efficient on cells in suspension without organic substrate and on attached cells to stainless steel (more than 5 $\log_{10}$ reduction). With organic matter, one cleaner and one sanitation agent were inhibited both by organic matter and low temperature. With organic matter at 5°C, efficient concentrations were multiplied by 10 to 20 compared to concentrations without organic matter at 20°C. Reduction on attached bacteria to polyurethane was between 2 and 3 $\log_{10}$ per cm^2 with all tested agents. On this surface, only complete sanitation procedures could eliminate more than 3 $\log_{10}$ per cm^2.

Suppliers of cleaning and sanitation agents give a range of concentrations and a contact time to obtain the best efficiency. To be efficient on polyurethane, cleaning and sanitation agents have to be used at the higher concentration recommended by suppliers.

P033 INFLUENCE OF SODIUM PYROPHOSPHATE ON THERMAL INACTIVATION OF *LISTERIA MONOCYTOGENES* IN PORK SLURRY AND GROUND PORK

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The thermal inactivation of *Listeria monocytogenes* in pork slurry and ground pork containing 0, 0.25 or 0.5% sodium pyrophosphate (SPP) was studied. Pork slurry or ground pork was inoculated with *L. monocytogenes* to give ~10^7 CFU per ml or gram, respectively. The inoculated samples were heated at 55, 57.5, 60 or 62.5°C and surviving cells were enumerated on Modified Oxford Medium. Decimal reduction (D)- values in pork slurry without SPP were 8.15, 2.57, 0.99, and 0.18 min for 55, 57.5, 60, and 62.5°C, respectively; D-values in ground pork ranged from 15.72 min at 55°C to 0.83 min at 62.5°C. Addition of 0.25% or 0.5% SPP dramatically decreased the heat resistance of *L. monocytogenes* in pork slurry ($P < 0.05$) but not in ground pork. The D-values in pork slurry + 0.25% SPP were 4.75, 1.72, and 0.40 min for 55, 57.5, and 60°, respectively; 7 $\log_{10}$ CFU/ml were inactivated by heating for 1 min at 62.5°C. Addition of 0.5% SPP further decreased D-values in pork slurry but not in ground pork. At 0 - 0.5% SPP, the Z values in pork slurry or ground pork were 5.47 to 4.80°C and 5.77 to 5.25°C, respectively. Results indicate that SPP was effective in decreasing the heat resistance of *L. monocytogenes* in pork slurry but not in ground pork. These findings support the use of actual food products as heating menstruums when assessing the influence of phosphates on thermal inactivation of *L. monocytogenes* in meats.

P034 WITHDRAWN

P035 EVALUATION OF SPRAY APPLICATION OF ACIDIFIED SODIUM CHLORITE ON FRANKFURTERS AND ITS EFFECT ON REDUCTION OF *LISTERIA MONOCYTOGENES*

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Sodium chlorite (1,200 ppm) acidified with 0.9% citric acid (ASC) was evaluated for its effectiveness in reducing *Listeria monocytogenes* on retail Little Smokies sausages. Sausages were inoculated with a cocktail of 5 strains of *L. monocytogenes* (ATCC 13932, 49594, 43256, 51414, and 7647). Inoculated sausages were subjected to no treatment, water wash treatment for 5 s (WW5), spray wash treatment with ASC for 10, 15, or 30 s (i.e. W10, W15, and W30), or dipping treatment with ASC for 10, 15, or 30 s (i.e. D10, D15, and D30). Following treatment, samples were microbiologically analyzed by plating on Modified Oxford agar (MOX), and incubated at 30°C for 48 h. Statistical analysis was conducted and significant differences were determined at the 5% level among the mean values. Results generally indicated that the ASC treatment

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of sausages was more effective in reduction of *L. monocytogenes* than WW5 treatment, with reductions increasing with longer ASC exposure times. From an initial inoculation level of 5.1 log CFU/g, counts were reduced by 1.2 log cycles using a 5 s water wash. No significant improvement in listerial reduction was observed with ASC application for 10 s over the water wash control. The ASC dip for 15 s provided a significantly better reduction (1.0 log cycle) compared to the 5 s water wash. Superior reductions ($P \leq 0.05$) were observed with an ASC exposure time of 30 s, with 1.1 and 1.6 log cycle reductions over the water control for spraying and dipping, respectively. Spray wash or dipping, treatments were comparable ($P \geq 0.05$) in antibacterial effectiveness against *L. monocytogenes*.

P036 BACTERICIDAL AND BACTERIOSTATIC EFFECT OF BOVINE LACTOFERRIN AND ITS PEPSIN HYDROLYSATE FOR FOODBORNE PATHOGENS

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Research has shown that bovine lactoferrin (bLf) and its pepsin hydrolysate lactoferricin (bLfcin), are bacteriostatic and bactericidal for many gram-negative and gram-positive foodborne pathogens. In this study, foodborne pathogens including *Listeria monocytogenes*, *Salmonella stanley*, *Escherichia coli* O157:H7 and *Staphylococcus aureus* (strains of human, bovine, and porcine origin) were evaluated. The minimum inhibitory (MIC) and minimum lethal concentration (MLC) of bLf and bLfcin for selected pathogens were evaluated in standard buffered broth systems. MIC and MLC were determined, following incubation at 37°C for 20 h (40 h for *L. monocytogenes*), by measuring the optical density of mid log cycle cultures spiked with bLf or bLfcin at varying concentrations. Results of those experiments indicate a range of 250 to 8000 µg/ml inhibited the growth of all test pathogens. Low concentrations of bLf and bLfcin were effect against *L. monocytogenes* and *E. coli* O157:H7, whereas *S. aureus* was recalcitrant to all but the highest concentration of bLf and bLfcin tested. In standard broth systems bLf was bactericidal to *L. monocytogenes*, *E. coli* O157:H7 and *S. stanley*, but bacteriostatic for *S. aureus*. Studies are in progress using model food systems, including milk (whole and skim) and liquid egg (whole and whites), to determine antimicrobial effects in comparison with antimicrobial agents in use. bLf or bLfcin may provide an alternative to the use of current antimicrobial agents.

P037 LIMITATIONS IN THE USE OF OZONE TO DISINFECT MAPLE SAP

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The heat processing (boiling) of the sap from the maple sugar tree to produce maple sugar and syrup is an important seasonal activity in parts of North America. The solutes in sap consist principally of sucrose, and microbial growth in sap during holding can adversely affect syrup quality. The use of ozone was investigated as a method of reducing the microbial levels in sap. Initial attempts to reduce aerobic plate counts (APC) of sap by introduction of a stream of ozone were, surprisingly, unsuccessful. The three predominant microbial species, as identified by colony morphology on nutrient agar, were therefore isolated to determine if each was inherently resistant to ozone. These represented two yeast, both *Candida* species, and *Pseudomonas fluorescens*. When suspended in buffer each was readily inactivated under ozone exposure conditions similar to those used for sap. However, addition of 3% sucrose to buffer markedly reduced ozone effectiveness for all three organisms. For example, ozone resulted in a $\log_{10}$ 2.5 reduction in viability within 15 min in buffer alone, whereas in the presence of sucrose a similar inactivation required approximately 35 min. Use of an ozone generator with a 10-fold greater rated output was only marginally effective, requiring 30 min to reduce the APC from $\log_{10}$ 6.0 to $\log_{10}$ 3.9 even though saturating ozone conditions were reached within 5 min. Previous investigators have noted the quenching effect of organic material on ozone action. Our results support these observations and suggest the inefficiency of ozone as a processing method for reducing the microbial levels in maple sap.

P038 EFFECT OF FREEZING ON THE ISOLATION AND SURVIVAL OF PLASMID-BEARING VIRULENT *YERSINIA ENTEROCOLITICA* IN PORK

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Freezing food prior to analysis may impair the isolation of low levels of pathogens. Pork chops (PC) artificially contaminated with 10, 1, and 0.5 CFU/cm^2 of plasmid-bearing virulent *Yersinia enterocolitica* strain GER O:3 (YEP$^+$) were placed in sterile petri dishes and held at -20°C for 24 h. The PC were swabbed when frozen or after thawing at room temperature (RT) or 4°C. Swabs were enriched in modified trypticase soy broth containing yeast extract and bile salts at 12°C for 24 h

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and incubated for another 24 h after the addition of 4 µg/ml of Irgasan. The YEP+ was isolated by Congo red (CR) binding on CR brain heart infusion agarose. The YEP+ was recovered under all conditions using an initial inoculum level of 10 CFU/cm^2, and at a level of 1 CFU/cm^2 in thawed, but not in the frozen PC. The YEP+ was not recovered from the PC inoculated with 0.5 CFU/cm^2, whereas YEP+ was recovered at this level from non-frozen PC. The ability of YEP+ to survive storage at -20°C was also studied in irradiated ground pork (GP). The initial inoculum level decreased from 7.4 to 7.1 $\log_{10}$ CFU/g after 12 weeks of frozen storage, with no evidence of freeze-thaw injury. Thus, freezing inhibited the recovery of YEP+ present at low levels in PC, whereas in the GP inoculated with high levels of YEP+, the organism survived during extended storage at -20°C.

P039 EFFECT OF GROWTH TEMPERATURE OR STARVATION ON THE RADIATION RESISTANCE OF *ESCHERICHIA COLI* O157:H7 IN A MODEL SYSTEM AND GROUND BEEF

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The effect of growth temperature or starvation on the resistance of *E. coli* O157:H7 to electron-beam irradiation in buffered peptone water (BPW) and ground beef was investigated. *E. coli* O157:H7 cells grown at 35°C (control) or 43°C in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE), or starved in minimal media at 35°C, were inoculated into BPW or ground beef to give approximately 10^7 CFU/ml or g, respectively. Cells grown at 43°C and then shifted to 35°C were inoculated into ground beef to give approximately 10^7 CFU/g. Inoculated samples were irradiated at 0.00, 0.50, and 1.00 kGy. Survivors were enumerated in trypticase soy agar supplemented with 0.6% yeast extract (TSAYE), after incubation at 30°C for 48 h. In BPW, 0.50 kGy reduced controls by 5.41 logs, whereas cells grown at 43°C or starved were reduced by 3.97 and 4.62 logs, respectively ($P < 0.05$). In ground beef, 0.50 kGy reduced controls by 4.76 logs, whereas cells that were grown at 43°C, starved, or endured a shift in growth temperature, were reduced by 3.95, 4.67, and 5.05 logs, respectively. A dose of 1.00 kGy resulted in greater than 5.0 log reductions in controls and stress-adapted *E. coli* O157:H7 in ground beef. These results suggest that *E. coli* O157:H7 cells grown at 43°C or starved develop increased radiation resistance in BPW and ground beef. However, the extent of this stress-induced radiation resistance is inadequate to allow survival of *E. coli* O157:H7 in refrigerated ground beef irradiated at 1.00 kGy.

P040 SUSCEPTIBILITIES OF *STAPHYLOCOCCUS AUREUS, LISTERIA* AND *SALMONELLA* ISOLATES ASSOCIATED WITH POULTRY PROCESSING TO SIX ANTIMICROBIAL AGENTS

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In the poultry industry, administration of antimicrobial agents at subtherapeutic levels via the feed and water is necessary, not only to control and prevent disease but also to accelerate weight gain and to improve feed efficiency. The broth microdilution method was used to determine the activities of antimicrobial agents used in the South African poultry industry (colistin, neomycin, chlortetracycline, oxytetracycline and tylosin) and vancomycin against 38 strains of *Staphylococcus* (*S.*) *aureus*, 25 *Listeria* (*L.*) *innocua*, 18 *L. monocytogenes*, and 62 strains belonging to six *Salmonella* (*S.*) serotypes (*S. agona*, *S. blockley*, *S. enteritidis*, *S. isangi*, *S. reading* and *S.* Typhimurium). The source of all the strains were samples associated with poultry processing. Colistin was most active against the gram-negative strains (MIC range of 0.5 - 16 µg/ml) while vancomycin was most active against the gram-positives (MIC range of 1 - 4 µg/ml). *S. aureus*, *L. innocua*, *L. monocytogenes*, *S. enteritidis* and *S. isangi* strains were susceptible to neomycin with MIC_{90}s not exceeding 8 µg/ml. High levels of resistance were recorded for all the strains to chlortetracycline and oxytetracycline (MIC_{90} range of 32 - >512 µg/ml) except for *L. monocytogenes* and *S. enteritidis* (MIC range of 1 - 4 µg/ml). The MIC_{90} of the *S. aureus* and *Salmonella* strains was >512 µg/ml for tylosin.

P041 INVASIVE ABILITY AND TOLERANCE OF ACID-ADAPTED AND NON-ADAPTED *SALMONELLA* TYPHIMURIUM DT104 TO STRESS CONDITIONS

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Salmonella Typhimurium DT104 (DT104) and non-DT104 strains were grown in tryptic soy broth without (non-adapted, NA) or containing 1% glucose (acid-adapted, AA) for 18 h at 37°C. The level of invasion of DT104 strains in J774 macrophage and Int407 intestinal cell lines was not higher than that of non-DT104 strains. Also there were no significant differences between AA and NA cells in invasion of J774 cells, except for DT104 strain 41 ($\log_{10}$ 5.42 for NA compared to 4.18 CFU/well for AA). In Int407 cells, significant differences in

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invasion were observed for three DT104 strains, (log_{10} 4.33, 3.43, and 1.88 for AA, compared log_{10} 5.35, 5.08, and 3.61 for NA, respectively). In related studies, survival of three strains of AA and NA DT104 was compared to survival of a non-DT104 (LT2) strain in 0.5 and 0.05% H_2O_2, 1 and 0.25% acetic acid, 15% NaCl, 15% NaCl containing 1% acetic acid, synthetic gastric fluid (SGF) at pH 2 and pH 3, and apple cider. For all four strains, acid adaptation did not result in increased survival in apple cider. After 15 days of storage at 4°C, reductions ranged from log_{10}1.96 to 4.1 CFU/ml with AA compared to log_{10} 0.48 to 1.34 CFU/ml with NA from a starting level of 1×10^7 CFU/ml of cider. Neither AA nor NA DT104 strains were more resistant to NaCl, acetic acid, H_2O_2, or SGF solutions than was strain LT2. The level of AA was not appreciably reduced after exposure to SGF; however, the level of NA decreased to non-detectable levels in SGF, pH 2 after 3 h of exposure. These results indicate that the DT104 strains tested did not display increased resistance to food environment stresses compared to a non-DT104 strain and that acid-adaptation does induce increased resistance to a low pH gastric environment.

P042 HEAT ADAPTATION INDUCED CROSS-PROTECTION AGAINST OSMOTIC STRESS IN *SALMONELLA* TYPHIMURIUM DT104

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Heat adaptation-induced protection of *Salmonella* Typhimurium DT104 to osmotic stress was investigated. Heat adaptation of five *Salmonella* strains was determined. Two *S.* Typhimurium DT104 strains were selected by determining extent of heat adaptation. Strain 8748A-1 and strain H3380 showed the greatest differences in heat resistance between nonadapted and heat-adapted (46°C) cells. These were $D_{60°C}$ 0.58 and 2.54 min for strain 8748A-1 and $D_{60°C}$ 0.42 and 1.55 min for strain H3380 for nonadapted and heat-adapted cells, respectively. Nonadapted and heat-adapted cells were then placed in glycerol, sodium chloride, or sucrose solution (a_w 0.87) at 30°C. Survivors and injured cells were enumerated by plating onto tryptose phosphate agar plus 0.1% w/w sodium pyruvate and onto MacConkey agar. Heat-adapted cells of both *S.* Typhimurium DT104 strains in glycerol showed significantly enhanced resistance to osmotic challenge throughout the 21-day test as compared with their nonadapted counterparts. Heat-adapted cells of strain 8748A-1 declined approximately 4 log cycles whereas nonadapted cells declined up to 7 log cycles after 21 days of exposure. In sucrose, significant differences were found after 6 and 9 days for strain 8748A-1 and strain H3380, respectively. Approximately 0.1% of the original heat-adapted cells of strain 8748A-1 was viable after 21 days, whereas nonadapted cells showed only 0.02% survival. However, there was no significant difference in the survival of nonadapted and heat-adapted cells in sodium chloride. The sucrose challenge provided the greatest protection of solutes tested. Heat adaptation at 46°C and subsequent exposure to glycerol or sucrose resulted in greater cell injury as compared with nonadapted treatment.

P043 MULTIPLE STRESS STUDIES IN *ARCOBACTER* SPECIES

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The foodborne pathogen *Arcobacter* is associated with gastritis and bacteremia in humans and has been detected in drinking water supplies and raw meats, including pork products. The use of hurdle technology combining multiple stress factors acting synergistically, as an intervention technique, would reduce the incidence of *Arcobacter* in raw and processed ground meats. Preliminary studies identified individual stress factors affecting growth of the organism, including incubation temperature, initial pH, sodium chloride, sodium nitrite and sodium tripolyphosphate (STPP) levels. *A. butzleri* strains were sensitive to lower levels of STPP and higher levels of nitrite as compared to some other foodborne pathogens. Consequently, a multiple stress modeling study was carried out to determine the interaction effects of the known individual stresses. The effects of incubation temperature (12-37°C), initial pH (6.0-7.5), sodium chloride (0-3.5%), sodium nitrite (0-180 μg/ml) and STPP (0-0.012%) on the microaerophilic growth of a three-strain mixture of *A. butzleri* in EMJH medium were determined. These five variable factors interacted, affecting the growth kinetics of the organism, with the effects being mainly observed with respect to lag phase durations (LPDs) and generation times (GTs). LPDs ranged from 24 h to 8 days, while the maximum population densities (MPDs) achieved were generally unaffected by the variables. Initial pH was the most significant determining factor affecting growth of *A. butzleri* strains and it had an increased inhibitory effect at higher NaCl and STPP concentrations and at suboptimal growth temperatures.

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P044 INFLUENCE OF FRUIT VARIETY, HARVEST TECHNIQUE, CULLING, AND STORAGE ON THE MICROBIAL COMPOSITION AND PATULIN CONTAMINATION OF UNPASTEURIZED APPLE CIDER

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Some disease outbreaks have been attributed to consumption of unpasteurized apple cider pressed from ground-picked apples. Therefore, we studied harvest and post-harvest control measures to determine their impact on microbiological loads in cider. Seven varieties (McIntosh, Gala, Golden Delicious, Red Delicious, Rome, Granny Smith, Fuji) were harvested from orchards in El Dorado County, CA. Apples were collected from trees or the ground, then processed immediately or stored at 4°C for 25-73 d. Apples were culled to exclude visible degradation and punctures. Apples were milled; then cider was extracted using pressure. Levels of aerobic microorganisms and of yeasts and molds were quantified in samples of apples (four composites of six), post-pressing pomace, and cider; patulin levels in cider were measured. Cider from fresh, tree-picked apples showed reduced aerobic bacterial counts from all varieties, compared with cider from fresh, dropped apples (mean +/- sd 3.4 ± 0.7 vs. 5.0 ± 0.3 log CFU/g). Culling reduced the tree-picked average to 2.9 ± 0.8. Differences among these three groups were significant ($P<0.01$). Aerobic microbial levels in composited apples and pomace, and yeast and mold counts, correlated with these results. Moreover, using stored apples did not change the microbial load significantly. Patulin was not detected in ciders from fresh, tree-picked or stored, tree-picked, culled fruit. Patulin was detected in cider from some varieties of fresh, dropped fruit [4.13-413.7 ng/ml] or stored unculled apples [2.9-177 ng/ml]. Results support eliminating dropped apples from apple ciders and juices, as a means of reducing consumers' risk from patulin.

P045 ORGANIC ACIDS AND HYDROGEN PEROXIDE INHIBIT MICROBIAL VIABILITY IN FRESH JUICES

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Foodborne illness outbreaks have been associated with unpasteurized fruit juice and cider. Two outbreaks were attributed to *Cryptosporidium parvum*. Organic acids and/or hydrogen peroxide can be added to fruit juices as an alternative non-thermal treatment to inhibit survival of *C. parvum* and *Listeria monocytogenes*. Addition of acids alters microbial response to acidic pH, and the acid, sugar, and phenolic composition of juices. The dominant organic acid of a juice was added at 1%, 2%, 3%, or 5% on a wt/wt basis (malic acid-apple cider, citric acid-orange juice, tartaric acid-grape juice). Addition of acid and/or hydrogen peroxide (1% acid + 1.5% H_2O_2 or 3% H_2O_2) resulted in a decrease in pH by up to 41% and up to a 12-fold increase in titratable acidity. Total phenolics, hydroxycinnamates, and flavonoids increased up to 2-fold in modified grape juices and decreased by up to 5-fold in apple and orange juices. Growth of *L. monocytogenes* demonstrated a 2-log reduction with addition of 3% citric acid in orange juice, compared to controls after 48 h at 4°C. Growth was inhibited after 4 days in 3% citric acid. *L. monocytogenes* was still viable after 10 days in 1% citric acid. Unmodified or modified (1%, 2%, 3% malic acid) apple cider did not support growth of *L. monocytogenes*. Currently, filtration is the only way to control *C. parvum* contamination in juices. Acids and hydrogen peroxide are used to inhibit the viability of *C. parvum oocysts*. Juice composition can be correlated with the survival or loss of microorganisms in fruit juices.

P046 SURVIVAL OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157:H7 STRAINS IN WOUNDED APPLE TISSUE DURING TEMPERATURE ABUSE

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This study determined the survival of enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) strains at different temperatures in wounded apple tissue. Red delicious apples were wounded with an artist knife (7 mm deep) and inoculated with 4μl of decimally diluted EHEC strain C7929 (apple cider isolate) or 43890 (human isolate). The inoculated apples were stored at a constant temperature of 37°C, 25°C, 8°C, or 4°C, or at 37°C for 24 h and then 4°C. A cylinder of the internal tissue from the site of the wound inward to the core of each apple was removed with a cork borer (1 cm in diameter) and divided into three equal parts of 1 cm each (top, middle and bottom) and bacterial counts were determined every week for 28 days. At day 0, the bacterial counts were 6.0 to 6.7 log CFU/g for both strains in the top portion, 3.5 to 4.7 log CFU/g in the middle portion, and 3.0 to 4.0 log CFU/g in the bottom. By days 21 and 28 at 37/4°C, the EHEC apple and human isolate both had higher viable counts of 6.5 to 7.13 log CFU/g than 4°C, in the top

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portion of the apple wound, while at 4°C the apple isolate counts were significantly greater (5.60 log CFU/g vs 2.31 to 4.12 log CFU/g) for the human isolate. Our study shows that EHEC strains can adapt and survive, and produce higher viable counts in wounded apple tissue when apples are temperature abused.

P047 LOSS OF FUMONISIN DURING THE CORN FLAKE PROCESS WITH AND WITHOUT SUGARS

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The stability of fumonisin B1 (FB1) in spiked and cultured flaking grits during a simulated corn flake process with and without sugars was investigated. The overall recovery of FB1 added to fumonisin-free corn grits at various stages of processing was 101.3±11.0%. No significance differences ($P < 0.05$) were found when spiked flaking grits were processed without sugars, with sucrose alone, and with sucrose in combination with maltose and high fructose corn syrup (HFCS). Corn flake processing of spiked grits without sugars resulted in 53.5% and 48.7% losses of FB1 after cooking and toasting, respectively. The percentage losses of FB1 in spiked grits after cooking and toasting with sucrose were 43.8% and 47.6%, respectively. About 44.1% and 48.7% FB1 was lost when cooked and toasted, respectively, with sucrose in combination with maltose and HFCS. Similar results were found when cultured grits were made into corn flakes. Loss of FB1 averaged 36% when cultured flaking grits were processed without sugars and 44.7% and 54% when processed with sucrose, alone and in combination with maltose and HFCS, respectively. As expected, more significant ($P < 0.05$) losses of FB1 were found when both spiked and cultured grits were processed with glucose, alone and in combination with maltose and HFCS. Greater reductions of FB1 in spiked (up to 89.2%) and cultured (up to 86.3%) grits were observed after toasting.

P048 PEROXIDATIVE STRESS ADAPTATION AND THERMAL CROSS-RESISTANCE IN *ESCHERICHIA COLI* O157:H7 933 SUBJECTED TO SUBLETHAL DOSES OF PEROXYACETIC ACID SANITIZER

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We hypothesized that *Escherichia coli* O157:H7 exposed to sublethal concentrations of peroxyacetic acid sanitizer would adapt to peroxidative stress and develop thermal cross resistance. Two methods of sublethal peroxyacetic acid (PAA) sanitizer exposure were utilized to represent contact scenarios: *acute* and *chronic*. Cultures were grown in Trypticase soy-yeast extract broth (*acute* & *controls*) or TSYEB + 0.0015% v/v PAA (*chronic*) for 24 h at 37°C. Cultures were centrifuged, resuspended in fresh media, and split for pre-challenge at 37°C for 1 h. Pre-challenge conditions were: acute, 0.1% v/v PAA; peroxide control, 10mM H_2O_2; acetic acid control, 6.7mM acetic acid; negative controls received no treatment. Chronic contact cultures received no pre-challenge. Next, all cultures were subjected to peroxidative (80mM H_2O_2) or thermal (54°C) challenge and enumerated on TSYE-pyruvate &/or TSYE-deoxycholate agar. Acute, chronic and peroxide control cultures showed substantially increased peroxidative tolerance (D_{80mM} >3 h) versus negative control (D_{80mM}=0.19 ±0.03 h). The inactivation rate of the acetic acid control (D_{80mM}=0.21 ± 0.05 h) was similar to the negative control rate. Acute ($D_{54°C}$=0.55 ± 0.07h) cultures did not exhibit increased thermal resistance versus the control ($D_{54°C}$= 0.54 ± 0.07h). Chronic cultures ($D_{54°C}$=0.94 ± 0.24h) were significantly (P<0.01) more thermo-resistant versus control. Chronic cultures ($\Delta D_{54°C}$=0.39h) also exhibited significantly (P<0.04) more thermal-induced injury than control ($\Delta D_{54°C}$=0.04h) and acute ($\Delta D_{54°C}$=0.05h) cultures, determined as difference in CFU/ mL between pyruvate and deoxycholate media. These data show that sublethal contact (acute & chronic) with PAA sanitizer induced peroxidative tolerance and/ or thermal cross-resistance in *E. coli* O157:H7.

P049 EFFECT OF INHIBITORS OF BRANCHED-CHAIN KETO ACID DEHYDROGENASE ON THE GROWTH, FATTY ACID COMPOSITION, AND ENZYME ACTIVITY OF *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes is a foodborne pathogen that is a cause for serious concern because of the high mortality associated with listerial infections, and significant costs of food contamination with the organism. Ready-to-eat foods that are stored at refrigeration temperatures for long periods pose a risk of *L. monocytogenes* infection, because the bacterium can grow at refrigeration temperatures. Addition of a chemical antimicrobial barrier to food may be an avenue for control of the growth of *L. monocytogenes*. It is believed that fatty acid anteiso C15:0 plays a critical role in the ability of

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L. monocytogenes to grow at refrigeration temperatures through imparting an essential fluidity to the membrane. A mutant deficient in branched-chain fatty acid synthesis is markedly impaired in growth at low temperatures. The effects of inhibitors of mammalian branched-chain keto acid dehydrogenase (BCKAD) activity on the growth, fatty acid composition, and BCKAD activity of *L. monocytogenes* were studied. The most potent inhibitors of growth were clofibric acid, sodium-4-(3-thienyl)-2-oxo-butenoate, and sodium-4-(2-thienyl)-2-oxo-buten-oate, and they were more inhibitory at 10°C than at 30°C. However, cells grown in the presence of clofibric acid or sodium-4-(3-thienyl)-2-oxo-butenoate were not deficient in branched-chain fatty acids, suggesting their biosynthesis might have been slowed but not switched to straight chain fatty acids, as in the cold-sensitive mutant. Colorimetric assays indicated that none of the compounds totally inhibited BCKAD activity in cell-free extracts of *L. monocytogenes*. The results suggest that searching for more potent inhibitors of BCKAD activity may be a fruitful direction for future research.

P050 *ZYGOSACCHAROMYCES BAILII* TIME-TO-GROWTH AS AFFECTED BY TEMPERATURE, WATER ACTIVITY, PH, AND ANTIMICROBIALS

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Zygosaccharomyces bailii is frequently implicated in the spoilage of high acid and/or high sugar foods, and can develop resistance to sorbate and benzoate. For foods where shelf stability relies either on acidity, weak acid antimicrobials, or combinations, of these *Z. bailii* time-to-growth must be determined. The effects of incubation temperature (15 or 25°C), water activity (a_w, 0.99, 0.98, or 0.97), pH (4.0, 3.5, or 3.0), and concentration (0, 250, 500, up to 1500 ppm) of potassium sorbate (KS) and/or sodium benzoate (NaB) were evaluated on the time-to-growth of *Z. bailii*. Laboratory media were made for every combination of the five factors, inoculated, incubated for up to a maximum of 30 days, and observed for gas production and/or signs of turbidity. Of the total of 882 combinations tested, *Z. bailii* survived and grew before 30 days of incubation in only 209. At 25°C, yeast growth was observed in 137 combinations of factors, although the rate of growth varied considerably, taking at pH 3.0, between 1 day (without antimicrobials) to >25 days (with 1000 ppm antimicrobial combinations). At 15°C, in only 72 combinations, growth was observed. Inhibitory combinations of factors included at least 250 ppm of KS, or 750 ppm of NaB. In the presence of subinhibitory antimicrobial concentrations, long times-to-growth were observed at 15°C, or with reduced a_w and pH. Our results can be used to assess the efficacy of potassium sorbate and sodium benzoate, used alone or combined, on the spoilage potential of chilled, acidified, and/or sugared food products.

P051 EFFECT OF SALT ON SURVIVAL OF *SHIGELLA FLEXNERI* AS AFFECTED BY TEMPERATURE AND PH

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Shigella, a major foodborne pathogen, survives in salt-containing environments. However, systematic data are scarce. We studied survival of *Shigella flexneri* 5348 in brain heart infusion broth (pH 4 to 6) containing 5 to 9% NaCl. Stationary phase cells were inoculated into media to give initial populations of 6 to 7 $\log_{10}$ CFU/ml and incubated at 4 to 37°C. A two-phase linear inactivation model was applied to plate count data to calculate the time for a 4-$\log_{10}$ decrease in the population (T_{4D}). Growth did not occur at pH 5. At pH 6, the bacteria grew (after an initial decrease in the population) in the presence of up to 6 and 7% NaCl at 19 and 28°C, respectively. In media containing 8% NaCl, T_{4D} values at pH 6, 5, and 4 were 32.4, 21.2, and 5.0 days, respectively, at 19°C, and 21.5, 11.6, and 7.3 days, respectively, at 28°C. Incubation at 37°C in pH 5 media containing 8% NaCl resulted in T_{4D} of 2.6 days, while at 4°C in pH 4 media containing 6% NaCl, the population decreased only about 2-$\log_{10}$ units after 38 days. Results show that *Shigella* is salt tolerant and suggest that salty foods may serve as vehicles for this bacterium.

P052 USE OF POLYSTYRENE FOAM NET CONTAINING SILVER-COATED CERAMIC TO EXTEND SHELF LIFE OF LONGISSIMUS STEAKS FROM KOREAN CATTLE

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Consumers and meat producers desire an improvement in the quality and safety of meat. Developing new packaging materials to extend shelf life of meat is one method to satisfy consumers and meat producers. The objective of this study was to develop a polystyrene (PS) foam net containing silver-coated ceramic (PSN), with antimicrobial properties, to extend shelf life of meat. Longissimus steaks (LS) from Korean cattle were wrapped with PSN or PSN without silver-coated ceramic (PSNW) and packaged in PS foam tray with high density polyethylene overwrap. Volatile basic nitrogen

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(VBN) values, thiobarbituric acid (TBA) values, and pH of LS were determined during storage at 5°C for 5 days. Color was expressed as Hunter L, a and b values. Total aerobic plate counts (APC) and coliform bacterial counts were determined by spiral platings. The pH of meat wrapped in PSN was slightly higher, but not significantly higher when compared with meat in PSNW and packaged without PSN (control). There were no significant differences in Hunter color values of the meat samples during storage. Drip loss from the meat in PSN was lowest for all treatments during storage. TBA and VBN values for meat in PSN were lower when compared with meat in PSNW and the control during storage. When compared with other samples, meat in PSN had the lowest APC and coliform bacterial count during storage. In conclusion, these results indicate that PSN can extend the shelf life of meat.

P053 IMPACT OF HEATING STRESS ON THE BEHAVIOR OF TWO *LISTERIA MONOCYTOGENES* STRAINS IN A BROTH WHICH MIMICS THE CAMEMBERT CHEESE COMPOSITION

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The occurrence of the foodborne pathogen *Listeria monocytogenes* in dairy food products is widely reported. This organism is able to survive under various environmental stress conditions. The aim of this work is to study the impact of heating stress on the growth of two *L. monocytogenes* strains (EGD and 306715) in Richard's broth, which mimics Camembert cheese composition.

After 15.5 h at 37°C in IMM broth (well-defined composition), cultures of strains were heated for 30 min at 56°C. The stress effect was immediately evaluated by plating on TSAYE and TSAYE supplemented with 4% NaCl. Unheated or heated cells were then inoculated in Richard's broth to follow the growth of bacteria at 25°C at pH 5.0 and at pH 7.0. These pH values correspond to the beginning and the end of ripening pH.

The heat treatment induced 15% to 70% survival, with 69% to 95% of stressed cells for the 306715 and EGD strains, respectively. For heated cells growing in Richard's broth at pH 7.0, the lag time was multiplied by 6 factor. At pH 5.0, a bactericidal effect was observed for stressed or unstressed cells. For the 306715 strain, a population level of 2 CFU/ml was reached respectively after 12 h or 50 h with or without stress. For the EGD strain, the population level was obtained after 40 h and not within 70 h for the control. The heat testing condition, similar to cleaning temperatures in dairy plants, allowed reduction of the growth or survival of *L. monocytogenes* cells in broth.

P054 UNRELATEDNESS OF NISIN RESISTANCE AND ANTIBIOTIC RESISTANCE IN *LISTERIA MONOCYTOGENES*

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Nisin, an antimicrobial peptide produced by *Lactococcus lactis*, is used as a preservative in a variety of food products. There is concern that the use of nisin in food can contribute to nisin-resistant mutants, which may be cross resistant with clinical antibiotics. To investigate the relationship between nisin resistance and antibiotic resistance, we determined the frequency of spontaneous antibiotic-resistant mutants for *Listeria monocytogenes* with or without pre-exposure to a slightly inhibitory concentration of nisin (20 IU/ml) for one hour. The frequency of resistance to chloramphenicol was 6.8×10^{-7} for wild type cells and 1.9×10^{-8} for cells pre-exposed to nisin. Similar frequencies of resistance to ampicillin were obtained (2.1×10^{-7} and 3.3×10^{-7}, respectively). Wild type cells were inhibited by between 380 and 550 IU/ml of nisin. In comparison, the antibiotic-resistant mutants had an MIC for nisin ranging from 413 to 553 IU/ml. To determine whether antibiotics and nisin work synergistically or additively, cells were exposed to minimally inhibitory concentrations, alone or in combination. After 21 h, the effect of either antibiotic used in conjunction with nisin was additive. These data indicate that antibiotic-resistant mutants are as sensitive to nisin as wild type cells and that exposure to nisin does not increase the frequency of antibiotic-resistant mutants. Therefore, nisin can safely be used in foods without contributing to microbial resistance of clinical antibiotics.

P055 CHANGES IN POPULATIONS AND ACID TOLERANCE OF *LISTERIA MONOCYTOGENES* IN FRESH BEEF DECONTAMINATION FLUIDS

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Pathogenic bacteria exposed to meat decontamination stress may survive, develop resistance, and pose health risks. The objective was to determine changes in populations and acid tolerance of *Listeria monocytogenes* (10^5 CFU/ml) in decontamination spray-washing fluids (water 10 or 85°C; solutions of 2% lactic or acetic acid, 55°C) of beef top round cuts. The washings were tested with or

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without prior filter-sterilization. The acid tolerance of the pathogen was determined by transferring cells to tryptic soy broth with yeast extract (pH 3.5) and to lactic (pH 2.5) or acetic acid (pH 3.2) washings. In acid washings, *L. monocytogenes* died off (<1 log CFU/ml) after 7, 2-7, and 1 day at 4, 10 and 35°C, respectively. In nonacid washings, the pathogen increased (1-2 log CFU/ml), irrespective of natural flora which, when present, outgrew (10^8 CFU/ml) *L. monocytogenes* after 1, 2 and 4 days at 35, 10 and 4°C, respectively. The pH of acid washings was unchanged, while that of nonacid washings decreased or increased depending on absence or presence of natural flora. These microbial and pH changes affected acid tolerance of *L. monocytogenes*. In sterile nonacid washings at 35°C, *L. monocytogenes* increased its acid tolerance by at least 1 log CFU/ml from day 1 to 8, while in nonsterile washings the organism was acid tolerant at day 1 (<1 log CFU/ml reduction) and became acid sensitive (3-5 log CFU/ml reduction) after 8 days. These results suggest that, depending on its growth rate and metabolic activity, the natural flora of a food environment may harden or weaken pathogens to acid stress and affect food safety.

P056 EVALUATION OF *LISTERIA MONOCYTOGENES* IN VACUUM-PACKED GRAVAD SALMON

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There is an international concern about the presence and behavior of *L. monocytogenes* in ready-to-eat fish products like gravad and cold-smoked fish. In this study, the behavior of *L. monocytogenes* naturally present in vacuum-packed gravad salmon (*Salmo salar*) and the population of acid-producing bacteria were evaluated. Samples of the product were collected the day of production and transferred to the lab, where they were stored under commercial refrigeration (7°C) for up to 90 days according to package stated shelf life. *L. monocytogenes* was quantified using the MPN technique, while acid-producing bacteria were quantified by the spread plate technique. Sensory evaluation of the samples (color, flavor and odor) were also done. Samples were analyzed every 15 days (Day 0, 15, 30, 45, 60, 75 and 90) and 3 trials were conducted. It was observed that the *L. monocytogenes* population varied from <0.03 to 4.27 MPN /g on day 0 to <0.03 to 0.92 MPN/g on day 60. Several samples had their sensory characteristics altered by day 60, and acid-producing bacteria population was high (around 6E -7E UFC/g) since the beginning. The results showed that even if *L. monocytogenes* grows in the product, dangerous populations (>100/g) will not be achieved. Acid-producing bacteria may play an important role in that.

P057 FATE OF *ESCHERICHIA COLI* O157:H7 IN CHANNEL CATFISH POND WATER

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Survival of *Escherichia coli* O157:H7 in channel catfish pond water in a controlled environment was investigated. Water sampled from three catfish ponds in South Georgia was inoculated with *E. coli* O157:H7, containing green fluorescent protein (gfp) and an ampicilin-resistant gene, at levels of 10^5, 10^6, and 10^7 CFU/ml. Water samples were incubated at 26°C for 16 days. The dissolved oxygen of pond water ranged from 5 to 7.5. To simulate the pond environment, tested water was aerated and fish food was added during the incubation. Water samples were analyzed daily and spiral plated on Brain Heart Infusion broth (BHI) containing 150 ug/ml ampicillin. Fluorescent colonies were counted using a portable long wave UV lamp. Approximate 6-log reduction in *E. coli* O157:H7 were observed after 3 days inoculation. Longer survival and less reduction of *E. coli* O157:H7 was observed in water inoculated at log 7 CFU/ml over the inoculation period. However, water from two of the three ponds sustained the survival of *E. coli* up to 15 days. A 1-3 log increase in bacterial counts was observed in all pond water after the third day, followed by a rapid decrease of 6 logs. When no *E. coli* O157:H7 was detected at the lowest dilution, samples were grown in enriched TSB. None of these were positive for *E. coli* growth. The results of this study imply that channel catfish can be contaminated with *E. coli* O157:H7 from the surrounding water.

P058 INTERNALIZATION OF *ESCHERICHIA COLI* OUTSIDE LABORATORY CONDITIONS

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Foodborne illnesses in unpasteurized apple cider have been attributed to the pathogenic bacterium *Escherichia coli* O157:H7. Contamination is likely to occur during the fruit growing and harvesting phases. In apple cider production where the entire apple is pressed, pathogens found within the apple core are a potential problem. Internalization of *E. coli* in apples under natural environmental conditions was addressed in this study using a controlled outdoor setting. A surrogate *E. coli* species, ATCC 25922, was used. The bacterial culture was applied to topsoil and spread evenly on a

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6 × 6-foot area. Red Delicious, Yellow Delicious, and Rome apples were placed randomly on the soil much like drop or windfall apples and it was noted whether the apple fell calyx up or down, or on its side. Apples were examined for the presence of *E. coli* and sampled on days 1, 3, 8, and 10. Skin, flesh, and inner and outer core samples were plated on MacConkey agar supplemented with cyclohex-imide and MUG. *E. coli* was found in the inner core and flesh samples of Yellow Delicious apples, ranging from 486 CFU/ml and 1270 CFU/ml in the inner core on days 1 and 8, and 538 CFU/ml and 683 CFU/ml in the flesh on days 1 and 8. Similar results were observed in the inner core and flesh of Red Delicious and Rome apples. Here internalization was found to occur outside laboratory conditions.

P059 LOCALIZATION AND TISSUE DAMAGE INDUCED BY ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157:H7 IN APPLE TISSUE

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Our study investigates the ability of entero-hemorrhagic *Escherichia coli* O157:H7 (EHEC) to spread in wounded apple tissue and the type of cellular matrix the bacteria invade. Red delicious apples, after being wounded with an artist knife (7 mm depth), were either inoculated with 4µl per wound of decimally diluted EHEC or submerged into EHEC suspended in sterile dH_2O and stored at 37°C for 24 h. Tissue printing was done by first placing membranes on top of infected apple halves, then removing and immunoprobing the membranes with an EHEC specific polyclonal antibody. Core samples of wounded apple tissue infected with EHEC were fixed with 2% glutaraldehyde and 2% paraformaldehyde for transmission electron microscopy (TEM). The immunoblots showed that EHEC traveled from the site of the wound down through the apple tissue to the core at day one for both EHEC treatments. Conversely, EHEC spread from the site of the wound between the epidermis and tissue toward the top of the apple when wounded apples were submerged in EHEC-contaminated dH_2O. In TEM, EHEC formed bacterial aggregates near the apple cell walls and single cells were attached to the cell wall surfaces. Cytoplasmic inclusion bodies and separation of the plasma membranes were observed in EHEC cells grown in apple tissue. EHEC induced in apple tissue degradation of mitochondria, release of ribosomes into the cytoplasma, and reduction in the apple cell wall density. Our study shows that EHEC can adapt to the apple tissue environment and cause degradation of the cellular components.

P060 MODELING THE SURVIVAL OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN UNCOOKED FERMENTED SALAMI

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Outbreaks of foodborne illness and deaths due to *E. coli* O157:H7 associated with the consumption of salami and other types of fermented sausage have caused Canada to introduce directives governing the production of these products. These directives outline process control requirements for each Canadian federally registered establishment. These directives require validation of the manufacturing process. Predictive modeling is gaining wider acceptance among processors and regulatory agencies as a alternative means to ensure product safety. In developing a predictive model for the behavior of *E. coli* O157:H7 in uncooked, fermented sausages, data was collected from commercial processes. Three models were developed that include different variables to describe *E. coli* O157:H7 reduction. The variables selected for inclusion in all models were significant at the $P<0.0001$ level. Model A included the variables a_w, pH, and time. Model B separated the processing stages into fermentation and drying. Variables of time, temperature, and pH were examined in the fermentation stage while time and a_w and their interaction was examined in the drying stage. The third model examined the variables a_w, time at pH 5.3 required to achieve a 2-D reduction of *E. coli* O157:H7, and their interactions. All models were validated. The predicted reduction in *E. coli* O157:H7 in uncooked fermented salami was compared to the observed reduction, and good correlation was found.

P061 GROWTH OF *ESCHERICHIA COLI* O157:H7 IN BIOFILMS WITH MICROORGANISMS ISOLATED FROM MEAT PROCESSING ENVIRONMENTS

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The growth of *Escherichia coli* O157:H7 with competitive strains obtained from meat processing plant environments was studied in biofilms on stainless steel coupons. *Staphylococcus*, *Micrococcus*, *Alcaligenes*, and *Enterobacter* were isolated from the environment of a meat plant and used for this study. *E. coli* O157:H7 and competitive strains were inoculated into two low-nutrient 0.2 and 1% tryptic

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soy broth with coupons and incubated at 21°C. Stainless steel coupons were transferred to fresh media every 48 h to produce the biofilm, until day 8. Coupons removed from broth for analysis were rinsed with sterile phosphate dilution buffer to remove unattached cells and scraped using a sterile teflon spatula in a sterile beaker containing phosphate buffer. Dilutions were plated using tryptic soy agar for total counts and violet red bile agar for *E. coli* counts. Growth of *E. coli* O157:H7 with competitive strains was observed in most biofilm experiments. Especially, *Alcaligenes* and *Staphylococcus* spp. stimulate the accumulation of *E. coli* O157:H7 in biofilms at 1% TSB. None of the competitive strains inhibited the growth of *E. coli* O157:H7 from the biofilm. The effect of nutrient level on the accumulation of *E. coli* O157: H7 was dependent on the type of competitive strains used to produce the biofilm.

P062 GROWTH AND SURVIVAL OF *ESCHERICHIA COLI* O157:H7 AND NONPATHOGENIC *E. COLI* IN CHEDDAR CHEESE CURDS

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Growth and survival of *E. coli* O157:H7 and nonpathogenic *E. coli* were compared during the manufacture and storage of 1.7% and 0.9% NaCl Cheddar cheese curds. For each duplicate vat, milk was inoculated with 3-log CFU/ml *E. coli* (*E. coli* O157:H7 strains F90 or F5854 or nonpathogenic *E. coli* 8104). *E. coli* were enumerated for inoculated milk, curd, and whey during manufacture, in finished curd, and in curds during storage at 21.1, 12.8, and 4.2°C for up to 3, 14, and 28 d, respectively, by plating on MacConkey Sorbitol agar. Additional trials evaluated the survival of strain F5854 in curds packaged with air, 100% CO_2, 100% N_2, or 60%CO_2/40%N_2. All three *E. coli* strains grew during manufacture of cheese curds. Average populations were 5.7-$\log_{10}$ CFU/g curd at packaging; populations were not significantly different among the three strains tested (P>0.05). At 3 d, populations of all *E. coli* strains were similar (5.51+0.20 $\log_{10}$ CFU/g) regardless of salt level or storage temperature. *E. coli* steadily decreased to 4.50 and 3.13-$\log_{10}$ CFU/g at 14 and 28 d, respectively. Packaging curd under modified atmospheres (MA) had no effect on *E. coli* O157:H7 survival through 14 d storage. However, curd packaged in air and stored at 4.2°C for 28 d had significantly lower counts than curd stored in CO_2 and/or N_2. These results suggest that storage temperature and NaCl levels are not significant factors in survival of *E. coli* in cheese curds but that MA packaging to increase shelf life may allow greater survival of *E. coli* O157:H7.

P063 SURVIVAL OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157:H7 IN RETAIL MUSTARD

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Escherichia coli O157:H7 survival in acid foods such as apple cider, fermented sausage, and mayonnaise is well documented. Researchers have determined that *E. coli* O157:H7 can survive in refrigerated acid foods for weeks. The potential of acid foods to serve as a vector of *E. coli* O157:H7 foodborne illness prompted this study to determine the fate of this organism in retail mustard containing acetic acid when stored at room and refrigerator temperatures. Various retail brands of dijon, yellow, and deli style mustard, pH ranging from 3.17 to 3.63, were inoculated individually with three test strains of *E. coli* O157:H7. Samples were inoculated with approximately 1.0×10^6 CFU/gram, incubated at room and refrigerated temperatures, and assayed for surviving test strains at predetermined time intervals. An aliquot was appropriately diluted and plated using Sorbitol MacConkey Agar (SMAC). When the test strain was not recoverable by direct plating, the sample was assayed by enrichment in modified Tryptic Soy Broth and recovered using SMAC. Growth of *E. coli* O157:H7 test strains was inhibited in all retail mustard styles. *E. coli* O157:H7 survived in dijon style mustard for six h at room and two days at refrigerated temperatures. Survival in yellow and deli style mustard was not detected beyond one hour. Overall, test strain survival was greater at refrigerated than room temperature. Retail mustard demonstrates the ability to effectively eliminate any chance contamination by this organism within 1 h to days, suggesting that these products are not a likely vector of *E. coli* O157:H7 foodborne illness.

P064 ENVIRONMENTAL CONDITIONS AFFECTING SURVIVAL OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* TYPHIMURIUM DT104 IN LAND-SPREAD MANURE

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Outbreaks of foodborne illness linked to fresh produce are increasing. To devise preventative measures, it is critical to understand conditions which affect the survival and/or growth of potential pathogens within the crop environment. There is little data available identifying critical control points

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(CCPs) in growing vegetable crops, although fertilization with manure is a likely CCP. This study profiles the decline of the pathogens *E. coli* O157:H7 and *S.* Typhimurium DT104 in land-spread bovine manure exposed to different soils, temperatures, and moisture regimes.

Two Wisconsin soil types were combined with inoculated manure and subjected to two regimens each of temperature, watering, and application. The pathogens were enumerated by selective plating and quantitated by PCR when CFUs could no longer be detected. Sample analysis was monthly for each combination of environments.

Following introduction into soil, the inoculants underwent rapid growth, up to a three log increase, before beginning to decline. Several trends became evident from our treatments:

(1) Bacterial death declined more rapidly at 25° than 10°C.

(2) O157:H7 declined more rapidly than DT104 under similar conditions.

(3) Neither soil type nor watering regime seemed to have appreciably affected the rate of decline.

(4) Mixing manure with soil hastened the rate of decline compared to top-spreading.

Our results suggest that the critical factors for the death of manure-borne pathogens in soil are temperature and application. Plowing manure into soil, to enhance competitive interactions with soil organisms, and warm weather application offer the best conditions for eliminating potential pathogens.

P065 EFFECT OF ANTACID ON SURVIVAL OF *VIBRIO VULNIFICUS* AND *VIBRIO VULNIFICUS* PHAGE IN A SIMULATED GASTROINTESTINAL MODEL

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V. vulnificus can cause primary septicemia or gastroenteritis after consumption of raw Gulf Coast oysters. Both *V. vulnificus* and phages lytic to *V. vulnificus* are abundant in Gulf Coast oysters. The objective of this study was to determine the effect of antacid on survival of *V. vulnificus* and its phages in a simulated (mechanical) human gastrointestinal model. Several strains of *V. vulnificus* or its phages (ca. 10^5 CFU/ml or PFU/ml) in sterile oyster homogenate were introduced individually into the gastric compartment containing simulated gastric juices. Then the mixture was incrementally pumped into the intestinal compartment containing simulated intestinal juices over 2 h. A 30 min resident time in the gastric compartment was required to kill all *V. vulnificus* strains, but *V. vulnificus* surviving the gastric compartment increased up to 2 logs after 9 h in the intestinal compartment. Phages were eliminated after 60 min in the gastric compartment, but phages entering the intestinal compartment remained viable after 9 h, with less than a 2 log reduction. Numbers of *V. vulnificus* decreased 0 to 2 logs in the gastric compartment containing antacid after 2 h and increased 1 to 3 logs in the intestinal compartment during 9 h. Phage numbers decreased 1 log in the model containing antacid after 9 h. The model suggests that the use of antacids may increase numbers of viable *V. vulnificus* cells entering the intestine and may increase the risk of infection.

P066 SURVIVAL OF *VIBRIO VULNIFICUS* IN RAW AND FRIED MUSSELS (*MYTILUS GALLOPROVINCIALIS*) BEING CONSUMED AS TRADITIONALLY IN TURKEY

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Thirty raw samples of mussels (*Mytilus galloprovincialis*) harvested, shucked and prepared for cooking and 30 fried samples cooked at 180-200°C for 5 min were analyzed for the presence of *Vibrio vulnificus*; this is the first report of *Vibrio vulnificus* in shellfish harvested from the Sea of Marmara, Turkey.

Vibrio spp. were isolated from samples, mainly after preenrichment in alkaline peptone water (APW) at 40°C followed by streaking on thiosulfate-citrate-bile salts-sucrose (TCBS) agar. *Vibrio* species grown at 42°C were detected in 73.3% of raw samples. *Vibrio vulnificus* was found in mussels in 8 of 30 (26.7%) shucked, raw samples analyzed. Other species found most frequently were *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio cholera*, and *Vibrio mimicus*, with rates of 36.7%, 20%, 6.7%, and 3.3%. None of the fried samples had *Vibrio* species, and the frying process had apparently destroyed *Vibrio* spp. in mussels.

P067 MICROBIAL POPULATION, CHEMICAL STATUS AND SHELF STABILITY OF SMOKED AND NON-SMOKED COUNTRY-CURED HAMS

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In response to the outbreaks associated with cured products, this research attempts to validate

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the safety of the Country-style ham processing procedures and demonstrate reductions of *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and *Escherichia coli* O157:H7 to safe levels following USDA guidelines. Four groups of 116 11-kg hams were surface inoculated with each microorganism, and then cured, equalized, and divided into smoked and non-smoked treatments, after which hams were aged up to six months. Three different hams per treatment were analyzed following each processing procedure to evaluate microbial population, chemical status, and presence of toxin during the aging time. *L. monocytogenes* populations decreased from 8.4 log_{10}CFU/cm^2 to < 1.0 log_{10} CFU/cm^2 after 178 days of aging. However *L. monocytogenes* growth was positive after an enrichment procedure on inoculated hams. *Salmonella* spp. population decreased from 7.3 log_{10}CFU/cm^2 to <1.0 log_{10}CFU/cm^2 after 122 days. *S. aureus* populations decreased from 8.4 log_{10}CFU/cm^2 to <1.0 log_{10}CFU/cm^2 after 178 days of aging. However, *Staphylococcus* enterotoxin was detected in some of the samples during the aging period. *E. coli* O157:H7 was reduced from an average of 8.4 log_{10}CFU/cm^2 to <1.0 log_{10}CFU/cm^2 after 94 days. These results indicate that the Country ham process can be effective in pathogen reduction. Nevertheless, *Staphylococcus* enterotoxin production is a concern, suggesting that additional investigations related to previous conditions and factors should be conducted.

P068 FATE OF BACTERIAL PATHOGENS INOCULATED ON FRESH PORK DURING SIMULATED TEMPERATURE ABUSE AT DISTRIBUTION

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Fresh meat products may be exposed to improper holding temperatures during transportation, during transit at loading and unloading points, and in coolers that are not properly operating or insulated. A study was designed to investigate the fate of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Salmonella* spp., *Yersinia enterocolitica* and *Listeria monocytogenes* inoculated on fresh pork products subjected to short-term, limited temperature abuse. Ground pork and pork loin chops samples were inoculated with mixed strain cultures of each organism and held in plastic bags at 0, 3.3, 6.7 or 10°C for 24 and 48 h. Duplicate samples were analyzed in each of the three replicates for aerobic plate counts, total coliform counts and for each pathogen using two selective agar media per pathogen. *Campylobacter jejuni* showed no major changes or minor reductions in populations during storage, while *L. monocytogenes* showed inconsequential variation in populations. *Salmonella* spp. showed slight increases (0.4 – 0.7 log CFU/cm^2 or g) at 10°C, while populations of *E. coli* O157:H7 showed minor fluctuations in pork chops and increased by 0.6 – 1.4 log CFU/g in ground pork stored at 10°C. Populations of *Y. enterocolitica* increased after 48 h at 6.7°C and 10°C in pork chops by 0.5 – 1.2 log CFU/cm^2 and at 0, 3.3, 6.7 and 10°C in ground pork by 0.1 – 2.1 log CFU/g. These results further emphasize the importance of minimizing microbial contamination at the production stage as well as maintenance of proper refrigeration temperature during handling, and should be useful in risk assessment studies for enhancement of fresh pork safety.

P069 COOLING RATE EFFECT ON OUTGROWTH OF *CLOSTRIDIUM PERFRINGENS* IN COOKED TURKEY PRODUCTS

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The potential for *Clostridium perfringens* spores to germinate and grow in cooked ready-to-eat turkey products was evaluated to determine a safe cooling rate through the critical temperatures of 48.9°C (120°F) through 12.8°C (55°F). Raw turkey deli breast roasts were inoculated with a cocktail of *C. perfringens* spores (NCTC 8238, NCTC 8239, and NCTC 10388) and cooked in a steam oven to an internal temperature of 72°C. The sample roasts were then cooled through the critical cooling range at rates yielding cooling times of 6, 8, and 10 h. Turkey roasts were analyzed for spore growth and multiplication using tryptose-sulfite-cycloserine agar (100) and anaerobic incubation at 37°C for 48 h. Cooling times of 6 and 8 h caused no proliferation of *C. perfringens* that would violate the USDA/FSIS safe cooling standard criteria, which would allow no more than a 1-log_{10} multiplication between the temperatures of 48.9°C and 12.8°C. A 9.6 h cooling period between the designated temperatures at a 95% confidence interval was determined to be adequate for nonproliferation of *C. perfringens*. On the other hand, a 95% tolerance interval would be more stringent in that it suggests no more than an 8.9 h cooling period. Tolerance intervals state that 95% of all our observations will not exceed the limit of one log_{10} increase in *C. perfringens*. This study indicated that in cooked ready-to-eat turkey deli breasts, a cooling period no greater than 8.9 h should be utilized to prevent possible *C. perfringens* foodborne outbreaks.

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P070 COMPARING ATTACHMENT STRENGTH, HEAT TOLERANCE AND ALKALI RESISTANCE OF PATHOGENIC AND NON-PATHOGENIC BACTERIA ON ORANGE SURFACES

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At fresh juice processing plants, non-pathogenic bacteria may be introduced as surrogate organisms for human pathogens to validate the effectiveness of disinfection treatments. The purpose of this study was to compare the attachment strength, thermal tolerance and chemical resistance of seven potential surrogate organisms (PSO) to human pathogens (*Salmonella* spp. and *Escherichia coli* O157:H7) on orange surfaces. Among the evaluated PSO, *E. coli* ATCC 8739 and *E. coli* ATCC 35218 showed a significantly lower attachment strength (SR = 0.04 and 0.07, respectively) on orange fruit surfaces in comparison to *Salmonella* spp. They were excluded from further evaluation. In thermal tolerance studies, the $D_{70°C}$ values of the tested PSO were either no different from or greater than the pathogens. However, the $D_{80°C}$ values of *E. coli* ATCC 25922 and *Enterobacter cloacae* ATCC 23355 were lower than that of *E. coli* O157:H7. In general, *E. coli* ATCC 11229 exhibited a higher level of alkali sensitivity than both pathogens; *L. plantarum* ATCC 14917 and, to a lesser extent, *L. fermentum* ATCC 9338 showed a significantly greater tolerance to the alkali and alkali cleaner treatments. These results suggest that non-pathogenic bacteria such as *E. coli* ATCC 11229, *L. fermentum* ATCC 9338, and *L. plantarum* ATCC 14917 may be utilized in fresh fruit research to represent pertinent pathogens in their thermal tolerance. Non-pathogenic strains of *E. coli* ATCC 25922 and *L. fermentum* ATCC 9338, on the other hand, may be selected as surrogate organisms to validate the efficacy of disinfection by high-pH fruit cleaners.

P071 POTENTIAL FOR TRANSFERENCE OF INOCULATED AND INDIGENOUS BACTERIA FROM THE NON-WOUNDED RIND OF MELONS TO THE INTERIOR EDIBLE FLESH

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The potential for bacteria, both inoculated and naturally acquired, to transfer from a non-wounded outer rind to the interior edible flesh of cantaloupe and honeydew melons was evaluated. The non-wounded rind of cantaloupe does not appear to be an absolute barrier to bacterial transference of artificially inoculated or indigenous bacterial contaminants. Comparative tests with laboratory strains *Salmonella* Typhimurium LT2 and *Pseudomonas fluorescens* A506 showed that transference to tissue 5mm and 10mm below the netted rind of cantaloupe is infrequent but detectable. The frequency of transference increases with the concentration of applied bacteria, time of storage, and temperature of storage. The frequency of transference to 5mm and 10mm below the netted rind is higher at the low netted and thin-rind ground spot. High rates of transference appeared to be associated with conditions that favor fruit softening and sub-rind penetration of fungi. Inoculated bacteria applied to the blossom-end scar or stem-end scar were occasionally detected in the sub-rind flesh after storage. For honeydew, apart from natural openings at the stem and blossom-end, transference to the edible flesh from a non-wounded surface was not demonstrated. Enhanced detection of *Salmonella* in sub-surface flesh was facilitated by the combination of immunomagnetic separation and a *Salmonella*-specific PCR system.

P072 SURVIVAL OF POLIOVIRUS ON FRESH PRODUCE

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This study was performed to ascertain the potential for enteric pathogenic viruses (using poliovirus as a model) to survive on various foodstuffs at temperatures commonly used for their household storage. The studies were performed using fresh produce stored over a period of up to two weeks, which was considered to represent the maximum time elapsing between purchase and consumption. The quantities of food used were those which would normally constitute a portion for consumption in the United Kingdom. Each food sample was seeded with viruses and analyzed at appropriate time intervals. Extraction and concentration of viruses from food was performed by elution, followed by differential centrifugation. Virus numbers in the final extracts were determined by quantal cell culture, and the decimal reduction times (D-value), or number of days taken for the initial virus numbers to decline by 90%, were calculated.

In summary, the results (Food (storage temperature), D-value)were: 30 g lettuce (4°C), 5.0 days; 10 g green onion (4°C), no decline; 90 g white cabbage (4°C), 6.0 days; 60 g raspberries (4°C), no decline; 100 g strawberries (-20°C), at least 5.6 days.

The results showed that viruses could persist on fresh fruit and vegetables for several days under the conditions commonly used for storage in households. Therefore, if contamination has occurred

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before purchase, there will always be a risk of viral infection from consumption of the food.

P073 CYTOTOXICITY AND BUFFERING CAPACITY OF AN ALKALINE TOLERANT DAIRY-ASSOCIATED *BACILLUS* ISOLATE

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A *Bacillus cereus*-like strain (*Bacillus*5), isolated from alkaline dairy wash solutions and capable of attachment to and growth on stainless steel surfaces at alkaline pH, was used in this study. Cytotoxicity of *Bacillus*5 was tested against McCoy mouse cells using fluorescent viability stains in conjunction with confocal scanning laser microscopy (CSLM), and scanning electron microscopy. The internal buffering capacity of *Bacillus*5 cells grown at pH 7 and 12 was also tested and compared to that of a control, *B. cereus* ATCC 10702. CSLM combined with fluorescent staining indicated that the McCoy mouse cells died after 24 h exposure to supernatants of *Bacillus*5. The cytotoxic effects of *Bacillus*5 appeared to be membrane active and resulted in cell necrosis. Furthermore, disrupted cell surfaces and leaking cells were observed in corresponding scanning electron micrographs, confirming CSLM observations. Generally, *Bacillus*5 cells in suspension had a higher pH when grown at pH 7 and 12 when compared to *B. cereus* ATCC 10702 cells, indicating improved tolerance to alkaline pH by *Bacillus*5. The internal alkaline buffering capacity for both *Bacillus*5 and *B. cereus* ATCC 10702 cells was, however, small indicating that the buffering capacity was associated with the outside of the cell wall. Growth under alkaline conditions of potentially cytotoxic *Bacillus* spp. attached to processing equipment may thus have food safety implications if it results in post-pasteurization contamination of dairy products.

P074 TWO NOVEL GENES RELATED TO LOW TEMPERATURE GROWTH OF *LISTERIA MONOCYTOGENES* AS IDENTIFIED USING TRANSPOSON-INDUCED COLD SENSITIVE MUTANTS CLD-14 AND CLD-27

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The foodborne pathogen *Listeria monocytogenes* is able to grow at refrigeration temperatures, and this can lead to contamination of food when stored refrigerated. We are interested in understanding the molecular mechanisms of psychrotrophy in *Listeria monocytogenes*, in the hope that this knowledge will eventually lead to new and improved methods of control of the organism. One approach has been to generate transposon Tn917 induced cold-sensitive mutants (including cld-14 and cld-27) to identify affected genes. Antibiotic selection of *E. coli* cells transformed with genomic DNA from cld-14 and cld-27, which is ligated after digestion with XbaI, leads to the cloning of the interrupted genes from these mutants. Sequencing analysis of the clone obtained from cld-14 indicated that a novel gene encoding a hypothetical protein within the yqfF locus was interrupted by Tn917, based on a homology search using the open reading frame of the gene. A Blast Search against the Micado Database indicated that the gene shares 66% homology over 345 bp with the *Bacillus subtilis* locus yqfF, which is located between the yqfG and phoH genes. Sequencing analysis of the available data from the gene interrupted by Tn917 in cld-27 is not yet definitive. Further sequence analysis will help explain the structure of the two new genes and their roles in low temperature growth.

P075 TRANSPOSON INSERTIONS IN BRANCHED-CHAIN ALPHA-KETO ACID DEHYDROGENASE REGION OF TWO COLD-SENSITIVE *LISTERIA MONOCYTOGENES* MUTANTS

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Listeria monocytogenes has the ability to grow at refrigeration temperatures, and two transposon Tn917-induced cold-sensitive mutants, incapable of growth at low temperature, had dramatically decreased contents of branched-chain fatty acids. Branched-chain fatty acids are believed to impart an essential fluidity to the *L. monocytogenes* membrane that allows growth at low temperatures. The DNA flanking the transposon insertions in mutants was cloned and sequenced, and the genes were analyzed. The results showed that there were four open reading frames (ORFs) in the region. The deduced amino acid sequences had high homology (53-74% identity) to the E1 alpha, E1 beta, E2, and E3 subunits of branched-chain alpha-keto acid dehydrogenase (BCKAD) in *Bacillus subtilis*. The organization of the three ORFs E1 alpha, E1 beta and E2 was similar to that of BCKAD region in *B. subtilis*. The transposon Tn917 in mutant cld-1 was located in the gene that encoded the E2 subunit, while in mutant cld-2 it was located in the gene that encoded the E3 subunit. However the typical -10 and - 35 regions were not found upstream of

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the genes. The inverted repeats located downstream of the E2 gene may function as a transcription termination structure. Interruption of BCKAD genes is consistent with the branched-chain fatty acid deficiency in the mutants.

P076 A RISK-BASED EVALUATION OF TRADITIONAL AND SOCIAL MARKETING METHODS OF FOOD HYGIENE EDUCATION

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The domestic environment is an important location for outbreaks of foodborne disease, and educating the consumer about food hygiene is therefore essential. The use of the social marketing approach for consumer education has proven to be successful in other areas of public health. The objective of this research was to evaluate food hygiene education based upon social marketing principles in comparison to more traditional health education methods.

Traditional interventions assessed included a leaflet and a video documentary. Use of the social marketing approach required identification of specific behavioral objectives and an in-depth understanding of a target audience leading to the development of consumer oriented highly focused intervention materials. Participants were observed using CCTV and their hygiene practices were scored while they prepared a set meal in a model domestic environment. Risk scores were gained before and after hygiene education interventions. The total mean risk score before intervention was >14000, representing many food handling malpractices and a consequent need for intervention. Traditional interventions based solely on the distribution of a leaflet or video did not produce a significant decrease ($P<0.05$) in overall risk score. Utilization of the social marketing approach reduced the total risk scores to >9000 immediately after intervention and to >8000 one month later. Scores for the specific behavioral objectives also decreased, but improvements in handwashing practices were short lived, while improved cleaning of utensils in contact with raw foods were more persistent. The approach described allows an objective evaluation of food hygiene education based on behavioral change. Immediate and long term assessments of the effects of specific intervention materials can also be gained, providing valuable information for further initiatives.

P077 FOODBORNE DISEASE REPORTING IN AMERICA: CLOSING THE GAPS IN OUR FEDERAL FOOD-SAFETY NET

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Foodborne illness causes as many as 5,000 deaths and 76 million illnesses annually. While the Centers for Disease Control and Prevention (CDC) and the states investigate outbreaks, there is no readily available list of outbreaks. Such a list is needed by policy makers, researchers, the food industry, and the public to monitor trends, issue public health alerts, change production practices, and, ultimately, prevent illnesses and deaths caused by contaminated foods.

To help fill that gap, CSPI compiled an inventory of 352 foodborne illness outbreaks that occurred between 1990 and 1999. Eggs were responsible for 123 outbreaks. Meat and poultry products caused 77 outbreaks. Produce caused 42 outbreaks, and seafood was responsible for 39 outbreaks.

CSPI's inventory of outbreaks is compiled from numerous documented sources, such as CDC's *Morbidity and Mortality Weekly Report*, medical journal articles, and abstracts from conferences. Journal articles were found by searching the National Library of Medicine's database using the names of foodborne pathogens as keywords.

While preventing foodborne illness is a complicated task requiring many changes, surveillance of outbreaks could be improved greatly if the following steps were taken:

(1) CDC should serve as the national clearinghouse that maintains a comprehensive inventory of outbreak investigations and issues timely reports on all outbreaks;

(2) States should report all foodborne illness outbreaks to CDC; and

(3) Congress should provide adequate funding to the CDC and the states under the National Food Safety Initiative to improve outbreak surveillance and release of information to the public.

P078 FOOD HANDLERS' BELIEFS ABOUT FOOD SAFETY PROCEDURES AND RISKS

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There is some doubt about the efficacy of existing training methods in changing food handler

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behavior. Some studies have indicated that food handlers' knowledge of food safety appears to improve following a food hygiene training course; however, this does not necessarily result in a behavioral change. Considering improper food-handling practices have been quoted as contributing to approximately 97% of foodborne illnesses in foodservice establishments, it is vital that researchers better understand the factors affecting foodhandler behavior before attempting behavioral change.

The beliefs of 114 foodhandlers in small to medium-sized food businesses in Wales were obtained using a face-to-face questionnaire administered at their premises. 62% of foodhandlers admitted to sometimes not carrying out all the food safety behaviors at every appropriate occasion and 6% stated that they often did not carry out all the food safety behaviors. Yet the mean perception of risk of someone contracting food poisoning from these businesses was very low (1.92 where 1= no risk at all and 10 = a great deal of risk), and foodhandlers also believed that they had a high level of control over whether or not they carried out food safety procedures (9.35 = mean where 1 = no control and 10 = complete control). The qualitative data revealed that lack of time appears to be the most quoted barrier which prevents foodhandlers from carrying out these procedures.

This research indicates a need for further research into the factors affecting foodhandler beliefs, attitudes, and behavior in the workplace. The results will have implications for the development of future training strategies in the food industry.

P079 THE REPEATABILITY AND REPRODUCABILITY OF FOOD SAFETY BEHAVIOR IN THE DOMESTIC ENVIRONMENT

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Worldwide incidence of foodborne disease has increased dramatically in recent years and data suggest that inadequate food handling behavior in the domestic environment may be an important factor. Most consumer studies have determined knowledge of food safety or self reported food safety practices. The small number of studies on actual practices have been based on single meal preparation, and information on the consistency of food hygiene implementation is lacking.

Participants were observed using CCTV in a model domestic environment. A risk-based scoring system was developed to differentiate between minor changes in food hygiene behavior. Risk scores were allocated according to bad practice, so a higher risk score represented increased food handling malpractices. The system was used to score participants' food hygiene practices repeatedly preparing the same meal (repeatability) and preparing 3 different meals (reproducibility). Results indicate no significant differences ($P < 0.05$) between the risk scores obtained following repeated preparation of the same meal. Variation in risk score increased with complexity of the meal, and the more complex meals resulted in significantly higher risk scores ($P < 0.05$). However, comparisons of 4 key hygiene practices indicated there was consistency of implementation between meals, i.e., if particular hygiene practices were used in preparing one meal they were usually used in preparation of other meals. The results suggest that important food handling behaviors may be habitual. The implications of the results will be discussed within the context of assessing food hygiene interventions based on behavior.

P080 PREVALENCE OF UNSAFE PRACTICES DURING PREPARATION OF HOMEMADE FOOD IN ARGENTINA

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The World Health Organization regards illness due to contaminated food as one of the most widespread health problems in the contemporary world. For infants, immunocompromised people, pregnant women, and the elderly, the consequences can be fatal. Consumers play an essential role in the prevention of foodborne diseases, both during food preparation and in deciding whether to have certain foods that may be contaminated with disease-causing organisms. Numerous reports describe what consumers can do to improve food safety in their own households. However, there is little information reporting the frequency of certain practices associated with sanitary risks in developing countries. Thus it is important to identify unhygienic preparation practices to intensify consumer awareness in those areas.

A written questionnaire was prepared to evaluate common errors in food handling, such as: personal practices (handwashing, cross contamination), insufficient cooking or reheating of food, hot

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and cold ingredient preparation and holding (time, temperature and product handling), general kitchen facilities, and consumption of high risk foods. In all of the 107 cases analyzed, at least one violation of safety rules was reported. Over half of the respondents consume food that includes raw eggs. About 20% "only sometimes" wash their hands before cooking and 32% neglects to wash cutting boards properly after using them with raw meat or poultry. Most of the people (71.6%) employ unacceptable reheating criteria.

The results of such study could prove useful to identify the most common hazardous practices or habits to intensify consumer education not only in Argentina but in other Latin American countries as well.

P081 EVALUATION OF A TARGETED INTERVENTION FOOD SAFETY PROGRAM FOR WOMEN WHO ARE PREGNANT AND/OR HAVE YOUNG CHILDREN

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Pregnant women and young children are at risk for serious outcomes of foodborne illness. The purpose of this study was to compare the effectiveness of using targeted food safety information to that of general messages for changing food-handling behaviors among women who were pregnant or had young children.

Pregnant women and mothers of preschoolers were recruited to complete a food safety survey (n=273). The survey questions covering four topics: food storage, kitchen sanitation, adequate cooking, and personal hygiene. Questions were based on surveys tested and validated in studies done at Washington State University, Cornell University, and the FDA. Participants were randomly divided into targeted information, general message, and control groups. The targeted information group received personalized mailings specifically addressing their risky food safety behaviors and educational materials. The general message group received only the educational materials. Controls received no materials.

Analysis of post-test surveys revealed frequency of temperature abuse of foods decreased in all groups, with the targeted intervention group more likely than the other groups to report behavior change. The targeted intervention group found it easy to adopt the recommendation to wash kitchen areas with soap and water following handling raw chicken, but they were resistant to adopt the use of sanitizer. Recommendations to check meat texture and color for doneness were adopted but the adoption rate for thermometer use was low. The preference for undercooked hamburgers and eggs was resistant to change in all groups. Adopting recommendations seems to be related to ease of following the procedure.

P082 COST, BENEFITS, AND ATTITUDES TOWARDS HACCP IMPLEMENTATION IN ENGLISH BUTCHERS' SHOPS

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In response to the widely publicized *E. coli* O157 outbreak from a Scottish butchers' shop in 1996, UK butchers have been encouraged to implement HACCP via government funded initiatives providing free training and consultancy.

In conjunction with the Meat and Livestock Commission, responsible for the English HACCP Initiative, postal questionnaires were distributed to a convenience sample of the first 1000 butchers to implement HACCP, achieving a 33.4% response rate. Managerial beliefs and attitudes towards the initiative were surveyed, with 73% of business managers agreeing that HACCP could not have been implemented without the assistance provided, although larger businesses were significantly less likely to agree ($P < 0.05$). Only 13% agreed that HACCP was difficult to apply to butchers' shops. Significant improvements were identified in managers' perceived HACCP-related knowledge and ability as a result of the training provided ($P < 0.05$). Businesses spent an average of £520 and 61 man-h implementing HACCP. Ongoing monitoring and documentation activities accounted for an average of 7.2% of the man-h available each week. Average weekly hygiene management costs increased by £8.45 and 5.4 man-h as a result of HACCP implementation.

These results demonstrate the contribution made by the subsidized training and consultancy provided in significantly improving managerial HACCP knowledge and ability and in making the implementation of HACCP a more financially viable option. With HACCP being increasingly recommended for all food businesses, the results of this study provide unique data against which to assess the potential impact of similar initiatives in other industry sectors.

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P083 **DEVELOPMENT OF A COMPETITIVE EXCLUSION PRODUCT TO REDUCE *ESCHERICHIA COLI* O157:H7 IN CATTLE**

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Several experiments were conducted to develop a competitive exclusion product containing lactic acid bacteria (LAB) to inhibit *E. coli* O157:H7 in cattle. Fecal samples from cattle that were not shedding *E. coli* O157:H7 were collected every three weeks over a period of twelve weeks. LAB to be screened for inhibitory activity towards *E. coli* O157:H7 were isolated from cattle feces by repeated plating on MRS and LBS agar. Six hundred eighty-six pure colonies were isolated by the end of twelve weeks and each isolate was tested for inhibition using an agar spot test. A four-strain mixture of *E. coli* O157:H7 was used to screen LAB for inhibitory action towards *E. coli* O157:H7. Three hundred fifty-five isolates (52%) showed significant inhibition towards *E. coli* O157:H7. The 75 isolates showing maximum inhibition were screened for acid and bile tolerance. For acid tolerance, growth was monitored at pH 2, 4, 5, during a 24-h period. For bile tolerance, growth was monitored in MRS broth with 0.05, 0.15 and 0.3% oxgall during a 24-h period. The majority (90%) of the isolates grew at all levels of acid and bile. Additionally, all isolates survived frozen storage at -70°C for 3 months. Isolates were identified using the API system. The following strains of LAB were most commonly identified, *Lactobacillus acidophillus*, *L. fermentum*, *L. delbreukii*, *L. salivarius*, and *L. cellobiosus*.

P084 **ISOLATION AND SELECTION OF LACTIC ACID BACTERIA FROM MEAT PRODUCTS TO INHIBIT FOODBORNE PATHOGENS**

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Strains of lactic acid bacteria (LAB) were isolated from raw poultry products and ready-to-eat (RTE) meat products. The inhibitory action towards foodborne pathogens was determined. The first set of LAB was isolated from ground turkey and chicken breast and the antagonistic action towards *Escherichia coli* O157:H7, *Salmonella* spp., and *Salmonella* Typhimurium DT104 at room temperature and 12°C was determined. The second set was isolated from pork-based RTE meat products and the antagonistic action towards *Listeria monocytogenes* was determined at 37°C, 12°C and 5°C. Methods that favored the isolation of LAB resulted in a total of 67 strains being obtained. Of the 67 strains, 49 were from the RTE pork products while 18 were from the raw poultry products. Agar spot tests indicated that 10 of the poultry isolates inhibited both *E. coli* O157:H7 and *Salmonella* spp. during growth at room temperature and during storage at 12°C. Of the 10 isolates, 9 were identified as *Lactococcus lactis* ssp. *cremoris* and the remaining one as *Streptococcus constelatus*. Of the 49 pork isolates, 15 of them inhibited *Listeria monocytogenes* during growth at 37°C and during storage at 12°C and 5°C. We selected the 6 of these isolates that resulted in the greatest inhibitory action. API identification indicated that 5 of these pork isolates were *Lactobacillus plantarum*, while the remaining isolate was *Lactobacillus brevis*. The chosen isolates could be added to raw and RTE meat products to inhibit foodborne pathogens during distribution and storage.

P085 **BIOCONTROL OF MOLD GROWTH USING *BACILLUS PUMILUS* AND *LACTOBACILLUS* SPECIES ISOLATED FROM FOODS**

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Antifungal activity of *Bacillus pumilus* and *Lactobacillus* species isolated from foods was studied. Several strains of *B. pumilus* and partially purified antifungal metabolites of *B. pumilus* inhibited mycelial growth of numerous species of *Aspergillus*, *Penicillium*, and *Fusarium*. All *Lactobacillus* strains tested exhibited a certain degree of inhibition of growth and spore production by test strains of *Fusarium*, *Penicillium*, and *Aspergillus*. The metabolites of both *B. pumilus* and *Lactobacillus* spp. were found to be heat stable and remained active after sterilization at 121°C for 30 min. The activity of *B. pumilus* was stable over a wide range of pH (2-10), whereas the activity of *Lactobacillus* spp. was stable only within a pH range from 3 to 6. However, it was concluded that the inhibition of mold growth was not due to organic acids produced by either *B. pumilus* or *Lactobacillus* species, since the inhibitory activity was still exhibited by supernatant fluids of neutral pH value. The metabolites of both *B. pumilus* and *Lactobacillus* strains, were resistant to hydrolysis by various proteases, peptidases, and other enzymes. The inhibition of mold strains was detected by both simultaneous and deferred antagonism assays, suggesting that the inhibitory activity was due to extracellular metabolites produced by *B. pumilus* and *Lactobacillus* species in cell-free supernatant fluids of cultured broth. The metabolites were produced over a wide range of temperature (25 to 37°C, 37 to 45°C) and

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pH (4 to 9, 6.2 to 8) growth of *B. pumilus* and *Lactobacillus* species, respectively.

P086 EMPLOYING *CITROBACTER RODENTIUM* AS A SURROGATE FOR *ESCHERICHIA COLI* O157:H7 IN A MOUSE MODEL TO INVESTIGATE THE EFFECTS OF THE PROBIOTIC *LACTOBACILLUS ACIDOPHILUS* ON PATHOGEN BINDING IN THE LARGE INTESTINE

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The pathogenesis of enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *E. coli* is quite similar. *Citrobacter rodentium* is a mouse homologue to EPEC. *C. rodentium* colonizes the distal portion of the colon and causes transmissible murine colonic hyperplasia (TMCH). The main hypothesis for our studies was that the probiotic bacterium *L. acidophilus* would attenuate hyperplasia caused by *C. rodentium*. Four groups of five mice each were given $\sim 10^9$ CFU/ml bacterial suspensions or phosphate buffered saline (PBS) orally on first day of a 13-day study. Group 1 (controls) received PBS, Group 2 received *C. rodentium*, Group 3 received *L. acidophilus*, and Group 4 received concurrently, *L. acidophilus* and *C. rodentium*. On day 13, mice were euthanized and a 4 cm segment of the distal large intestine was excised and weighed to determine the degree of hyperplasia. The average colon segment weights (mg) for the groups were: Group 1, 78.8 ± 4.7; Group 2, 102.3 ± 12.3; Group 3, 71.8 ± 4.5; Group 4, 130.6 ± 11.5 ($P<0.05$ versus control). A second study employed two groups of five mice each; both groups received *L. acidophilus* daily for 20 consecutive days. Additionally, on day 7, Group A received PBS and Group B received *C. rodentium*. The average sample weights (mg) on day 20 were: Group A, 74.4 ± 5.7; Group B, 94.4 ± 13.2. These data indicate that an in vivo murine model employing *C. rodentium* as a surrogate for *E. coli* O157:H7 will be beneficial to elucidate the pathogenesis of *C. rodentium* and potential protection by probiotic bacteria.

P087 PURIFICATION AND CHARACTERIZATION OF AN ANTILISTERIAL BACTERIOCIN PRODUCED BY *LEUCONOSTOC* SP. W65

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Listeria species are among the most prevalent foodborne pathogen since those species have the ability to withstand various environmental conditions such as low pH, refrigeration conditions, and high salt concentration. The purpose of this study was to characterize an antilisterial substance produced by *Leuconostoc* sp. W65 and to evaluate individual effects of pH, temperature, and time on the inhibitory activity using Response Surface Methodology. *Leuconostoc* sp. W65 was grown and maintained for 48 h at 20°C in BHI medium, and *Listeria* strains were grown on TSB at 37°C. Bacteriocin was purified by ammonium sulfate precipitation, hydrophobic interaction chromatography, and tricine-SDS PAGE from the culture supernatant of *Leuconostoc* sp.W65. The antimicrobial substance produced by *Leuconostoc* sp. W65 was inactivated by pronase-E and protease-K, suggesting it to be proteinaceous in nature. The bacteriocin inhibited the growth of *Listeria monocytogenes*, *L. innocua*, *L. ivanovii*, whereas other pathogens, including Gram-negative bacteria, were not susceptible. Compositional analysis originally estimated the peptide was 56-57 amino acids in length without asparagine, glutamine, and tryptophane. Bacteriocin production in BHI broth reached a maximum activity of 800 AU/ml during the early stationary phase after 48 h incubation at 20°C. In the presence of 25 and 50 AU/ml bacteriocin, *Listeria* spp. showed 5- and 6-log reductions of *L. ivanovii* respectively after 5 h. These results suggest that the bacteriocin produced by *Leuconostoc* sp. W65 is potentially useful in controlling *Listeria*, which is one of the most prevalent pathogens in minimally processed food products.

P088 RESISTANCE OF *LISTERIA MONOCYTOGENES* TO BACTERIOCINS OF LACTIC ACID BACTERIA

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Listeria monocytogenes is a foodborne pathogen; its physiological characteristics make this species difficult to control in food. Bacteriocins of lactic acid bacteria are natural antimicrobial peptides and have been proposed for preventing the growth of *L. monocytogenes* in food. Nisin, produced by some *Lactococcus lactis* subsp. *lactis* strains, belongs to the class I bacteriocins. Curvaticin 13, a bacteriocin produced by *Lactobacillus curvatus* SB13, could be a pediocin-like bacteriocin (class IIa). Experiments were realized in trypticase soy broth supplemented with 0.6% yeast extract (pH 6.5). Both bacteriocins had a transitory bactericidal effect against *L. monocytogenes* ATCC 15313. The regrowth was not due

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to the loss of the bacteriocin activity. Surviving cells to nisin or curvaticin 13 were more resistant to the respective bacteriocin than the parental strain. Survivors to curvaticin 13 were resistant to the class IIa bacteriocins (carnocin CP5, pediocin AcH) but remained sensitive to nisin. The behavior of nisin (1,000 IU/ml)- or curvaticin 13 (640 AU/ml)-resistant variants (Nis1000, Curv640) was investigated in the presence of nisin or curvaticin 13 at 22°C and 37°C, and compared to that of the parental strain. Nisin effectiveness was the same at both temperatures, whereas curvaticin 13 displayed a faster bactericidal action at 37°C. Nis1000 cells were less sensitive to curvaticin 13 than the parental strain, whereas Curv640 cells were more sensitive to nisin than the parental strain. However, the combination of both bacteriocins avoided the regrowth of bacteriocin-resistant cells.

P089 BOTULINAL TOXIN PRODUCTION IN REDUCED-FAT AND FAT-FREE PASTEURIZED PROCESS CHEESE PRODUCTS

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The effects of cheese-base type, fat, 0.05% monolaurin, 1.5% Cheddar enzyme modified cheese (EMC), 1.5% sodium lactate, and 3% b-glucan fat replacer on botulinal toxin production in pasteurized process cheese products (PPCP) were evaluated. To evaluate the effect of cheese-base type, PPCP were formulated using full-fat Cheddar (FF), 30% reduced-fat Cheddar (RF), or skim milk cheese (SC), respectively, and standardized to 59% moisture, pH 5.8, 3 or 4% total salts, and 15% fat using anhydrous milk fat. Subsequent trials evaluated the effect of the adjunct ingredients in PPCP made with SC, RF and FF cheese (<1, 10, and 20% final fat level, respectively). Botulinal toxin production was delayed several days in 15%-fat PPCP formulated with SC compared with RF or FF cheese; however, the effect was not statistically significant ($P>0.05$). When fat levels were not standardized, toxin production was significantly delayed in products made with SC compared with RF or FF cheese. Reducing fat in SC-PPCP formulations from 15 to <1% resulted in a 2-week delay for toxin production. Sodium lactate significantly delayed toxin production for all cheese types tested, whereas the fat-replacer did not delay growth. Monolaurin and EMC significantly delayed toxin production in SC products but had less effect in RF and FF products. These results verify that reduced-fat PPCP manufactured with fat-free and reduced-fat cheese may exhibit greater stability than full-fat products and that safety may be enhanced by using certain adjunct ingredients.

P090 ANTIMICROBIAL ACTIVITY OF SEVERAL SPICES AND ORGANIC ACID SOLUTIONS TESTED AGAINST *ARCOBACTER BUTZLERI*

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As advancements in food processing and distribution arise, there is continued interest in the use of GRAS substances which exert antimicrobial effects against foodborne pathogens.

While the inhibitory effects of antimicrobials like spices and organic acids have long been studied against traditional pathogenic bacteria, no research to date has examined their effects on the emerging foodborne pathogen, *Arcobacter butzleri.* This study focused on the antimicrobial effects of fourteen spice aquaresins, ten oleoresins, four essential oils, and three organic acids tested against two strains of *A. butzleri.* Minimum inhibitory concentration (MIC) values were obtained for each spice and organic acid solution using a standardized antimicrobial disk susceptibility protocol. Spices were tested at concentrations ranging from 0.05 to 50.00%. While many of the spices failed to inhibit growth at concentrations of 50.00%, it was discovered that cinnamon aquaresin (1.56%) and oleoresin (3.13%) and pimento leaf essential oil (3.13%) were most effective against both strains.

Additional spices which demonstrated antimicrobial activity included clove aquaresin and oleoresin, barbecue aquaresin, oregano aquaresin and oleoresin and the essential oil of sweet basil. All three of the organic acids tested (acetic, citric and lactic) exhibited antimicrobial activity at all concentrations tested (0.5 to 5.0%). These results should be considered by food scientists and processors when improving current food applications and implementing hurdle technologies aimed at minimizing human illness associated with *A. butzleri.*

P091 TRANS-2-HEXENAL AS AN ANTIMICROBIAL AGENT

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The natural volatile compound, Trans-2-hexenal is produced by leaf and fruit parts as a defense mechanism against injury. It has been found to be highly effective in reducing mold growth on strawberries, blackberries and grapes. The possible use of this compound as an effective agent against bacterial pathogens on various produce items was studied, and a method for its use as an alternative fumigant was developed. In vitro studies showed

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species such as *Listeria monocytogenes*, *E. coli* O157:H7, *Salmonella* Typhimurium, *Salmonella enteritidis*, *Shigella flexneri*, and *Bacillus cereus* can be effectively controlled by this natural agent. Studies using table grapes (Thompson seedless) with a surface inoculum of 7.0 $\log_{10}$ CFU following exposure to 25, 50 or 100 microliters of compound, demonstrated a population reduction of >99.5%. This reduction can be consistently achieved by exposure to trans-2-hexenal for a minimum of 24 h at 25 microliters and 12-24 h for 50 and 100 microliter volumes, respectively. The compound concentration in the vapor phase was highest at approximately 12 h following initial exposure at room temperature. These results indicate that trans-2-hexenal can be used to control pathogens on grapes. Additional studies are necessary to determine the compound's effectiveness in other types of produce.

P092 CARVACROL, CITRAL, EUGENOL, THYMOL, VANILLIN, POTASSIUM SORBATE AND SODIUM BENZOATE INHIBITORY CONCENTRATIONS FOR *ASPERGILLUS FLAVUS* AT SELECTED WATER ACTIVITIES AND PHS

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The antimicrobial activities of natural compounds from several spices used as flavoring agents in foods have been recognized for many years. However, data on the effect these compounds in combination with other factors on mold's growth are scarce. Our objective was to determine the inhibitory concentrations of selected antimicrobials from natural (vanillin, thymol, eugenol, carvacrol or citral) or synthetic (potassium sorbate or sodium benzoate) origin on *Aspergillus flavus* in potato-dextrose agar (PDA) formulated at selected water activity (a_w, 0.99 or 0.95) and pH (4.5 or 3.5). PDA was prepared with sucrose and hydrochloric acid to adjust a_w and pH. Selected amounts (100, 200, 300, up to 2000 ppm) of vanillin, thymol, eugenol, carvacrol, citral, potassium sorbate (KS) or sodium benzoate (NaB) were added. Triplicate plates for each antimicrobial agent and concentration were inoculated, incubated at 25°C, and observed during 60 days. Minimal inhibitory concentration (MIC) was defined as the minimum required to inhibit growth. MICs varied from 200 ppm of NaB (a_w 0.99, pH 3.5), carvacrol or KS (a_w 0.95, pH 3.5) to 1800 ppm of citral (a_w 0.99, both pHs). Important differences in the inhibitory concentrations were found among antimicrobials. In general, MICs of compounds from natural origin were less pH dependent than MICs of synthetic antimicrobials. Considerable differences in mold sensitivity to antimicrobials were observed. Therefore, it is possible to select antimicrobials on a rational basis and find further applications in real foods for natural antimicrobials in combination with a_w and pH reduction.

P093 ANTIMICROBIAL EFFECT OF HONEY ON HYDRATED BATTER MIX

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Sixteen samples of honey products from five different floral sources were purchased to determine their inhibitory effect on the growth of *Staphylococcus aureus* in both a laboratory medium and hydrated batter mix system. Brain heart infusion (BHI) broth and hydrated batter mix (1000 CPI) mixed with honey for final concentrations of 0, 5, 10, and 20% were inoculated with *S. aureus* (ATCC 13565) at 10^5 CFU/ml. Baird-Parker agar (BPA) mixed with Bacto EY Tellurite enrichment was used as medium for enumerating *S. aureus* by using an Autoplate 4000 Spiral Plater. The plates were incubated at 37°C for the BHI and at 4 and 25°C for the batter mix. Plating was done at 0, 6, 12 and 24 h for estimating the antibacterial action during the 24-h period. Total plate counts of the honey products ranged from 2.19 to 3.5 log CFU/g. Out of sixteen honey samples, only three had significantly lower *S. aureus* counts (by 3 logs) when 20% honey was incorporated into BHI. Hydrated batter mix showed a significantly lower *S. aureus* count (by 2 logs) after 24-h incubation at 25°C regardless of the honey concentrations in these samples. However, only three batter mix samples incorporated with 10 and 20% honey showed a significant antimicrobial effect when batter was incubated at 4°C. The results indicated that honey had an inhibitory effect on the growth of *S. aureus* in hydrated batter mix stored at room temperature. Since hydrated batter mix has been identified as a CCP for battered seafood products, incorporation of honey may help control the potential hazard.

P094 NATURAL ANTIMICROBIALS AS POTENTIAL REPLACEMENTS FOR CALCIUM PROPIONATE IN BREAD

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Pilot test bakes were carried out to identify potential alternatives to calcium propionate in bread preservation. Two preservative regimes thus identified were evaluated by triplicate brown bread test bakes. Combinations of acetic acid (AA) and

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lactic acid (LA) and of acetic acid and calcium lactate (CL) were compared to calcium propionate (CP). Baked loaves were stored at 30°C and analyzed up to 10 days post-baking by plate counting, viable staining, pH measurement, and minimum mold-free shelf-life (MMFSL) determination. Bacterial colonies were isolated from duplicate plates of the highest dilutions showing growth of all samples and characterized. Compared to antimicrobials used singly (0.1%AA, 0.25%LA or 0.375%CL), the combination treatments of 0.25%LA + 0.1%AA and 0.375%CL + 0.1%AA significantly increased rope-free shelf life (RFSL). Absence of statistically significant differences in RFSL achieved by these two combinations and 0.2%CP confirmed their suitability as potential alternatives to CP. The combination of 0.25%LA + 0.1%AA achieved an increase in MMFSL equivalent to that of 0.2%CP, while 0.375%CL + 0.1%AA only showed marginal increases in the MMFSL compared to untreated controls. Single antimicrobial treatments and 0.25%LA + 0.1%AA were noticeably less inhibitory to yeast activity than 0.2%CP, while the combination treatment of 0.375%CL + 0.1%AA marginally increased yeast activity. *Bacillus subtilis* was predominantly isolated from plate counts of samples from all treatments.

P095 EFFECT OF NATURAL ANTIMICROBIALS ON BAKERS' YEAST

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The main function of Baker's yeast fermentation (*Saccharomyces cerevisiae*) in bread production is to leaven dough by producing carbon dioxide. Consequently, preservative regimes aimed at retarding the microbiological spoilage of bread should produce a minimal reduction in Baker's yeast activity. This study evaluated the effect of selected natural antimicrobials on Baker's yeast activity using a laboratory test procedure as a preliminary means of assessing *in situ* suitability. Antimicrobials (based on flour weight and 100% active ingredient) used were acetic acid (0.025%-0.2%), lactic acid (0.125% -1.0%), calcium lactate (0.375%-1.5%) and a lactate-containing cocktail (0.25%-1.5%), which were compared to calcium propionate (0.5%-0.25%) as current standard. Antimicrobials were evaluated using a standardized dough fermentometer test, and each test was repeated on six separate occasions. All antimicrobial treatments were inhibitory to yeast activity, and decreases in yeast activity were generally noted as a function of increasing concentrations. The lactate-containing cocktail, however, showed no relationship between concentration and yeast activity reductions. Calcium propionate resulted in the highest yeast activity reduction of all the antimicrobial treatments tested. Calcium lactate was less inhibitory than calcium propionate, and acetic acid was noticeably less inhibitory to yeast activity than calcium propionate or calcium lactate, followed by lactic acid and the lactate-containing cocktail in decreasing order. It was concluded that all natural antimicrobials tested represented potential replacements for calcium propionate. These preliminary results remain to be assessed in terms of cost effectiveness and *in situ* efficacy of the natural antimicrobials.

P096 PREVALENCE OF *PSEUDOMONAS* SPP. IN PROCESS WATER, RECYCLED WATER AND DAIRY PRODUCTS

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Pseudomonas spp. are commonly found in water. However, the prevalence of *Pseudomonas* spp. in the dairy factory environment, in both foods and plant water, has not previously been investigated.

In this study, 100 samples were tested for *Pseudomonas* spp. For food samples, each was enriched for 48 h in TECRA *Pseudomonas* Enrichment Broth (TPEB) and then tested in the TECRA *Pseudomonas* VIA, as well as streaking onto selective agar. The foods tested included cream, butter, milk, cheese, yogurt, and dairy desserts. For the water samples, a membrane filtration technique was used, with each membrane cut in half and then tested after enrichment for 48 h in modified Letheen broth and TPEB. All samples were tested in the TECRA *Pseudomonas* VIA as well as being streaked onto selective agar. The water samples included process water from a dairy factory as well as recycled water.

The results for foods showed that of the 84 samples tested, 18 were found to contain *Pseudomonas* spp. The VIA detected all of these samples, as well as an additional three positives which could not be confirmed. For the water samples, of the 16 samples tested, 12 were found positive using the modified Letheen broth as the enrichment medium, compared to 10 with the TPEB. All of these samples were also positive with the VIA.

This study shows that the TECRA *Pseudomonas* VIA can effectively be used to detect *Pseudomonas* spp. in both food and water in the dairy factory. Presumptive results are obtained within 48 h and no false negatives were observed in this study. For water samples, the modified Letheen broth gave better recovery of *Pseudomonas*, probably due to the less selective nature of this broth.

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P097 POPULATION CHANGES OF PATHOGENIC BACTERIA INOCULATED IN FRESH PORK FOLLOWING CHILLED STORAGE AND SIMULATED CONSUMER TEMPERATURE ABUSE

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This study investigated the responses of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Salmonella* spp., *Yersinia enterocolitica*, and *Listeria monocytogenes* during cold (0°C) storage under vacuum and subsequent aerobic temperature abuse (15.6, 21.1 and 26.7°C for 3 or 6 h) in inoculated ground pork and pork loin chops. The samples were inoculated with composite strains of each organism, vacuum packaged, and stored at 0°C for 18 days (ground pork) and 20 days (pork loin chops). Following storage, individual samples were repackaged into styrofoam trays simulating retail display packages. One set of samples was abused immediately following storage, while another set was held overnight (4.4°C) before temperature abuse. *Campylobacter jejuni* showed reductions (0.5 – 1.5 log CFU/g or cm^2) during vacuum storage at 0°C, while subsequent aerobic abuse counts fluctuated slightly in ground pork and increased (approximately $\leq$1.4 log CFU/cm^2) in pork chops. *Escherichia coli* O157:H7 populations fluctuated slightly in pork chops, but increased ($\leq$ 1.4 log CFU/g) in abused ground pork. Populations of *L. monocytogenes* tended to increase more in ground pork than in pork chops, while slight increases (0.4 – 0.8 log CFU/g) of *Salmonella* spp. were detected in ground pork after 6 h at 26.7°C. *Yersinia enterocolitica* increased in ground pork under vacuum at 0°C (18 days) and after 6 h at abusive temperatures. The results verify that temperature abuse may promote proliferation of pathogens and demonstrate the importance of consumer education in safe food handling practices. Furthermore, the results should be useful in risk assessment studies.

P098 PREVALENCE OF *LISTERIA MONOCYTOGENES, SALMONELLA* TYPHIMURIUM AND *YERSINIA ENTEROCOLITICA* ON INCOMING HOGS AND FRESH PORK DURING AND AFTER SLAUGHTER

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A total of 185 samples were collected during three visits to a small slaughterhouse. Eight hogs were randomly selected and sampled by swabbing before stunning, after scalding/singeing, after evisceration, after final carcass washing and after chilling. Environmental samples were collected from receiving (i.e., truck, holding pens), processing (i.e., dehairing machine, band saw, drains) and storage areas (i.e., cooler walls). Five composite environmental samples of manure, feed, trough water and pen areas were collected from the two farms supplying hogs to the slaughterhouse. All samples were examined for *Salmonella* and *Yersinia enterocolitica* using standard FDA methods. The USDA procedure was used to detect *L. monocytogenes*. The VIDAS automated system (bioMérieux, St. Louis, MO) was used to initially screen for *Salmonella* and *L. monocytogenes*. *L. monocytogenes* was present in 18 of 195 samples, 16 of which were environmental. All six samples from trailers off-loading hogs for slaughter yielded *Listeria*. In addition, all three samples from the dehairing machine were positive. *Listeria* was never recovered from any carcass swabs after evisceration in the post-slaughter processing area. However, one floor drain sample from the meat-processing/packing area was positive. *Salmonella* Typhimurium was detected in 11 samples, seven of which were environmental, including three samples from hog trailers. Only one carcass sample yielded *Salmonella* in the post-slaughter processing area. However, *Salmonella* was detected in two floor drains from the meat-processing/packing area examined the following day. One composite sample taken from the back of hogs on the farm also tested positive. No *Yersinia* spp. were identified.

P099 LEVELS OF MICROBIAL CONTAMINATION IN UNITED STATES PORK RETAIL PRODUCTS

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To determine the extent of microbiological contamination, 384 samples of retail pork were collected from 24 stores in six cities, including: (a)whole-muscle, store-packaged pork, (b)fresh, store-packaged ground pork and/or pork sausage, (c)pre-packaged ground pork and/or pork sausage, and (d)whole-muscle, enhanced (injected/marinated; 60% store-packaged, 40% pre-packaged) pork. Additional samples (120) of freshly-ground pork and/or pork sausage were collected from two hot-boning sow/boar sausage plants, two slaughter/fabrication plants, and two further-processing plants. Samples were analyzed for Aerobic Plate Counts (APC), Total Coliform Counts (TCC), and

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Escherichia coli Counts (ECC) (log CFU/g) and incidence of *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni/coli*, and *Yersinia enterocolitica*. APC and TCC were highest ($P < 0.05$) for store-ground pork, while whole-muscle, enhanced products and pre-packaged ground products had the lowest ($P < 0.05$) APC. APC and TCC were higher ($P < 0.05$) in samples from slaughter/fabrication plants compared to hot-boning and further-processing plants. ECC were lower ($P < 0.05$) in samples from further-processing plants. *Listeria monocytogenes* was detected in 26.7% of plant samples and in 19.8% of retail samples and was present more frequently in ground products. *Yersinia enterocolitica* was detected most often in whole-muscle, store-packaged cuts (19.8%) and store-ground product (11.5%). *Salmonella* spp. was present in 9.6% of retail samples and 5.8% of plant samples, while *C. jejuni/coli* was found in 1.3% of retail samples and 6.7% of plant samples. Pork products exposed to more handling appeared to have lower microbiological quality. These results should be useful in risk assessment efforts to enhance food safety.

P100 MICROBIAL CONTAMINATION OCCURRING ON LAMB CARCASSES PROCESSED IN THE UNITED STATES

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Lamb carcasses (n = 5,042) were sampled from six major lamb packing facilities in the United States over three days during each of two visits ("Winter"/October-February; "Spring"/March-June) in order to develop a microbiological baseline for the incidence (presence/absence) of *Salmonella* spp. and for populations of *Escherichia coli* after 24 h of chilling following slaughter. Samples were also analyzed for Aerobic Plate Counts (APC) and Total Coliform Counts (TCC). Additionally, incidence (presence/absence) of *Campylobacter jejuni/coli* on lamb carcasses (n = 2,226) was determined during the slaughtering process and in the cooler. All samples were obtained by sponge sampling the muscle-adipose tissue surface of the flank, breast and leg of lamb carcasses (100 sq cm per site; 300 sq. cm. total). Incidence of *Salmonella* spp. in samples collected from chilled carcasses was 1.5% for both seasons combined, with 1.9% and 1.2% of "Winter" and "Spring" samples being positive, respectively. Mean (log CFU/sq cm) APC, TCC, and ECC on chilled lamb carcasses across both seasons were 4.42, 1.18 and 0.70, respectively. APC were lower ($P < 0.05$) in samples collected in the "Spring" versus "Winter", while TCC were higher ($P < 0.05$) in samples collected in the "Spring". There was no difference ($P > 0.05$) between ECC from samples collected in the "Spring" versus "Winter". Only 7 out of 2,226 total samples (0.3%) tested positive for *C. jejuni/coli*, across all sampling sites. These results should be useful to the lamb industry and regulatory authorities as new regulatory requirements for meat inspection become effective.

P101 SAMPLING OF DAIRY CATTLE FOR *LISTERIA MONOCYTOGENES*

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Regulatory agencies are seeking baseline data to determine the occurrence of foodborne pathogens from on-farm sites. *Listeria monocytogenes* (LM) is a major concern to the agrifood industry. This study was conducted to determine factors affecting the occurrence and recovery of *Listeria* spp., particularly LM from dairy cattle. Isolation and confirmation of LM was performed according to the FDA Bacteriological Analytical Manual (AOAC) with modifications in isolation protocol. Cows (n=30) were sampled monthly for six months. LM was isolated from foremilk (20%), teat (20%), hair (20%) and oral samples (20%), but not from anal samples. At least one of the four sites (foremilk, teat, hair, and oral) was confirmed positive for LM in over 60% of the cattle. Optimal recovery of LM was achieved by sampling multiple sites on the animal, since only one site was positive on the majority of the animals. *Listeria* species, excluding LM, were isolated from all cows and all locations on the animal. The most common *Listeria* spp. isolated were *L. seeligeri* or *L. ivanovii*, since 56% of the dairy cattle tested positive for either species. These data show that LM frequently exists on the bodies of dairy cows and may occur exclusively on a single site on the animal. Preliminary data showed that feed bunk and grain samples were positive for LM. The development of a stepwise "on-farm" pathogen reduction program to control LM in dairy cattle and milk products will require a comprehensive sampling program, which can consistently detect the presence of LM.

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P102 INCIDENCE AND ANTIBIOTIC RESISTANCE OF *SALMONELLA* SPP. CULTURES ISOLATED FROM ANIMAL HIDE AND BEEF CARCASSES

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Antibiotics necessary for treatment of infectious diseases have been used worldwide for years. However, emergence of multidrug-resistant bacteria, like penta-resistant *Salmonella enterica* subsp. *enterica* serovar Typhimurium definitive phage type 104 (104 ST), is a major cause of disease treatment failure and has become a serious public health concern. This study evaluated the incidence and resistance of *Salmonella* isolates found on animal hides and beef carcasses to ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamides (Su), and tetracycline (T). *Salmonella* isolates were obtained from sponge swab samples of external animal hides and carcass surfaces in eight geographically dispersed, beef packing plants. In each plant, samples were collected at two in-plant sampling sites, with hide samples obtained prior to the dehiding process and carcass samples obtained prior to chilling. Overall, out of 639 samples collected, 53 samples yielded 521 isolates that were confirmed as positive *Salmonella* spp. Of the total samples yielding confirmed positive isolates, 49 originated from 319 animal hide samples and 4 originated from 320 carcass samples, resulting in incidence rates of 15.4 and 1.3%, respectively. Of the total samples that yielded *Salmonella* spp. isolates, 27 (50.9%) yielded isolates resistant to at least one of the screened antibiotics. Multiple resistant isolates originated from 10 of the 27 samples (37.0%), with 8 of the 10 multiple-resistant samples (80.0%) yielding isolates with penta-resistant profiles. The significant incidence of single and multiple antibiotic resistance among environmental *Salmonella* isolates collected from hides and carcasses warrants antibiotic administration regulation in an attempt to reduce subsequent environmental pressures.

P103 SURVEILLANCE OF *ARCOBACTER* IN VARIOUS ENVIRONMENTAL SOURCES

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The occurrence of the foodborne pathogen *Campylobacter jejuni* has been documented for several years and has been shown to be one of the primary causes of foodborne illness throughout the world. A closely related organism, *Arcobacter*, has been found in similar environmental niches. Unfortunately, there is little known about the prevalence and pathogenicity of this microorganism. The objective of this study was to examine different food and environmental sources (poultry, clinical samples from patients exhibiting diarrhea, cabbage, and water) for the presence of *Arcobacter*. Seventy poultry samples were taken from a central Texas processing facility, consisting of line trimmings as well as finished products, and examined for the presence of *Arcobacter*. Two hundred and forty stool samples taken from individuals exhibiting loose stool or diarrhea were tested for the presence of this organism. One hundred and forty-five samples consisting of cabbage, river water, irrigation water, and wash water from two farms located in the Rio Grand Valley of Texas were also tested. In addition, these water isolates were examined for the presence of the *C. jejuni* cytolethal distending toxin genes. A total of 59 *Arcobacter* isolates were found from poultry. Thirteen were isolated from water samples and none were isolated from the cabbage samples. Out of the 210 clinical samples analyzed, one *Arcobacter* isolate was recovered. No characteristic size DNA fragments were amplifed using primers for the identification of the *C. jejuni* toxin; however, a 300bp fragment was observed. The virulence and the relatedness of these isolated strains is unknown. However, by obtaining these isolates from this and other studies, it is our plan to conduct DNA fingerprinting to determine whether common sources of contamination exist.

P104 PRESENCE OF *CAMPYLOBACTER, ESCHERICHIA COLI* AND *SALMONELLA* IN RETAIL MEATS

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Retail meat samples (beef, pork, turkey and chicken) were randomly obtained from 40 stores of four food supermarket chains in the Maryland suburban of Washington metropolitan area to assess the presence of *Campylobacter*, *Escherichia coli* and *Salmonella*. Methods described in the FDA Bacterial Analytical Manual were used to isolate the organisms. *Campylobacter* isolates were further confirmed using a PCR assay specific for *C. coli*, *C. jejuni*, and *C. lari*, whereas *E. coli* and *Salmonella* were confirmed using API system. Results showed that most (84%) of the chicken samples (n=80) contained *Campylobacter* and that all of the 40 stores had *Campylobacter*-contaminated chickens. Approximately 23% of the 77 turkey samples also yielded *Campylobacter*. Fewer pork (3.8%) and beef (6.3%) samples contained *Campylobacter*. Of the 100

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chicken samples, 45 (45%) yielded *E. coli.* Beef, pork and turkey samples were positive for *E. coli* at rates of 23%, 23.7%, 16.5%, respectively. However, only 14 (4%) of the 327 retail meat samples tested were positive for *Salmonella*. The study revealed that retail meats are often contaminated with enteric pathogens and can be potential vehicles in transmitting foodborne illness.

P105 ANTIBIOTIC RESISTANCE PATTERN OF *CAMPYLOBACTER* SPP. ISOLATED FROM BROILERS PROCESSED IN AIR AND IMMERSION CHILL PROCESSING FACILITIES

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Campylobacter spp. isolates were collected from chicken broilers after chilling. Broilers were chilled in either an air-chilled or immersion-chilled process in two separate commercial processing facilities. Isolates were tested for resistance against nalidixic acid using a disk diffusion assay. Nalidixic acid resistant isolates were further tested for resistance against fluoroquinolones and other antibiotics. The majority of the isolates from broilers from both processing facilities were identified as *Campylobacter coli*. The remaining isolates were *Campylobacter jejuni*. Of the isolates from the immersion-chilled broilers, 46% were resistant to nalidixic acid. Of those isolates, 55% were also resistant to cepra-floxacin, 44% to grepafloxacin, and 38% to tetracycline. Few isolates were resistant to erythromycin and levofloxacin. Isolates from the air-chilled broilers were less resistant, with only 15% of them showing resistance to nalidixic acid. Of those isolates, 66% were resistant to erythromycin and 33% to ceprafloxacin. However, all were sensitive to tetracycline, levofloxacin, grepafloxacin, and ceprafloxacin. Farm production and processing techniques are being evaluated to determine the origin of the resistance.

P106 CHARACTERIZATION OF ANTIBIOTIC RESISTANCE IN SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*

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Shiga toxin-producing *Escherichia coli* (STEC) strains, including 125 *E. coli* O157:H7 and 29 other STEC, were screened for their antibiotic resistance. The resistant strains (ca. 59) were further examined for resistance to 17 antibiotics used in the National Antibiotic Susceptibility Monitoring Program (NASMP). Many of the strains are resistant to up to nine antibiotics. The multiple antibiotic-resistant STEC were analyzed using PCR assays for the presence of integrons, which are mobile DNA elements with a specific structure consisting of two conserved segments flanking a central region (cassettes) that often encodes antibiotic resistance. One *E. coli* O157:H7 and 10 other STEC isolates displayed class 1 integrons of 1 and 2 kb in size, respectively. Sequence analysis showed that the integron of *E. coli* O157:H7 exhibited ca. 86% homology with a previously described integron in the GenBank database, whereas the integron of an *E. coli* O111:H8 demonstrate 100% homology with the same GenBank sequence. The *E. coli* O157:H7 integron was transferable by conjugation. Southern hybridization analysis also indicated that the integron was carried on both plasmid and chromosome. The study suggests that STEC have developed resistance to multiple antibiotics and that integrons may contribute to acquisition and dissemination of antibiotic resistance genes in STEC and other Gram-negative bacterial pathogens.

P107 EVIDENCE OF TOXIN PRODUCTION BY *BACILLUS* STRAINS ISOLATED FROM STREET-VENDED FOODS IN JOHANNESBURG, SOUTH AFRICA

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Twenty-one strains each of *Bacillus* (B.) *cereus*, *B. subtilis* and *B. licheniformis* were isolated during microbiological surveys of street-vended foods. Supernatants of each strain were tested for cytotoxicity towards McCoy mouse cells using a 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT)-based assay, confocal scanning laser microscopy (CSLM) and scanning electron microscopy (SEM). Results of the MTT-based assay showed that 48% of *B. cereus*, 19% of *B. subtilis*, and 33% *of B. licheniformis* strains produced compounds that exhibited cytotoxic effects against McCoy cells. For *all B. cereus* strains, the compounds that exhibited cytotoxic effects were inactivated by heat treatment at 121°C for 15 min. For some *B. subtilis* (10%) and *B. licheniformis* (24%) strains, however, cytotoxic effects persisted following heat treatment at 121°C for 15 min. Results of the MTT-based assay correlated with CSLM and SEM observations.

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McCoy cells treated with *Bacillus* supernatants that exhibited cytotoxic effects by the MTT-based assay exhibited red fluorescence (indicative of cell death) and leaking of the cell contents, in contrast to control cells, which exhibited green fluorescence (indicative of cell viability) and no structural damage. Similarly, SEM indicated that McCoy cells treated with cytotoxic *Bacillus* supernatants had disintegrating membranes and leaked cell contents.

P108 MICROBIOLOGICAL QUALITY OF BOTTLED WATER

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A total of 152 samples of bottled water were purchased in supermarkets in southeastern Pennsylvania and examined for heterotrophic bacteria, coliforms, fecal coliforms, and fungi, using the membrane filter method. Coliforms were detected using M-endo Agar, the heterotrophic bacteria were detected by use of m-HPC Agar, and the fungi were detected using the Difco Special Yeast and Mold Medium supplemented with Chloranphenicol and Streptomycin. Coliforms were detected in 10% of samples; however, no fecal coliforms were found. Heterotrophic bacteria were detected in 48% of samples. Although there was no apparent fungal growth in the water samples, various fungi, especially molds, were detected in 39% of the samples. Identification of randomly isolated bacteria included *Escherichia coli*, *Enterobacter aerogenes*, *Citrobacter*, *Pseudomonas aeroginosa*, and *Serratia liquifaciens*. The dominant mold genera isolated from the water samples included *Penicillium*, *Alternaria*, *Cladosporium* and *Acremonium*.

P109 IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF AMINE-PRODUCING STRAINS OF *STRENOTROPHOMONAS MALTOPHILIA* ISOLATED FROM WHITE MUSCLE OF FRESH AND FROZEN ALBACORE TUNA (*THUNNUS ALALUNGA*)

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Three strains – one of them psychrotrophic and two mesophilic – of *Strenotrophomonas maltophilia* – an emerging pathogen involved in an increasing number of clinical syndromes – were obtained from fresh and frozen-stored (6 months at -25°C) albacore tuna in a routine screening of biogenic amine-forming bacteria. Phenotyping assays, including the investigation of up to 40 biochemical tests and 15 susceptibility assays, revealed slight differences among the three strains. Genotyping experiments based on RFLP analysis and Southern blot hybridizations indicated that strains 15MF and $26MC_6$ were different and not multiple isolates of a single strain.

All three strains of *S. maltophilia* showed histidine-decarboxylase activity when assayed in a 2% histidine-containing broth and further analyzed by HPLC. Although all three strains produced less than 25 ppm of histamine, they showed strong lysine-decarboxylating activity, cadaverine being produced at concentrations ranging from 1,736 to 4,821 ppm after 48 h. Strong secretion of extracellular lipase and protease was also observed in all three strains. The psychrotrophic strain *S. maltophilia* $5PC_6$ was able to produce both extracellular lipase and protease even at refrigeration temperatures. The potential enhancing effect of cadaverine on histamine toxicity, together with the intrinsic pathogenic mechanisms of *S. maltophilia*, in which extracellular protease production has been described, and its resistance to a large number of antimicrobial agents, underlines the interest of this pathogen to be considered in studies aimed at evaluating the safety of fish products.

P110 MICROBIAL ECOLOGY OF MUFFINS BASED ON CASSAVA AND OTHER NON-WHEAT FLOURS

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Four replicate test bakes were carried out to evaluate the microbial ecology of two muffin mixes made with different combinations of cassava, soya, sorghum, sago, and potato flour. Muffins were stored at 30°C and analyzed for 10 d post-baking by duplicate aerobic plate counts, spore counts and mold counts. Viable staining was carried out to determine *in situ* bacterial growth, and minimum mold-free shelf life was determined. Raw materials were analyzed microbiologically as above on the day of baking. Characterization of predominant bacterial and mold isolates from raw materials and muffins was carried out using standard methods. For raw materials, the highest aerobic plate counts were obtained for soya flour followed by, in decreasing order, sorghum, cassava, sago, and potato flour. Spore counts of the raw materials did not exceed 3.5 log CFU/g. The highest mold counts were recorded for sago flour, while mould counts below 2.5 log CFU/g were determined for the other four flours. Both bacterial and mold counts of the two muffin

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types were similar and viable staining confirmed these results. The rope-free shelf life of both muffin types was *ca.* 1 day, while the minimum mold-free shelf life was *ca.* 3 d. Predominant bacterial isolates from the raw materials and muffins were *Bacillus* (83 and 99%, respectively). Predominant mold isolates from raw materials were *Gloeoesporium* (42%), *Penicillium* (13%) and *Mucorales* (13%) and from muffins were *Penicillium* (37%) and *Aspergillus* (37%).

P111 EVALUATION OF UNIVERSAL PREENRICHMENT BROTH FOR GROWTH OF HEAT-INJURED PATHOGENS

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Studies were done to determine recovery and growth rates of heat-injured *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *S. enteritidis* and *Listeria monocytogenes* in Universal Preenrichment Broth (UPB). Bacterial cells were heat-injured at 57.2°C and inoculated at populations of ca. 0.2 to 63 injured cells per gram into raw ground beef, fresh chicken, lettuce, and environmental sponge samples. Enrichment cultures were sampled at 1, 2, 3, 4, 5, 6, and 24 h of inoculation, and pathogens were enumerated on appropriate selective media. Results revealed that recovery and growth of pathogens during the first six h of enrichment was not sufficient to assure adequate numbers of bacteria ($>10^3$ CFU/ml) for detection by most immunoassays or PCR assays. Cells often required 3 to 4 h for recovery before growth was initiated. *Salmonella, E. coli* O157:H7, or *L. monocytogenes* cell populations in enrichment cultures with ground beef or lettuce at 6 h were 0.5 to 2.9 $\log_{10}$ CFU/ml. At 24 h of incubation, cell counts of enrichment samples for the three pathogens from all food and environmental sponge samples ranged from 4.0 to 8.3 log CFU/ml. Enrichment in UPB at 37°C of foods or environmental sponge samples containing heat-injured cells of *Salmonella, E. coli* O157:H7, or *L. monocytogenes* provides, at 24 h but not at 6 h of incubation, sufficient cell populations for detection by rapid immunoassay or PCR assay procedures.

P112 CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* FROM COLD-SMOKED FISH PLANT BY PULSED-FIELD GEL ELECTROPHORESIS (PFGE)

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For twenty years, *Listeria monocytogenes* has been considered a foodborne pathogen. It is an ubiquitous bacterium in the food industry. In order to determine contamination ways of finished products, the aim of this study was to study DNA polymorphism of 79 *Listeria monocytogenes* strains collected on one year period in fish plant. Thirty-eight of the strains were isolated from the environment, the others from fresh fish (13), intermediate products (7) and finished products (21). Among 79 genotypable *Listeria monocytogenes* isolates, 10 different patterns after *Apa*I restriction were identified. Ten different profiles were, equally, obtained with *Sma*I digestion. The pulsotype combination gave 11 profiles. Each PFGE type was found to contain from one to 60 isolates. With two enzymes, one major combined-pulsotype was observed. It represents 75% of total strains. This pulsotype was found everywhere (fresh salmon (2/13), intermediate products (5/7), finished products (15/21) and environment (25/38). Two strains present in finished products were not found in environment and entire fish. But these strains are similar to dominant pulsotype, with a similary level superior to 85%. The fresh fish is source of genetic diversity in *Listeria monocytogenes* strains. Six different pulsotypes were found in salmon. No dominant pattern was observed. Each PFGE-profile contained one to four strains. It is not the case for smoked salmon fillets, where the contamination by *Listeria monocytogenes* was homogeneous. The environment is the main source of contamination. It contains a *Listeria monocytogenes* pulsotype which presents a high faculty of adaptation to hostile environment and cleaning and sanitaring procedures.

P113 *LISTERIA MONOCYTOGENES* DETECTION IN FOOD USING AN ELISA-BASED METHOD

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As one of the major foodborne pathogens, *Listeria monocytogenes* needs to be quickly and easily monitored in the food chain. A new method, so-called Transia Plate *Listeria monocytogenes*, combining a two-step enrichment done either in Palcam or Fraser broths and an ELISA test, has been developed for the food application.

The ELISA test detected all the different serotypes of *Listeria monocytogenes* (31 strains tested) and did not show any crossreactivity towards the other *Listeria* species (n = 28) such as *Listeria innocua* (n = 18). Among thirteen *Listeria monocytogenes* strains, the limit of detection of the ELISA test was between 10^6 and 10^7 CFU/ml.

The studies of spiked food samples (pasteurized cheese, raw milk, rillettes, smoked salmon, and wrapped cabbages) contaminated by different levels of *Listeria monocytogenes* serotypes (1/2a, 1/2b and 4b) showed the limit of detection of the method was

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as low as 3 CFU/25 g of food. Finally, when screening naturally contaminated food samples (75 samples, mainly fish, cheese and vegetable), this new ELISA-based method offered equivalent results to the ISO method 11290-1.

P114 FACTORS AFFECTING THE ISOLATION AND ENUMERATION OF *ESCHERICHIA COLI* O157:H7 ON ALFALFA SEEDS

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Isolation of *Escherichia coli* O157:H7 from alfalfa seeds used to produce sprouts that were later implicated in human illness has been difficult, perhaps due to spotty and very low level contamination and inaccessibility of the pathogen trapped in protected areas of the seed coat. We investigated some treatments that may facilitate release of *E. coli* O157:H7 from seeds. The influence of soaking time (0, 1, 3, 5, or 15 h at 4 or 21°C), homogenization (blending and stomaching for 1 or 2 min), washing method (shaking for 5 min), and addition of surfactants (0.1, 0.5, or 1.0% Tween 80 and Span) to wash water was investigated. Soaking for 1 h or longer at 4 or 21°C, and blending or stomaching for 1 or 2 min gave maximum release of cells. The effect of surfactants on the isolation of *E. coli* O157:H7 was temperature dependent. At 21°C, wash water containing 1.0% Span resulted in maximum numbers of cells released from seeds, while at 37°C, 0.1 or 0.5% Tween 80 was more effective. However, compared to the water control, the increase in number of cells released from seeds by any detergent treatment was not more than 0.5 $\log_{10}$ CFU/g. *E. coli* O157:H7, inoculated on seeds at 2 $\log_{10}$ CFU/g, was detected after storage for 8 weeks at 37°C. The pathogen was also recovered from sprouts produced from these seeds.

P115 EFFICACY OF VARIOUS NON-SELECTIVE RESUSCITATION MEDIA FOR INCREASED DETECTION OF HEAT-INJURED *ESCHERICHIA COLI* O157:H7

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The objective of this investigation was to evaluate the efficacy of various resuscitation broth systems for increasing the detection of heat-injured *Escherichia coli* O157:H7 on Sorbitol MacConkey Agar (SMA). Trypticase Soy Broth (TSB) was inoculated with a three-strain mixture of *E. coli* O157:H7 to give a final concentration of ~10^7 CFU/ml, sealed in thermal death time (TDT) tubes, and heated at 57.5°C. At 0, 6, and 8 min, aliquots of the heated cell suspensions were surface-plated directly onto both Trypticase Soy Agar (TSA) and SMA or dispensed into tubes of TSB or Phenol Red Sorbitol Broth (PRSB), alone or in combination with 0.05% cysteine (cys), and/or purging with nitrogen gas (N_2). The efficacy of each broth system to allow repair of injured *E. coli* O157:H7 was assessed by plating aliquots of inoculated broth onto both TSA and SMA after 2 h of incubation at 25°C. At 6 and 8 min of heating, 96.3% and 98.8% of the cell population, respectively, were injured. At 8 min of heating, only 49.8% of *E. coli* O157:H7 were detected by direct plating; whereas, TSB or PRSB alone increased detection to 63.2% and 67.0%, respectively ($P < 0.05$). Addition of cys + N_2 to TSB or PRSB further increased detection to 75.3% and 76.1%, respectively ($P < 0.05$). The results of this research strongly support the need for resuscitation procedures when testing for *E. coli* O157:H7 in heat processed food products.

P116 PHOSPHATE BUFFER INCREASES RECOVERY OF *ESCHERICHIA COLI* O157:H7 FROM FROZEN APPLE JUICE

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It is common practice to dilute food products in 0.1% peptone. However, this diluent may not be appropriate for injured organisms in acidic foods. Apple juice (pH 3.6) was inoculated with approximately 1×10^7 CFU/ml of *E. coli* O157:H7 and stored at 25±2°C (control) or frozen to -20±2°C for 24 h to induce injury prior to sampling. Unfrozen or thawed juice was diluted 1:1 or 1:10 in 0.1% peptone water or 0.1 M phosphate buffer (pH 7.2). Juice samples were plated onto tryptic soy agar with 0.1% sodium pyruvate (TSAP) to measure survival or onto sorbitol MacConkey agar (SMA) to indicate injury. There was no difference in plate counts on TSAP or SMA for control samples held in peptone or phosphate buffer for up to 45 min. Populations of *E. coli* declined rapidly when frozen samples were diluted in peptone. In less than 20 min, *E. coli* underwent a >1 log reduction on TSAP and was reduced to below the limit of detection (40 to 200 CFU/ml) on SMA. In contrast, frozen and thawed *E. coli* diluted in phosphate buffer showed an insignificant decrease in viability on TSAP and less than 0.6 log reduction on SMA during a 45 min storage period. The acidity of apple juice appears to interfere with the recovery of freeze/thaw injured *E. coli* O157:H7 during sampling. Using phosphate buffer as a diluent results in superior recovery of these organisms on both selective and non-selective plating media.

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P117 EVALUATION OF FDA/BAM AND RAPID METHODS FOR ENUMERATION AND DETECTION OF *ESCHERICHIA COLI* O157:H7 FROM FARM ANIMAL ENVIRONMENTS

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FDA/BAM methods and rapid tests were evaluated for their efficacy to recover or detect *E. coli* O157:H7, inoculated at high (10^4 CFU/g) and low (10 CFU/g) levels, from cattle, swine, and chicken environments (various feed, bedding, feces, and water samples). High inocula were recovered by direct plating on sorbitol MacConkey (SMAC), SMAC supplemented with cefixime and tellurite (CT-SMAC), hemorrhagic colitis (HC), modified eosin methylene blue (MEMB), and Rainbow O157 (plus novobiocin and tellurite) agars. Low inocula were recovered after 24 h enrichment at 37°C in modified tryptic soy broth (mTSB), EHEC enrichment broth (EEB), and modified *E. coli* broth (mEC) followed by streaking on selective media described above and by using Dynabeads, EHEC-Tek, and Reveal O157 test systems. Rainbow O157 and CT-SMAC recovered high inocula from 18 of 24 samples, although Rainbow O157 provided poorer numerical recovery of *E. coli* O157:H7 than other selective media ($P < 0.05$). Generally, recovery was next best on MEMB, followed by SMAC and HC. For enrichment, low inocula were recovered best (18 of 48 samples) with mTSB streaked onto CT-SMAC. Reveal O157 and EHEC-Tek provided positive results in 20 of 24 and18 of 24 samples, respectively, although false positives in controls were frequent with Reveal O157. Dynabeads performed best when plated on Rainbow O157 (10 of 24 positive). These results suggest that some methods used to detect *E. coli* O157:H7 in foods may be useful for detecting the pathogen in heavily contaminated environments associated with farm animals.

P118 RAPID AND SENSITIVE IDENTIFICATION OF VIABLE *ESCHERICHIA COLI* O157:H7 IN FOOD BY REVERSE TRANSCRIPTION PCR

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Escherichia coli O157:H7 continues to be the causative agent of worldwide foodborne illness. Infection can progress from diarrhea to the potentially life-threatening disorder hemolytic uremic syndrome. Foods associated with outbreaks have included beef, yogurt, mayonnaise, apple cider, fruits and vegetables. Its severe symptoms combined with its putative low infectious dose (<100 cells) justify ranking *E. coli* O157:H7 among the most serious of known foodborne pathogens. Many new, sensitive tests for screening the pathogen in food have been developed, but most of those methods (including PCR amplification techniques) require a long detection time, because they involve culturing in enrichment and/or selective media. Rapid methods, which address objectives of the Food Safety initiative, are lacking.

An alternative sensitive, specific, and rapid method for detection of viable pathogens is Reverse Transcriptase PCR (RT-PCR). The objective of our research was to develop and optimize a RT-PCR assay for detection of live *E. coli* O157:H7 present in food and environmental matrixes. The presented assay targets specifically flagella H7 and *rfb*E O-antigen transcript genes, and can be modified to simultaneously detect expression of the virulence genes, *stx*1 and *stx*2. Protocol includes immunomagnetic separation/concentration of *E. coli* O157:H7, extraction of mRNA, reverse transcription to synthesize cDNA, and PCR using cDNA as the template in the reaction. The assay can be completed within 8-12 h. Results indicate that RT-PCR is a sensitive, convenient method for specific and rapid detection of *E. coli* O157:H7 in food and environmental samples.

P119 COMPARISON OF SELECTIVE MEDIA FOR EVALUATING SURVIVAL OF *ESCHERICHIA COLI* O157:H7 IN FRUIT JUICES

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Survival of *E. coli* O157:H7 in apple, orange, white grape, red grape, and cranberry juices was evaluated, with efficacy of recovery determined on tryptic soy (TSA), sorbitol MacConkey (SMAC), SMAC supplemented with cefixime and tellurite (CTSMAC), hemorrhagic colitis (HC), Rainbow O157, and modified eosin methylene blue (MEMB) agars. A three-strain mixture of *E. coli* O157:H7 was used to inoculate (ca. 7 log CFU/ml) juices, which were stored at 4°C. Samples were withdrawn at 4-day intervals, neutralized with 1.0 N NaOH, serially diluted in 0.1% peptone water, and surface plated onto TSA, SMAC, CTSMAC, HC, Rainbow O157, and MEMB. When not detected by direct plating, samples were enriched for *E. coli* O157:H7 in modified (bile salts and novobiocin) trypic soy

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broth. The organism remained viable in cranberry, apple, red grape, orange, and white grape juices for <1, 52, 52, 70, and >80 days, respectively. Efficacy of media for recovery of *E. coli* O157:H7 from orange, white grape, red grape, and cranberry juices followed the order: TSA = Rainbow O157 > HC = SMAC > CTSMAC = MEMB ($P < 0.05$). The order of recovery from apple juice was TSA > Rainbow O157 > SMAC > CTSMAC = MEMB ($P <0.05$), with recovery on HC equivalent to that of Rainbow O157 and SMAC ($P >0.05$). These results demonstrate the importance of preventing contamination of fresh or processed juice with *E. coli* O157:H7, since the high acidity of these products does not preclude survival of this pathogen. Additionally, careful selection of recovery media is essential for optimizing recovery of acid-stressed cells.

P120 WITHDRAWN

P121 MEDIA EVALUATION FOR RECOVERY OF INJURED CELLS OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP.

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Escherichia coli O157:H7, *Salmonella* spp, and *Salmonella* Typhimurium DT-104 were stressed with lactic acid and cell-free supernatants from lactic acid bacteria (LAB) and plated on three different media to determine if injured cells were recovered. Acid stress conditions were created by adjusting the pH of a cocktail mixture of the pathogen to 3.50 with lactic acid and holding for 18 h. The pathogen cocktail was also stressed with a cell-free supernatant of *Lactobacillus lactis* (pH = 3.90) in a 4:6 ratio. Both non-stressed and stressed cocktail cultures were plated on Trypticase soy agar (TSA) and violet red bile agar (VRBA) for *E. coli* and XLT4 for *Salmonella*. Repair of injured cells was evaluated by pour plating the stressed cells on a 5 ml thin layer of TSA and allowing a 2 h room temperature incubation followed by overlaying with VRBA or XLT4. There were significant reductions in populations of both pathogens under both stress conditions when plating was done on non-selective media. Acid-injured *E. coli* O157:H7 was recovered significantly less on both recovery and selective media compared with TSA. Numbers of cells of stressed *Salmonella* spp. plated on recovery media were not significantly different from numbers obtained on TSA, while those plated on selective media were significantly less than those on recovery media. Supernatant-stressed cells for all *Salmonella* spp. were recovered similarly on TSA, selective and recovery media. This method was successful for the recovery of *Salmonella* spp. injured cells, but it was not for injured *E. coli* O157:H7.

P122 COMPARISON OF SELECTIVE ENRICHMENT MEDIA TO RECOVER *SALMONELLA* FROM ACIDIFIED BARBECUE AND LIQUID NON-DAIRY PRODUCTS

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The FDA-BAM method and AOAC approved methods do not have guidelines for detection of *Salmonella* from acidified barbecue (BBQ) and liquid non-dairy (LND) products. Also, standard methods for recovering *Salmonella* from foods typically include selenite cystine (SC) broth, which contains toxic chemicals requiring special handling and disposal care. The objective of this study was to develop a validated method to isolate and detect *Salmonella* in BBQ and LND products without utilizing SC broth. Individual samples of commercial BBQ and LND products were separately inoculated with 4 different strains of *Salmonella* at two levels of inoculum, 10^2 and 10^6 CFU/g. After pre-enrichment in lactose broth or buffered peptone water (BPW) at 35°C for 18 h, selective enrichment was carried out in Rappaport-Vassiliadis (RV) broth at 42°C, SC broth at 35°C, and tetrathionate (TT) broth at 42°C for 6 h each. A 1-ml aliquot from each selective medium was transferred to M-broth, incubated at 42°C for 18 h. Thereafter, an enzyme-linked fluorescent assay (VIDAS; bioMérieux) was carried out according to manufacturer's instructions to detect presence of *Salmonella*. Pre-enrichment with BPW at 35°C for 18 h and selective enrichment in RV broth at 42°C for 6 h allowed for recovery of all *Salmonella* strains in both BBQ and LND products.

P123 EVALUATION OF A LATERAL FLOW DEVICE FOR DETECTING *SALMONELLA ENTERITIDIS* IN RAW EGGS AND CHICKEN FECES

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The Centers for Disease Control and Prevention (CDC) estimates there were 300,000 cases of *Salmonella enteritidis* (SE) in 1997. Egg products were associated with many of the cases. To address this problem, many producers implemented flock surveillance of the SE situation at their facility. A rapid and simple method for detecting SE from poultry samples is critical for the effective implementation of such testing strategies.

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A lateral flow device for the detection of *Salmonella enteritidis* utilized in this study was manufactured by Neogen Corporation, Lansing, MI. The test panel is a presumptive qualitative test system that detects only members of Group D1 *Salmonella* species. A series of studies were conducted to optimize the test procedure for raw eggs and poultry environmental samples. Detection of SE was 100% in raw egg pools inoculated with SE 1-10 CFU/ml of egg, incubated in buffered peptone water (BPW) or tetrathionate broth (TT) at 1:10 ratio, and incubated for 24 h at 37°C. The panels detected 100% of chicken manure samples initially seeded with 1 CFU SE/g and enriched in tetrathionate broth for 24 h at 37°C. *Salmonella enteritidis* did not grow as well in straight fecal samples and, in many cases, after 24 h enrichment did not attain the minimal levels of organism detectable by the panels. The false positive ratio was less than 0.04% in the fecal samples from chickens infected with *Salmonella* Typhimurium or Kentucky. The test kit detected 94% and 100% of enriched fecal samples from SE-infected birds at 4 and 7 days post infection (PI), respectively, but detection decreased to 62% and 83% at 14 and 20 days PI. Treatment of the tetrathionate enrichment prior to administration to the panels can affect panel detection sensitivity. The panels identified fewer positive samples if the enrichments were shaken first, while autoclaving the enrichment samples first dramatically increased SE detection.

The developed lateral flow test kit could provide a simple, rapid, and inexpensive method for egg producers and processors to test specifically for *Salmonella* group D1 serovars, such as *Salmonella enteritidis*, in environmental and egg samples.

P124 IMPROVED ISOLATION OF *SALMONELLA* FROM CHOCOLATE

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Contaminated chocolate and other cocoa-containing products have caused many large outbreaks of salmonellosis in recent years. Isolation of *Salmonella* from contaminated chocolate is more difficult than for most processed foods. The bactericidal effect on *Salmonella* of naturally occurring substances in cocoa powder is well documented and reference isolation methods have protocol modifications to help neutralize the toxic components. Despite these modifications, *Salmonella* have been proven to enter a viable but non-culturable state in certain cocoa-containing products.

In this study, 2 independent laboratories compared the performance of the ISO *Salmonella* reference method (ISO 6579) with that of the new Oxoid SPRINT *Salmonella* timed release enrichment method. A total of 83 chocolate, chocolate containing, and chocolate raw material samples was tested, 26 of which were deliberately spiked with *Salmonella*. The new SPRINT *Salmonella* method, which has an optimized resuscitation stage, was significantly ($P \leq 0.0078$) more sensitive and faster than the reference method (21/83 *versus* 13/83 positive samples, respectively). These results illustrate the enhanced sensitivity of SPRINT *Salmonella*, with results also being provided 24 h sooner than the reference method.

P125 RECOVERY OF *SALMONELLA* FROM ARTIFICIALLY CONTAMINATED DAIRY FEEDS

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Methods to recover *Salmonella* from feeds need to be compared and improved to assist in implementation of pathogen control programs. The objective of this study was to systematically evaluate and optimize current methods for the recovery of *Salmonella* from feeds. Pre-enrichment in lactose broth (LB) or direct selective enrichment (DE) in Rappaport Vassiliadis (RV) at 42°C, Tetrathionate Broth at 35°C (TT35), 42°C (TT42), and Selenite Cystine (SC) at 35°C, in combination with plating on Brilliant Green (BGA), Bismuth Sulfite (BSA), Hektoen (HE), and Xylose Lysine Tergitol (XLT) agar media, were evaluated. Silage and grain samples were inoculated with 10 to 20 *Salmonella* cells per gram. Ten sub-samples were analyzed for *Salmonella* using 32 combinations of selected media (n=320). All *Salmonella* isolates were confirmed biochemically and serologically. Optimal recovery of *Salmonella* (80 to 100%) from the grain samples was obtained using direct enrichment in TT42, TT35, or SC followed by differential plating on BSA, XLT, and BSA, respectively. Pre-enrichment is not recommended for grain samples. However, excellent recovery of *Salmonella* (80-100%) from the silage samples was obtained with pre-enrichment followed by enrichment in all four media followed by plating on BGA, BSA, HE or XLT. The recovery of *Salmonella* in silage was only achieved by re-adjusting the pH during both pre-enrichment and enrichment. Use of optimal isolation protocols is essential when documentation of *Salmonella* is needed in feeds, since some methods will lead to erroneous reporting of low occurrence of *Salmonella*.

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P126 SELECTIVE AND DIFFERENTIAL PROPERTIES OF CHROMOGENIC MEDIA FOR ISOLATION OF SALMONELLAE FROM FOODSTUFFS

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The ability of chromogenic media to differentiate salmonellae from competitive flora is based on a number of different biochemical reactions. The suitability of different chromogenic mechanisms employed in a number of media was examined for ability to correctly identify salmonellas and to differentiate salmonellas from other organisms commonly found in foodstuffs. Twenty-five grams of minced beef, chicken, raw egg, and bean sprouts was separately inoculated with 1-100 cells of 23 different *Salmonella* serotypes to include the 20 serotypes most commonly associated with food poisoning in the UK. Five media, Rambach Agar (Merck), SM ID Agar (bioMérieux), Harlequin ABC Agar (IDG), CHROMagar *Salmonella*, and *Salmonella* Chromogenic Agar (Oxoid), were inoculated after pre-enrichment overnight in Buffered Peptone Water and selective enrichment of the foods in Rappaport-Vassiliadis and Selenite Cystine Broths. Plates were examined after overnight incubation for colonies typical for the medium of *Salmonella*. Identification was confirmed by latex agglutination. The identification of other organisms growing on the media was confirmed using biochemical reactions. In addition, 61 *Salmonella* serotypes were inoculated at low dilution directly onto the agars. The results showed that media based on detection of α galactosidase activity or propylene glycol hydrolysis by salmonellas failed to correctly identify all the *Salmonella* tested, including *Salmonella gallinarum* and *pullorum*. Detection of ß galactosidase activity was found to be an effective method of differentiating non-salmonellas. *Proteus* spp. and pseudomonads grew on all media tested except *Salmonella* Chromogenic Agar, in some cases the growth made correct interpretation of the plates difficult.

P127 DETECTION OF *CAMPYLOBACTER JEJUNI* IN DAIRY SILAGE

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Silage is reported to be a reservoir for *Listeria monocytogenes* and *Yersinia enterocolitica* on dairy farms and may be a possible route for these pathogens to enter the dairy supply. The occurrence of *Campylobacter jejuni* in silage has not been fully investigated. The objective of the study was to determine if silage could be a source of *C. jejuni* on dairy farms. Recovery of *C. jejuni* from silage was determined using a combination of four enrichment and four plating methods (n=16), according to the FDA Bacteriological Analytical Manual (BAM). Five replications were performed for all samples. Corn silage samples (25 g) from bunker silos were examined using *Campylobacter* Enrichment Broth (CEB), *Campylobacter* Enrichment Broth + Rifampicin (CEB-R), Bolton Broth (BB), and Bolton Broth + Rifampicin (BB-R), in combination with selective plating on Modified *Campylobacter* Blood-Free Selective Agar (CCDA) and Abeyta-Hunt-Bark Agar (AHB). Enrichments were incubated under microaerobic conditions and streaked for isolation to CCDA and AHB after 48 h. *Campylobacter*-like colonies were confirmed as *C. jejuni* by conventional biochemical methods. Recovery of *C. jejuni* from silage ranged from 20-100% depending on recovery protocol and sample source. Optimal recovery (100%) from silage was obtained using either BB or CEB enrichment, followed by selective plating to either AHB or CCDA. Addition of oxyrase to enrichment or plating media did not improve recovery of *C. jejuni*. Recovery of *C. jejuni* from silage indicates that it may serve as a reservoir for *C. jejuni* and that silage should be included in an on-farm pathogen reduction plan.

P128 A COMPARISON OF ISOLATION PROTOCOLS FOR RECOVERY OF *CAMPYLOBACTER JEJUNI* FROM CATTLE FECES

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The development of protocols to provide reliable data on recovery and occurrence of *Campylobacter jejuni* in animals and animal production environments is needed so that on-farm foodborne pathogens control programs may be implemented to improve food safety. *C. jejuni* is considered a significant cause of gastroenteritis in humans. Bovine feces are a potential vehicle for transmitting *C. jejuni* to humans. FDA\BAM methodology was investigated as a procedure for the recovery of *C. jejuni* from feces of healthy feedlot dairy cattle. Fecal specimen were inoculated with five strains of *C. jejuni* (log 3 CFU/g) and examined by direct selective plating and selective-enrichment-plating using *Campylobacter* Enrichment Broth (CEB), *Campylobacter* Enrichment Broth + Rifampicin (CEB-R), Bolton Broth (BB), Bolton Broth + Rifampicin (BB-R), Modified *Campylo-*

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bacter Blood-Free Selective Agar (mCCDA) and Abeyta-Hunt-Bark Agar (AHB). A protocol using 1:10 dilution enrichments was also evaluated. Plates and tubes were incubated under microaerobic conditions. Enrichments were streaked for isolation to CCDA and AHB plates after 48 h. *Campylobacter*-like colonies were confirmed as *C. jejuni* biochemically. Recovery of *C. jejuni* ranged from 0-100% depending on choice of isolation protocol. Optimal recovery (100%) from fresh manure (< 2 h) was obtained from direct plating onto CCDA. Optimal recovery (100%) from rectal swabs and fresh manure was obtained using diluted (1:10) CEB enrichment, followed by selective plating to AHB or CCDA. Optimal recovery (100%) from fresh manure was obtained using diluted (1:10) CEB-R enrichment inoculated to AHB. Proper choice of enrichment and plating protocols is essential for consistent recovery of *C. jejuni* from bovine feces.

P129 A RAPID METHOD TO IDENTIFY AND ENUMERATE FOODBORNE PATHOGENS USING MACHINE VISION

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The objective of this research was to develop and evaluate a rapid method to detect and enumerate foodborne bacteria using image processing and statistical modeling techniques. A pure culture of *Salmonella* Typhimurium was used in our experiments. *Salmonella* cells were isolated using rabbit-anti-*Salmonella* (RAS) coated magnetic beads. Also, the bacterial cells were stained with fluorescein isothiocyanate (FITC) labeled goat-anti *Salmonella* (GAS). The mixture of FITC-GAS and RAS-beads were filtered through a polycarbonate membrane that was mounted later on a glass slide. Pictures of bacterial cells were acquired using a CCD camera attached to a motorized fluorescence microscope. A composite image was obtained from multiple images of the sample that were scanned one micron of distance apart in the Z plane of a single field of view. A shape boundary modeling technique, based on the use of circular autoregressive model parameters, was used. In addition, we used a minimum-distance classifier known as the feature weighting (FW) method. The FW classifier was trained using ten images belonging to each shape class (rod shape and circle shape). Also, a disconnecting algorithm was used to separate the contours of touching bacterial cells. Test experimental results showed that the model parameters could be used as a descriptor of shape boundaries detected in digitized binary images of bacterial cells. The recognition and enumeration accuracy improved with model order equal to 3. The computer performed the recognition and counting tasks in approximately 5 s/each field of view.

P130 DETECTION OF GUAIACOL PRODUCED BY *ALICYCLOBACILLUS ACIDOTERRESTRIS* IN APPLE JUICE BY SENSORY AND CHEMICAL ANALYSES

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Spoilage of fruit juice by *Alicyclobacillus acidoterrestris* is characterized by a distinct medicinal or antiseptic off odor attributed to guaiacol, a metabolic by-product of the bacterium. The objective of this study was to determine the threshold for recognition of guaiacol in apple juice by sensory evaluation, and the population of *A. acidoterrestris* and incubation time at 21 and 37°C necessary for guaiacol to be detected by chemical analysis. The best estimate threshold (BET) for recognition of guaiacol by an experienced 19-member panel was 2.23 ppb. Apple juice was inoculated with *A. acidoterrestris* spores (2.98 $\log_{10}$ CFU/ml) and stored at 21 or 37°C for up to 61 days. The panel detected ($P \leq 0.01$) guaiacol in inoculated juice stored at 37°C within 8 days. At three of four sampling times (ranging from 13 to 61 days), at which the sensory panel detected ($P \leq 0.001$) guaiacol, concentrations of 8.1 - 11.4 ppb were detected by chromatographic analysis. The panel detected ($P \leq 0.1$ or $P \leq 0.01$) guaiacol in five samples stored at 21 or 37°C for 8 - 61 days in which the compound was not detected by chromatographic analysis. It appears that guaiacol content in apple juice inoculated with *A. acidoterrestris* is not always correlated with numbers of cells, and the limit of sensitivity of chromatographic quantitation of the compound is higher than the BET.

P131 SAMPLING TECHNIQUE EFFICACY FOR *ARCOBACTER BUTZLERI* FROM LIVE CHICKENS

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Arcobacter butzleri is a causative agent of human enteritis that has been recently differentiated from the genus *Campylobacter*. Previous work suggests that its transmission to humans is likely through a foodborne route, with a substantial tendency to be located on poultry carcasses. For reducing the incidence of this pathogen on commercial poultry, improved protocols are needed to sample and identify *A. butzleri* from infected birds prior to slaughter. The purpose of this study was to design a protocol to sample live chickens that were artificially infected *per os* with *A. butzleri* and housed in simulated on-farm conditions. This

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sampling protocol would identify an optimum sampling technique for the qualitative recovery of *A. butzleri*. We tested three sampling techniques commonly used to determine the microbiological quality of poultry: cloacal swabs, fecal samples, and environmental surface (drag) swabs collected when birds were 3, 5, or 7 weeks old. These samples were cultured in Johnson-Murano enrichment broth and analyzed by polymerase chain reaction. Results indicate that environmental surface (drag) swabs yielded the highest recovery percentage from artificially infected chickens. A detection rate between 75% to 100% was observed for each sampling period (age of chicken).

P132 DETECTION OF COLIFORMS ON FOOD CONTACT SURFACES

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Assessing the hygienic status of food preparation areas and the efficacy of cleaning is an important component of food safety management systems. Of particular concern for many food companies, especially those involved in the production of ready-to-eat foods, is the presence of coliforms, which can be used as indicator organisms. When testing for coliforms, the speed with which results can be obtained and the minimum detection limit are both of great importance. Two newly developed rapid coliform tests were compared to 3 traditional coliform enumeration techniques as well as ATP bioluminescence.

Serial dilutions of known levels of 3 different strains of coliform bacteria were inoculated onto food grade stainless steel squares. Surfaces were sampled immediately after inoculation while still wet or after 60 min when completely dry, using standardized techniques or the manufacturer's instructions.

Additional tests were carried out directly on the serial dilution (i.e., not applied to the surfaces).

Using dipslides and the two rapid tests, it was possible to detect < 1 CFU/cm^2 from a wet surface. Swabbing using spread and pour plates proved to be less sensitive, detecting 1 CFU/cm^2 and 3 CFU/cm^2, respectively. However, when directly inoculated each of the tests was capable of detecting lower levels of coliforms, suggesting that no test could pick up all of the cells present on the surface. Drying played an important part in further reducing the recovery of coliforms from the surface. As a result the detection limit of all the coliform tests increased to > 10^4 CFU/cm^2.

ATP bioluminescence gave results immediately, but its detection limit was approximately 10 CFU/cm^2 and 10^3 CFU/cm^2 from a wet and dry surface, respectively. All 3 traditional techniques required at least a 24 h incubation period before any results could be obtained however, one newly developed rapid test gave results within 18 h and the other after only 5 h.

The results of this investigation strongly suggest that when testing for coliforms, detectability need not be at the expense of speed. The newly developed rapid tests evaluated in this investigation could prove a valuable tool in the hygienic assessment of food contact surfaces.

P133 DETECTION OF ZEARALENONE BY FLUORESCENCE POLARIZATION IMMUNOASSAY AND ITS APPLICATION TO CORN

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A homogeneous fluorescence polarization immunoassay (FPIA) was developed to detect zearalenone (ZEA) using a TDx analyzer in photocheck mode (Abbott Labs). To use immunogen, which produces monoclonal antibody, zearalenone was converted to 6′-(*o*-carboxymethyl) oxime zearalenone (ZEA-oxime) and conjugated with bovine serum albumin. The tracer, fluorescein isothiocyanate (FITC)-labeled ZEA, was synthesized with ZEA-oxime and ethylenediamine fluorescein thiocarbamyl (EDF). By thin layer chromatography, synthesized ZEA-oxime-EDF was separated into four different spots on R*f* 0.825(#1), 0.625(#2), 0.5(#3), 0.375(#4). The separated different combinations of tracers were investigated in the FPIA system. The #2 spot had good sensitivity to assay. ZEA was diluted with methanol and then quantified with homogeneous competitive binding FPIA. The ZEA detection limit was 10 ng/ml in standard solution. And a simple procedure was devised for the screening of ZEA in corn, using FPIA. Crushed corn samples (5 g) were extracted with 25 ml of methanol by vortexing for 30 min and applied to FPIA. Average recoveries from the corn spiked with ZEA at levels of 1, 10, and 100 ng/ml were 90, 85, and 95%, respectively. Sixty-six samples were detected the ZEA contamination by FPIA. Among 66 imported and domestic corn samples, 10 (15.2%) contained over 30 ng/g of ZEA, with an average of 46.51 ng/g. In this immunoassay, separation step was not required and the total time for an assay for 10 samples was approximately 10 min. So this FPIA system is a useful tool for screening and detecting ZEA in corn.

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P134 SCREENING OF DEOXYNIVALENOL-PRODUCING FUNGI FROM GREENHOUSE HORTICULTURE SOILS AND PRODUCTS BY ALP/NADP METHOD

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In order to evaluate the safety of greenhouse horticulture products in Korea, we carried out this work by screening of *Fusarium* species, which produce deoxinivalenol (DON) from greenhouse horticulture in Western Gyeongnam and Northern Gyeongbuk, Korea. For this study, high sensitive enzyme-linked immunosorbent assay, ALP/NADP method, was applied to detection of DON by enzyme amplification system. From 192 samples of greenhouse horticulture soil and its products, 103 isolates of *Fusarium* species were obtained. The isolates were cultured at 28°C for 15 days and the cultured mediums were extracted by ethyl acetate. The production of DON was verified by thin layer chromatography (TLC). As the results of TLC, 8 strains were identified as DON producing strain. We screened potential producers of DON by ALP/NADP. The levels of DON production were shown from 0.007 to 1.21 μg/ml of YES medium. The maximum DON producing strain, No. 32-D-3, was isolated from soil in Namhae, Korea. In conclusion, the results indicate that DON producing fungi contaminated greenhouse horticulture products in Korea. Therefore, further studies are required to accumulate more detailed data about the contamination of DON in various cereals.

P135 A COMPARISON OF METHODS FOR MONITORING FOOD CONTACT SURFACE CLEANLINESS

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A number of methods exist with which to monitor food contact surface cleanliness, yet relatively little is known about the efficiency with which these methods recover bacterial contamination under different environmental conditions. In this study, the minimum bacterial detection limits of the Biotrace Clean-Trace Rapid Cleanliness Test, agar-contact dip slides, and traditional hygiene swabbing were determined. Areas (100cm^2) of food grade stainless steel were separately inoculated with known levels of *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 7644) and *Pseudomonas aeruginosa* (ATCC 27853). Surfaces were sampled immediately after inoculation while still wet, or after 60 min when completely dry. For all organisms, the minimum detection limit of the ATP Clean-Trace Rapid Cleanliness Test was 10^4 CFU/100cm^2 ($P < 0.05$) and was the same for wet and dry surfaces. Both organism type and surface status (i.e., wet or dry) influenced the minimum detection limits of microbiological methods, which ranged from 10^1 CFU/100cm^2 to 10^5 CFU/100cm^2 on wet surfaces and from 10^2 CFU/100cm^2 to 10^8 CFU/100cm^2 on dry surfaces. Hygiene swabbing percentage recovery rates for all organisms were found to be less than 0.1% on dry surfaces but significantly higher on wet surfaces. The results are discussed within the context of hygiene monitoring within the food industry.

P136 SPREADSHEET TOOL FOR RECORDING AND EVALUATING MICROBIOLOGICAL ENVIRONMENTAL SAMPLING DATA

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Processors increasingly rely on microbial sampling of the plant environment to determine if their products or processes are at risk of containing or transmitting pathogens. Sampling and analytical tests may be conducted for specific pathogens such as *Salmonella* or *Listeria*. Aerobic plate counts and ATP bioluminescence assays are also used to determine areas that need additional cleaning and sanitation. Often, an effort is made to report and react to isolated test results through additional sanitation procedures. But, a continual evaluation of an environmental sampling plan should be performed to determine if there are trends in microbial detection. The evaluation of the sampling plan and the test data over extended times may lead to changes in the test sample frequency, location and analysis performed, or in the plant's corrective actions. A spreadsheet template was developed for recording environmental sampling data. This template provides a format for recording sample collection day, date, shift, hour, plant area location, sample location, analytical test (qualitative or quantitative), and test results. A data set of 2,000 samples was constructed and analyzed using the "Pivot Table" feature in Microsoft Excel. This feature creates an interactive data table that quickly summarizes large data sets or subsets. A written instructional guide and diskette has been developed that explains how to use and design Pivot Tables to extract useful information for summarizing the data for environmental sample collection and sample analyses. The guide includes recordkeeping formats and ten examples of useful Pivot Table designs.

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P137 REVERSE DOT-BLOT DNA/DNA HYBRIDIZATION METHOD FOR THE DETECTION OF BACTERIA INVOLVED IN AMINE FORMATION IN ALBACORE TUNA (*THUNNUS ALALUNGA*)

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The development of rapid and sensitive DNA-based methods aimed at detecting and identifying pathogenic and/or spoilage bacteria in foodstuffs is gaining increased importance. Among these methods, DNA/DNA hybridization has lower detection limits than PCR and is less affected by the presence of potentially-interfering substances in such complex samples. Besides, probe detection methods seldom yield false-positive results due to the presence of residual amounts of DNA or dead cells.

Amine-producing bacteria are responsible for the biosynthesis and secretion of specific amino acid decarboxylases in the muscle of certain scombroid fish species. While histamine is responsible for scombroid poisoning, the role of cadaverine and putrescine as potentiators of histamine toxicity has also been documented. Total DNA extracts from amine-producing strains of *Morganella morganii*, *Strenotrophomonas maltophilia*, *Hafnia alvei*, *Enterobacter aerogenes* and *Klebsiella planticola* were obtained, and equivalent amounts of their DNAs were spotted onto Nylon-N+ blotting membranes. The specificity of the dot-blot hybridization method was checked by probing the blots with genomic DNAs from *M. morganii*, *S. maltophilia* and *E. aerogenes*, with no significant cross-reaction among the five species being observed. Additionally, portions of white muscle of albacore tuna were obtained, sterilized at 121°C, and artificially contaminated with pure cultures of *M. morganii* or *S. maltophilia*. Then, bacterial DNA from the inoculated muscle was isolated, labeled with fluorescein, and used as a probe in DNA/DNA hybridizations, being detection carried out under high-stringency conditions. Each species was successfully identified and the detection limits for *M. morganii* and *S. maltophilia* — major histamine and cadaverine producers, respectively, in albacore tuna — were also determined.

P138 THE USE OF MALDI-TOF AND NANOSPRAY-ION TRAP MASS SPECTROMETRY TO THE CHARACTERIZATION OF SPECIFIC PROTEINS SEPARATED BY TWO-DIMENSIONAL ELECTROPHORESIS: APPLICATION OF PROTEOMICS TO THE CONTROL OF SPECIES SUBSTITUTION IN FISH PRODUCTS

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Food labelling regulations aimed at detecting fraudulent substitutions of species in commercial products have become mandatory. Up to now, several techniques have been used with identification purposes in fish products. These techniques are based on either DNA-analysis or protein analysis. While isoelectric focusing, SDS-PAGE and HPLC have been traditionally considered for achieving specific protein profiles and amino acid composition, little attention has been paid to the identification and characterization of specific proteins from seafood products at the molecular level. We have combined the high-resolving power of two-dimensional electrophoresis with the potency of peptide analysis by mass spectrometry (MS) for the detailed molecular characterization of parvalbumins extracted from the muscle of five hake species of recognized commercial value. Peptide mass maps were obtained by "in-gel" tryptic digestion of protein bands followed by MALDI-TOF analysis, and individual species were sequenced by nano-spray-ion trap MS. In this manner, parvalbumin sequences were characterized in all five hake species, and a specific peptide was sequenced from *Merluccius australis* which was not present in the other species. A specific 18 kDa-protein from *M. merluccius* was also sequenced, revealing a 97% sequence identity with rat nucleotide diphosphate kinase A (EC 2.7.4.6., SWISS-PROT Q05982). The combination of high-resolution two-dimensional electrophoresis and mass spectrometry, which provides the basis for the emerging technology of Proteomics, proved to be a reproducible and sensitive strategy for the molecular characterization of peptides in fish products for both identification and characterization purposes.

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P139 DETECTION OF *SHIGELLA* USING A DIGOXIGENIN-LABELED POLYNUCLEOTIDE DNA PROBE

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There are four species of *Shigella* responsible for foodborne disease in humans, especially in food products that do not receive a terminal heat process prior to consumption. Low numbers of these organisms can cause infectious disease but current methods for the isolation and detection of these organisms are poor. A 620-bp fragment of the invasion plasmid antigen H (ipaH) gene from *S. flexneri* was amplified using the PCR and digoxigenin incorporated into the fragment during amplification. This labeled fragment was then used as a DNA probe to detect the ipaH gene in chromosomal DNA preparations and in *S. boydii*, *S. flexneri*, *S. sonnei*, and *S. dysenteriae* colonies lifted from agar plates onto nylon membranes. The target DNA hybridized with the digoxigenin-labeled probe was detected using an anti-digoxigenin antibody conjugated to alkaline phosphatase. A colorimetric substrate was used to detect low numbers of *Shigella* and enteroinvasive *E. coli* after enrichment and isolation on agar plates. The probe also was used to confirm that non-digoxigenin labeled 620-bp PCR fragments amplified using a *Shigella* PCR protocol were ipaH gene fragments.

TECHNICAL SESSIONS

*Presenter

T01 SURVIVAL AND HEAT RESISTANCE OF ALKALI-STRESSED *LISTERIA MONOCYTOGENES*

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A strain of *Listeria monocytogenes* serotype 4b isolated from a food processing plant was exposed to alkaline stress and evaluated for survivability and heat resistance. Cells were suspended in tryptose phosphate broth (TPB) at pH 7.3 or TPB adjusted with 1 N NaOH to pH 9.0, 10.0, 11.0, 12.0, or 13.0 ± 0.2 and incubated at 37°C for 15 or 45 min. Populations of alkali-stressed and non-stressed cells incubated in pH-adjusted TPB at 4 or 21°C were monitored for up to 144 h. At both temperatures, populations of *L. monocytogenes* remained at 8.6 – 9.7 $\log_{10}$ CFU/ml of TPB adjusted to pH 7.3, 9.0, and 10.0 for up to 144 h. Populations gradually declined in TPB at pH 11.0, while viable cells were not detected in TPB at pH 12.0 or 13.0 after incubation for 144 h. Thermotolerance of cells held in TPB at pH 7.3 – 13.0 for 15 or 45 min was determined using the capillary tube method. $D_{56°C}$ values of cells previously incubated in TPB at pH 7.3 for 15 or 45 min were 5.43 and 4.74 min, respectively, and did not differ significantly ($P < 0.05$) from $D_{56°C}$ values of cells treated at pH 9.0, 10.0, or 11.0. However, $D_{56°C}$ values of cells incubated in TPB at pH 12.0 for 15 and 45 min were 7.10 and 12.1 min, respectively, and differed significantly from that of cells exposed to pH 7.3. Alkali-stress response in *L. monocytogenes* may induce an increased resistance to otherwise lethal thermal processing conditions.

T02 *LISTERIA MONOCYTOGENES* IN UHT MILK: A CASE STUDY

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Land O' Lakes recently averted a potential *Listeria monocytogenes* outbreak using AOAC approved methods and a new rapid *Listeria monocytogenes* specific screening test in a continuous effort to maintain the highest degree of food safety in all Land O' Lakes products. The analytical laboratory discovered *Listeria monocytogenes* in a co-packer produced UHT milk product and utilized bioMérieux VIDAS products LIS and LMO in conjunction with AOAC approved *Listeria* detection methods to screen for both *Listeria* species and *Listeria monocytogenes* from the same enrichment broth. Using the LIS screen allowed the laboratory to obtain presumptive positive results for *Listeria* species. Subsequent analysis of the presumptive enrichments using the LMO allowed for presumptive identification of the cultures as *Listeria monocytogenes*. Both results were available within 48 h of sample receipt. All samples that screened positive for both *Listeria* and *L. monocytogenes*, using LIS and LMO tests respectively, were confirmed biochemically and by genetic fingerprint patterns as *L. monocytogenes*. As a result of these rapid methods in *Listeria* detection and confirmation, the company was the first to detect a serious contamination issue that involved a multi-brand, multi-company product recall, and was thereby able to avert a potential outbreak situation by early detection of contaminated product and prompt removal from the food supply.

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T03 THE ABILITY OF SUBLETHALLY HEAT-INJURED *LISTERIA MONOCYTOGENES* CELLS TO COMPETE WITH A COMMERCIAL MESOPHILIC LACTIC ACID STARTER CULTURE DURING MILK FERMENTATION

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Overnight tryptose broth cultures of three *L. monocytogenes* (30 ml) strains were centrifuged, suspended in 200 ml of tryptose phosphate broth, and heated at 56°C/20 min and 64°C/2 min to obtain low heat-injured (LHI) and high heat-injured (HHI) cells, respectively, showing >99% injury. Flasks containing 200 ml of UHT milk were tempered to 88°F, inoculated to contain 10^4-10^6 LHI, HHI, or healthy unheated *L. monocytogenes* cells and 0.5, 1.0, or 2.0% of a commercial *Streptococcus lactis/ Streptococcus cremoris* starter culture (Chr. Hansen). Additional flasks containing the starter culture alone and *L. monocytogenes* alone served as controls. Numbers of both healthy and injured *L. monocytogenes* cells were determined initially using tryptose phosphate agar w/o 4.0% added NaCl and at selected intervals during the 24 h fermentation period along with the numbers of starter organisms. Populations of LHI and HHI cells increased 2.8 and 3.13 logs after 6 h of fermentation using a 0.5% starter culture inoculum as compared to 2.77 and 2.3 logs using a 2.0% starter culture inoculum. *Listeria* attained stable populations of ~10^9 CFU/ml after 8 h of fermentation regardless of starter culture levels. After 24 h of fermentation, ~93% of the non-injured population became injured. In starter-free controls, >80% of both HHI and LHI cells were repaired within 10 h of incubation. Presence of *L. monocytogenes* did not adversely affect the growth of the starter culture at any inoculation levels. Overall, the growth of heat-injured *Listeria* was generally suppressed by the starter culture, particularly at higher inoculum levels.

T04 GROWTH OF *LISTERIA MONOCYTOGENES* AND *ESCHERICHIA COLI* O157:H7 IS ENHANCED IN READY-TO-EAT LETTUCE WASHED IN WARM WATER

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Ready-to-eat lettuce is routinely washed in cold chlorinated water. This treatment reduces total microbial populations by 1 log CFU/g. Research in our laboratory has shown that reductions approaching 3 log CFU/g are possible in warm (47°C) chlorinated water. The effect of warm water washes on the survival and growth of pathogens such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 during subsequent storage were previously unknown. Lettuce was dipped in cold (1°C) and warm (47°C) chlorinated water (100 µg /ml total chlorine) before and after inoculation with both pathogens. The lettuce was packed in oxygen permeable film bags (OTR: 6000-8000 cc/m2/24 h) and stored under ideal (1°C) and abusive (12°C) temperatures. Total microbial populations were reduced by approximately 1 log CFU/g in cold chlorinated water, and 2.5 log CFU/g in warm chlorinated water. *L. monocytogenes* and *E. coli* O157:H7 populations did not increase in warm water washed lettuce after 14 days in storage at 1°C but decreased in lettuce washed in cold water. Growth of both microorganisms was limited in lettuce processed in cold water and stored at 12°C. In contrast, extensive growth was observed in lettuce washed in warm chlorinated water, particularly for *L. monocytogenes*. These results suggest that survival and growth of pathogens may be enhanced in ready-to-eat lettuce washed in warm water.

T05 A SURVEY OF UNITED STATES ORCHARDS TO IDENTIFY POTENTIAL SOURCES OF *ESCHERICHIA COLI* O157:H7

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The association of unpasteurized apple cider with *Escherichia coli* O157:H7 foodborne illness has led to increased interest in potential reservoirs of this pathogen in the orchard. Fourteen United States' orchards were surveyed in autumn 1999 to determine the incidence and prevalence of *E. coli* O157: H7, generic *E. coli* and other microflora. Fruit samples (n=67) (8 apple and 2 pear varieties), soil, water and fecal samples were collected. Samples were plated on (a) Tryptic Soy Agar for total aerobic count (b) *E. coli* Petrifilm for total coliforms and generic *E. coli*, and (c) Yeast and Mold Petrifilm. Samples positive for coliforms were enriched for *E. coli* O157:H7. Fruit was also tested for internalization of microflora by aseptically removing the core, stem, and calyx areas and individually plating these samples as listed above.

No *E. coli* O157:H7 was found. Generic *E. coli* was detected in soil and water, and in 6% of fruit samples, collected from areas associated with fecal contamination. Coliforms were found in 43.7% of fruit samples. Total aerobic counts and total coliforms were higher in dropped and damaged fruit ($P < 0.05$) and in orchards associated with fecal contamination or proximity to pastures ($P < 0.05$). Yeasts and molds were internalized in 96.7% of samples, aerobic bacteria in 89.6%, and coliforms in 33.5%. No internalized *E. coli* was found.

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The data suggest that dropped and/or damaged fruit should not be included in fruit destined for the production of unpasteurized juice. Orchards should be located away from potential sources of contamination, such as pastures.

T06 ATTACHMENT OF *ESCHERICHIA COLI* O157:H7 TO THE EPIDERMIS AND INTERNAL STRUCTURES OF APPLES AS DEMONSTRATED BY CONFOCAL SCANNING LASER MICROSCOPY

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Confocal scanning laser microscopy (CSLM) was used to demonstrate the attachment of *Escherichia coli* O157:H7 transformed with a plasmid encoding for green fluorescent protein (GFP) to the surface and within the internal structures of unwaxed apples. Apples at 4°C or 25°C were inoculated with a cell suspension at 2°C or 25°C. The effect of a negative temperature differential (warm apple, cold inoculum), a positive differential (cold apple, warm inoculum), and no differential (warm apple, warm inoculum), with or without a pressure differential, on infiltration and attachment of cells was determined. Three-dimensional CSLM micrographs of apples subjected to all combinations of test parameters showed preferential cellular attachment on the tissue surfaces in puncture wounds, on discontinuities in the waxy cuticle, and in lenticels, where *E. coli* O157:H7 was observed 40 μm below the surface. Infiltration through the floral tube and attachment to seeds, cartilaginous endocarp, and internal trichomes were influenced by temperature differential, with a negative differential leading to greater attachment. Pressure differentials had no effect on infiltration or attachment of *E. coli* O157:H7. Results support the probability that *E. coli* O157:H7 within lenticels and internal structures may evade decontamination treatment, especially if apples had been washed in contaminated water with a negative temperature differential.

T07 QUINOLONE RESISTANCE AMONG CLINICAL AND FOOD ISOLATES OF *CAMPYLOBACTER* SPP.

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Increasing resistance to quinolones among *Campylobacter* isolates from humans has been reported in Europe, Asia and the United States. We evaluated resistance to quinolones among *Campylobacter* isolates from humans and foods during a 2-year period. All 144 clinical isolates and 39 food isolates were tested for resistance to ciprofloxacin, gentamycin, erythromycin, ampicillin, enrofloxacin, norfloxacin, tetracycline, naladixic acid and cephalothin. In addition, a questionnaire was administered in order to determine risk factors for campylobacteriosis, including consumption of chicken, contact with well water and animals, recent restaurant visits, foreign travel, and time of the year.

The proportion of quinolone-resistant *Campylobacter* isolates from humans was higher (13.9%) than that among food isolates (2.6%). The number of infections acquired domestically was higher than those associated with foreign travel, with 62.4 and 37.6% attributed to domestic infections and foreign travel, respectively. Infection with *Campylobacter* spp. was highly associated (85.1%) with the consumption of chicken; 42.6% of cases also reported having visited a restaurant up to five days prior to the development of symptoms. In addition, 45.5 and 8.9% of cases reported having contact with pets and well water, respectively. Around 54 and 23% of the total cases were reported in the summer (May to September) and winter months (January through April), respectively.

Further characterization of these isolates is being carried out using a modified pulsed-field gel electrophoresis (PFGE) protocol and the *Sma* I restriction enzyme. Analysis of profiles will help to determine the common pulsotypes causing human illness in Canada.

T08 THE SURVIVAL AND CULTURABILITY OF *CAMPYLOBACTER JEJUNI* MICRO-COLONIES UNDER MODIFIED ATMOSPHERES AT 4°C AND 8°C USING A MODEL FOOD SYSTEM

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Packaging of meat in a modified atmosphere (MA) extends the shelf life during refrigerated storage, inhibiting the growth of spoilage bacteria, but at the same time, the survival of *Campylobacter jejuni* is said to improve. The morphology and culturability of *C. jejuni* cells in micro-colonies was investigated when incubated at refrigeration temperatures under MAs using a solid-phase model food system.

C. jejuni colonies were grown on agar coated microscope slides, and survival of the colonies was determined at 4°C and 8°C under 5% O_2: 10% CO_2: 85% N_2 (MA_1); 30% CO_2: 70% N_2 (MA_2) and

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compared with survival under aerobic conditions over 14 d. Computer image analysis (IA) was used to monitor micro-colony survival, growth and cell morphology (rod to coccoid ratio). Culturability of the colonies previously stored under the various MAs was determined by re-incubating under ideal conditions at 37°C to enhance resuscitation.

The percentage of rod to coccoid cells varied from 100% to 94% (4°C) and 95% (8°C) under all MAs. When re-incubated under ideal conditions, growth ceased following 1d and 3d for cells previously stored under aerobic conditions and MA_1, respectively (4°C and 8°C). After this period the cells appeared as single rods (MA_1) and predominantly coccoid in morphology (aerobic). Survival was prolonged when incubated under MA_2 and cells continued to double following 6d to 8d at 4°C and 8°C.

The direct method of detection employed provided beneficial data towards determining the complex mode of action of this organism within foods. Use of a solid-phase model food demonstrated the resuscitation potential of the organism and its ability to adapt its metabolism to different environments. Foods packaged under MAs containing CO_2 and consumed without further heating may be identified as high-risk products.

T09 SURVIVAL OF *CAMPYLOBACTER JEJUNI* IN BIOFILMS ISOLATED FROM CHICKEN HOUSES

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Campylobacter jejuni is a thermophilic and oxygen intolerant pathogen associated with poultry. Biofilms may be a source of *C. jejuni* in poultry house water systems as biofilms may protect constituent microorganisms from environmental stress. In this study, viability of *C. jejuni* in biofilms of gram-positive chicken house isolates (P1, Y1, W1) and a *Pseudomonas* sp. was determined using a cultural method (modified Brucella agar) and direct viable count (DVC). Two-day biofilms grown on polyvinyl chloride coupons in R2A broth at 12°C and room temperature were incubated with *C. jejuni* for a 6-h attachment period. Media were refreshed every 24 h. Biofilms of P1, Y1 and *Pseudomonas* increased attachment ($P < 0.01$) of *C. jejuni* (4.74, 4.62 and 4.78 log cell/cm^2, respectively) compared to W1 and control without biofilm (4.31 and 4.22 log cell/cm^2, respectively). This may be due to their ability to produce more biofilm or differences in the chemical nature of the biofilms. On day 7, isolates P1, Y1 and *Pseudomonas* produced biofilms of 5.40, 7.00 and 21.45 percent coverage, respectively, compared to 4.87% by W1. Viable *C. jejuni* within biofilms were reduced ($P < 0.05$) in number with time with greatest reduction occurring in the biofilm-free control. The number of viable *C. jejuni* determined by DVC was greater than that determined by the cultural method indicating that *C. jejuni* may form a viable but nonculturable state within biofilm. Both DVC and cultural method indicated that biofilms enhance ($P < 0.01$) survival of *C. jejuni* over 7-day incubation.

T10 COMPARATIVE TOLERANCE OF *SALMONELLA* TYPHIMURIUM DT104 TO HEAT AND DESICCATION

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S. Typhimurium DT 104 (DT 104) is a concern in the US. Since its survival in foods is not well characterized, we compared its thermal and dessication inactivation kinetics to non-DT 104. Strains from the US and UK were grown to stationary phase at pH 4.7, 7.0, and 8.7. Time required to inactivate 4 logs (4D60° = 2.3 min) was identical ($P > 0.05$), for all pH values and strains. Survival in brain heart infusion (BHI) broth, desiccated at 28°C, was similar ($P > 0.05$) for DT 104 (D = 26±6 h) and non-DT 104 (D = 22±6 h). Equine whole blood and whole egg were more protective than BHI. In blood, US DT104 (D = 616±167 h) was significantly ($P < 0.05$) more resistant to desiccation than UK strains (D = 307±158), but neither was different ($P > 0.05$) from non-DT 104 (D = 463±173 h). In egg, the overall mean desiccation D value (1180±540 h) was greater than in blood; no ($P > 0.05$) difference was observed for the three strain types. D60 values of isolates that were previously dried in BHI at 28°C fell from 0.65 to 0.23 min after 28 d of drying, and there were no differences ($P > 0.05$) among the isolates. Results indicate that DT 104 is no more resistant than non-DT 104, for thermal or drying inactivation. A secondary observation is the greater resistance of US isolates compared with their UK counterparts.

T11 ROUTES OF INFILTRATION, SURVIVAL, AND GROWTH OF *SALMONELLA ENTERICA* SEROVAR HARTFORD AND *ESCHERICHIA COLI* O157:H7 IN ORANGES

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Infiltration, survival, and growth potential of selected human pathogens in oranges were investigated. The infiltration potential was assessed in two ways: (1) by applying 0.1 ml of a 7-log CFU/ml

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suspension of *Escherichia coli* O157:H7 onto the stem scar, and (2) by creating small holes of specific diameter (0.95 mm) in the peel and immersing the orange in a 5-log CFU/ml suspension of *E. coli* O157:H7. The oranges were then subjected to temperature decrease, surface sanitization, juice expression, and microbial analysis of the number of *E. coli* O157:H7 in the resulting juice. Survival and growth studies were performed with *E. coli* O157:H7 and *Salmonella enterica* Hartford by injecting pathogens into simulated puncture wounds created at various locations on oranges, incubating fruit for 5-days at either 4° or 21°C, then juicing and enumerating the number of bacteria in the juice sample. Bacteria were recovered on non-selective and selective media. Approximately 3.6% of treated oranges internalized pathogens through the stem scar, at a ratio of 0.001 to 0.0001 of the challenge levels. Fruit with 0.95 mm holes in peels internalized pathogens at a frequency of 30%. Pathogen growth occurred in oranges at 21°C, but not at 4°C. Results demonstrated the potential for human pathogen internalization and growth within oranges. Producers and processors can prevent contamination and control exposure of picked oranges to warm internal/cold external conditions. More importantly, technologies need to be developed to detect and remove fruit with peels having small punctures of sufficient size to allow the internalization of pathogens.

T12 A DESCRIPTIVE ANALYSIS OF GIARDIASIS CASES REPORTED IN ONTARIO, 1990-1997

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Ontario human cases of giardiasis were described using notifiable disease data from the Ontario Ministry of Health for the years 1990-1997 inclusive. The annual average crude rate of disease was 27.1 cases per 100,000 population. Children under five years of age had the highest incidence of disease. Males, especially in the rural setting, were less likely to be infected than females. Only 1% of cases were reportedly related to male homosexual contact. Three deaths were reported. The most frequently reported symptom was diarrhea (50% of cases), while 27% reported cramps or abdominal pain and 10% nausea or vomiting. The proportion of cases occurring between July and October inclusive (40%) was significantly higher than expected (33%) assuming no seasonal variation ($P < .001$). The proportion of rural cases observed (13.7%) was significantly lower than expected (16.7%) based on the Ontario population ($P < .001$). The home was identified as the risk setting in 41% and travel in 39% of cases where a risk setting was reported (n=11,940). The most frequently reported sources of infection were water (particularly unfiltered) and person-to-person contact. These findings suggest that a high proportion of cases occur in urban versus rural areas, and unfiltered water and person-to-person contact are important sources of infection.

T13 DEVELOPMENT OF A STANDARD METHOD TO DETECT PARASITIC PROTOZOA ON FRESH VEGETABLES

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Foodborne outbreaks of parasitic protozoan infection have been documented in both developed and developing countries. Such outbreaks may occur following consumption of either local or imported surface-contaminated produce, particularly those which receive minimal treatment prior to consumption. Currently, no standard method exists in the United Kingdom for detecting the transmissive stages [oocysts and cysts] in/on foods. We therefore intend to develop a standard method for eluting, concentrating and identifying oocysts of *Cryptosporidium parvum* and *Cyclospora cayetanensis*, as well as cysts of *Giardia intestinalis*, from fresh vegetables. Our initial work has been performed on lettuce seeded with *C. parvum* oocysts, and various extraction parameters have been evaluated, which have led to the production of a workable detection method.

Small numbers of oocysts, seeded onto lettuce leaves, are eluted by pulsification in glycine buffer pH 5.5 and concentrated by centrifugation at 4,000 × g for 30 min. Further concentration and purification is performed by immunomagnetic separation (IMS). Following IMS, the magnetisable bead is desorbed from the bead-oocyst complex and the oocysts air-dried onto microscope slides. Oocysts are visualized by fluorescence microscopy using genus-specific FITC-labelled monoclonal antibodies and the nuclear fluorogen 4'6-diamidino-2-phenyl indole (DAPI). With 30 g lettuce samples seeded with 100 oocysts, recoveries of around 75 % could be achieved. Varying the pH of this extraction buffer may further improve recovery.

The final method will be validated through interlaboratory trial, for use in accredited analytical laboratories.

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T14 DEVELOPMENT OF CUSTOM IDENTIFICATION PATTERNS FOR *SALMONELLA* BASED ON THE USE OF THE RESTRICTION ENZYME PVUII WITH AN AUTOMATED RIBOTYPING SYSTEM

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The RiboPrinter® Microbial Characterization System is an automated ribotyping instrument. It generates, analyzes, and stores rRNA operon fingerprints (RiboPrint® patterns) of bacteria. The standard method for sample identification and characterization employs EcoRI restriction endonuclease for digestion of bacterial DNA. The resultant EcoRI rRNA operon fingerprint is compared to a dynamic database of RiboPrint® patterns for characterization, and also to a fixed identification database for providing classical taxonomy for *Salmonella* and other bacterial genera. The reagents used for sample DNA preparation and digestion by the system are, however, compatible with a variety of discrete restriction endonucleases. Of those tested, PvuII has been particularly useful for additional characterization of *Salmonella*. A set of 100 Custom Identification patterns was created from *Salmonella* samples characterized using PvuII. Such sets can be created and augmented by individual users and readily shared with other sites. The current set includes approximately 60 serotypes including nine patterns for *Salmonella ser.* Typhimurium, five patterns for *S. ser. Montevideo* and four patterns for *S. ser. Oranienburg*. The set includes identification patterns for all of the twenty most prevalent human and veterinary serotypes. This set of patterns should greatly increase the capacity of the system to provide classical identification for *Salmonella* characterized on the system, further extending the utility of automated ribotyping.

T15 THE DEVELOPMENT AND TESTING OF AN INSTRUMENT FOR THE HOMOGENEOUS DETECTION OF PCR PRODUCTS

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The BAX® for Screening family of PCR assays for foodborne pathogens (Qualicon, Inc.) determines the presence or absence of a specific target based on DNA amplification and gel electrophoresis. All primers, polymerase, and deoxynucleotides necessary for PCR as well as a positive control and an intercalating dye are incorporated into a single tablet. An instrument was developed to integrate the amplification and detection steps, further simplifying the process. This instrument uses an array of 96 blue LEDs as the excitation source and a photomultiplier tube to detect the emitted fluorescent signal. A *Salmonella* assay was used to test the system. Eight foods were inoculated at four different levels with three separate strains of *Salmonella* and processed using the manufacturer's recommended sample preparation procedure. Each inoculation level was replicated six times. An additional 288 uninoculated samples were also tested. The instrument conducted thermal cycling on the samples and then performed melting curve analysis to determine the presence or absence of the *Salmonella* target. Following melting curve analysis, gels were run for each sample to confirm the melting curve result. There was 98% agreement for the inoculated samples between the two detection methods in this study of 864 samples. Field testing of the instrument is under way. We will report on the results of studies of assays for *Salmonella* and *Listeria*. Such integrated amplification and detection instrument will greatly improve the ease of use of PCR-based screening assays.

T16 EVALUATION OF IMMUNO-CONCENTRATION PROCEDURE TO DETECT SALMONELLAE IN POULTRY SAMPLES

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Two immuno-concentration methods were compared to the USDA, FSIS cultural method for the detection of salmonellae in processed chicken rinse and poultry feed samples. The VIDAS Immuno-concentration *Salmonella* (ICS) assay is an automated process for the selective enrichment of salmonellae for use on the VIDAS system. The immuno-concentration involves reacting 800 l of an 18-24 h pre-enrichment with a cocktail of monoclonal and polyclonal antibodies specific to both O and H antigens which have been coated onto a solid phase receptacle (SPR), followed by washing and releasing steps. Both ICS procedures and the USDA procedure begin with the sample being preenriched in buffered peptone water for 18 to 24 h. Following preenrichment and ICS immuno-concentration, test samples were analyzed by two separate methods. For Method 1, released salmonellae were post enriched in a non-selective growth medium for 5-6 h, followed by immunoassay detection (24-30 h test). For Method 2, released salmonellae were plated on designated selective agar plates (42 to 48 h test). The USDA, FSIS method is a 72 h procedure. In these studies, 3 replications of 20 broiler carcass rinse samples and 1 set of 20 inoculated feed samples were evaluated by each of the methods. For broiler rinse samples, 12/60 (20%) and 9/60 (15%) of samples were found positive by Methods 1 and 2, respectively, compared

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to 11/60 (18%) by the USDA, FSIS procedure. One hundred percent (20/20) of inoculated feed samples were positive by all methods. The VIDAS ICS followed by automated ELISA is an automated procedure which accurately detected salmonellae from poultry samples in 24 h, saving 1 to 2 days over other traditional ELISA and cultural procedures.

T17 RAPID ENUMERATION OF *LACTOBACILLUS* SPP. IN SALAD DRESSINGS USING THE BIOSYS

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Lactobacillus spp. are common spoilage organisms of salad dressings, causing off flavors, off odors, and gassing in these products. Common practice in production facilities is to hold product in inventory until the results of routine testing for these organisms are complete, often up to 96 h. A new system for the enumeration of microorganisms, based on optical detection of acid production using a pH indicator, the BioSys (MicroSys, Inc.), can provide an estimation of the level of these organisms in a salad dressing within 24-48 h. Various types of viscous and pourable salad dressings were inoculated with homofermentative and heterofermentative lactobacilli and tested using a modified MRS broth medium containing bromcresol green in the BioSys instrument. Acid production by the lactobacilli caused a color change in the medium, which resulted in an increase in transmittance at 585 nm. The time for detection was inversely proportional to the initial levels of organisms in the product. Depending on the type of salad dressing tested, 10,000 lactobacilli per gram were detected within 10-18 h and 10 CFU/g within 17-36 h. No interference was seen from *Bacillus* spp. or other non-lactic bacteria. These reults demonstrate this method to be a useful screening tool for salad dressing quality, allowing for more rapid release of product to the market.

T18 PAPER KITS FOR THE RAPID ENUMERATION OF TOTAL AND COLIFORMS/ *ESCHERICHIA COLI*

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Paper test kits were developed for 24 h total bacterial count (PKT, 3 formulas) and 12 h coliforms/*E. coli* counts (PKC, 10 formulas). The performances were tested against pure cultures at various concentrations of *Escherichia coli*, *Enterobacter aerogenes*, *Citrobacter freundii*, *C. diversus*, *C. amonaticus*, *Klebsiella pneumoniae*, *Salmonella* Typhimurium, *S. typhi*, *S. paratyphi*, *S. derby*, *Shigella flexneri*, *Bacillus cereus*, and *Staphylococcus aureus*. The suspensions of bacterial culture in 0.85% sterile normal saline water were loaded on these paper kits and incubated at 30°C for total count and at 37°C for coliforms/*E. coli* counts. Simple regression analysis of the data showed a strong correlation between the paper kits and conventional methods, with R2 of 0.98 for PKTs and 0.97 for PKCs ($P<.05$). Non coliform cultures such as *Salmonella* Typhimurium, *S. typhi*, *S. derby*, *S. paratyphi A*, *Shigella flexneri*, *Bacillus cereus*, and *S. aureus* could not grow on PKC formulas. The PKTs and PKCs formulas were equally good to detect the respective organisms. Some PKCs formulas were able to chromogenically identify *Enterobacter*, *Salmonella* from coliforms and *E. coli*. PKTs and PKCs were designed to be very simple, easy and suitable tools for use in field-work by trained and untrained personnel and are promising to be alternative methods for examination of microbial quality of food products.

T19 INOCULUM SIZE OF *CLOSTRIDIUM BOTULINUM* 56A SPORES INFLUENCES TIME-TO-DETECTION AND PERCENT GROWTH-POSITIVE SAMPLES

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Clostridium botulinum is a deadly foodborne pathogen. The influence of inoculum size on the growth kinetics of *C. botulinum* 56A spores and percentage of samples that became positive for growth was studied at 30°C in a complete factorial design with 3 levels of each factor (inoculum size, pH, and sodium chloride). Forty four samples for each of the nine pH-salt combinations were inoculated at target levels of 1, 100, or 10,000 spores. Growth was followed hourly as A620 and the data were fitted to the Gompertz equation. Polynomial regression was used to analyze the effect of inoculum size, pH and sodium chloride on the percentage of growth-positive samples, maximum growth rate, and lag time. The lag time and percent growth-positive samples were significantly affected by inoculum size and its quadratic term. When inoculum size increased from 1 to 100 spores/ sample, the percent growth-positive samples increased and the lag time decreased. When the inoculum was 1,000spores/sample or higher, there was little additional effect. The effect was attributed to quorum sensing. The maximum growth rate was independent of inoculum levels. Results obtained at 22°C and 15°C were compared with those at 30°C.

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T20 ESTIMATING THE GROWTH OF *LISTERIA MONOCYTOGENES* AND *YERSINIA ENTEROCOLITICA* MICRO-COLONIES UNDER MODIFIED ATMOSPHERES AT 4°C AND 8°C USING A MODEL FOOD SYSTEM

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Modified atmosphere packaging (MAP) extends the shelf life of foods by altering the gaseous environment. This may present a safety hazard due to suppression of normal spoilage flora, allowing psychrotrophic pathogens to proliferate. Models describing bacterial behaviour in foods are based on experimental data from growth of relatively large numbers of bacteria usually grown in liquid culture. Growth of bacterial colonies on solid media surfaces may provide a more accurate reflection of the practical situation. A solid-phase model food system was used to estimate the growth of *Listeria monocytogenes* and *Yersinia enterocolitica*.

Colony growth on agar coated microscope slides incubated at 4°C and 8°C under a MA of 5% O_2: 10% CO_2: 85% N_2 was compared with aerobic growth. Computer image analysis (IA) was used to record colony growth and estimate dynamic growth data.

At 4°C, identical growth rates (μ) were obtained under the MA and aerobic conditions *for L. monocytogenes*. At 8°C, a significant difference ($P < 0.05$) was observed between the growth rates. Increasing temperature increased the rate of growth under both MAs. A significant decrease ($P < 0.05$) in μ was observed for *Y. enterocolitica* at 4°C and 8°C under the MA. The application of a MA at 4 or 8°C had no effect on the lag phase of the bacteria.

IA provided a useful tool allowing growth of bacterial micro-colonies to be observed over time. The use of area to estimate cell size provided an accurate means of determining the number of cells present within micro-colonies. The method employed gave reproducible dynamic growth data and provided an insight into the lag of the organisms studied.

T21 THE DEVELOPMENT OF A QUANTITATIVE ASSAY FOR THE DETECTION OF GENETICALLY MODIFIED SOY PROTEIN

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The introduction of genetically modified crops such as soy beans has created a demand for tests that can quantify the amount of modified product in crops and foods. We developed an assay for quantifying the genetically modified content of soy protein. The closed-tube homogeneous PCR process uses a commercial detection system and DNA intercalating dye, SYBR Green-I. During each thermal cycle, fluorescence data were collected at an intermediate temperature between the extension and denaturation steps. As the specific PCR product is generated, the dye intercalates into the product and the total fluorescence signal increases. Because there is no detectable product during the early stages of PCR, the signal during this period became a baseline fluorescence value. Regression analysis of the fluorescence increase determines the cycle at which the increase exceeded 5% of the baseline fluorescence. This became the threshold cycle (CT). Standards with known levels of genetically modified DNA (0.5%-5.0%) were amplified and the fluorescence signal recorded after each cycle. A curve was generated based on the linear regression fit of CT versus the log of percent genetically modified material. Unknown sample CT values were plotted against the standard curve and a genetically modified percentage was determined. Using a similar technique, the amount of total soy DNA was quantified by targeting an amplification reaction to the lectin coding region of soybean DNA. The ratio of the amount of the modified insert to the total amount of soy DNA allows an accurate percentage of genetically modified material to be calculated.

T22 A COMPARISON OF THE TRADITIONAL THREE-TUBE MOST PROBABLE NUMBER (MPN) METHOD WITH THE PETRIFILM, SIMPLATE, BACTOMETER CONDUCTANCE, AND BIOSYS OPTICAL METHODS FOR ENUMERATING *ESCHERICHIA COLI* FROM BROILER CARCASSES AND GROUND BEEF

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Petrifilm™ and SimPlate™, and the rapid microbiological methods BioSys optical and Bactometer conductance methods were compared to the MPN procedure for enumerating *Escherichia coli* (*E. coli*) from meat and poultry products. Fresh ground beef and chicken carcasses were evaluated in each of three replicate trials. Ground beef and carcasses were randomly separated into five groups containing five ground beef samples or carcasses. Two ground beef samples or carcasses out of the first group were sampled and analyzed immediately using Petrifilm™ (PEC) or SimPlate™ (SEC). The remaining four groups of ground beef or carcasses were sampled and analyzed using Petrifilm™,

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SimPlate™, and BioSys optical (BDT) and Bactometer conductance measurements (CDT) after temperature abuse at 37°C for 2, 4, 6, or 8 h. The correlation coefficients for the regression lines comparing *E. coli* MPN to PEC, SEC, BDT and CDT for ground beef were 0.93, 0.91, -0.93, -0.96, and for poultry carcasses were 0.95, 0.94, -0.91, and -0.90. Although PEC and SEC performed well, *E. coli* were not able to be enumerated from16.7 and 10% of samples, respectively. Using the BioSys optical and Bactometer conductance methods, results for samples that contained high levels of *E. coli* were able to be obtained within one working shift, rather than the 48 h required to conduct PEC or SEC, or 5 days required to conduct the MPN procedure. These methods would allow processors to test products and obtain results before shipping, avoiding the cost and loss of reputation associated with a foodborne illness outbreak.

T23 EVALUATION OF THE BIOSYS OPTICAL METHOD FOR RAPIDLY ENUMERATING POPULATIONS OF AEROBIC BACTERIA, COLIFORMS, AND *ESCHERICHIA COLI* FROM GROUND BEEF

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The BioSys optical method for enumerating aerobic bacteria, coliforms, and *E. coli* from ground beef was evaluated. Beef samples were analyzed immediately or after temperature abuse for various periods of time. Each ground beef sample was tested using aerobic plate counts (APC), violet red bile agar (coliforms), and the three-tube most probable number (MPN - *E. coli*) methods. In addition, each sample was tested using the BioSys for the following: total viable counts (TVC) by mixing 2 ml of ground beef homogenate (25 g into 225 ml of sterile 1% buffered peptone water) with 8 ml of nutrient medium containing brom-cresol purple (BCP), and placing into a test vial, and monitoring at 35°C; coliforms by mixing 5 ml of homogenate with 5 ml of Coliform Medium (CM) and monitoring at 35°C; *E. coli* by mixing 5 ml of homogenate with 5 ml of double strength Coliform Medium with 2% dextrose and monitoring at 42°C. Correlation coefficients for the regression lines comparing APC to BioSys TVC detection times (DT), VRB to BioSys coliform DT, and MPN to BioSys *E. coli* DT were -0.92, -0.95, and -0.96, and the line equations were $\log_{10}$ colony forming units (CFU)/ml = 8.21 -0.41 × DT, $\log_{10}$ CFU/ml = 8.47 - 0.61 × DT, and $\log_{10}$ CFU/ml = 9.09 - 0.90 × DT, respectively.

T24 A SURVEY OF *CAMPYLOBACTER* DIVERSITY IN POULTRY SAMPLES USING A NETWORK OF AUTOMATED RIBOTYPING SYSTEMS WITH THE RESTRICTION ENZYME PSTI

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The RiboPrinter® Microbial Characterization System (Qualicon, Inc.), an automated ribotyping instrument, can process high volumes of samples in support of large-scale epidemiological typing studies. It also provides the reproducibility, discrimination and standardization to allow multiple reporting sites to generate and share typing data without having to share the bacterial isolates. This evaluation assessed the ability of the system in two such studies. In the United States, two cooperating laboratories each ran the same set of 124 blind-coded *Campylobacter jejuni* and *C. coli* isolates. The isolates were independently ribotyped (one replicate) with the restriction enzyme PstI at each site and then the two data sets were merged electronically and further characterized using a standard visual refinement method. The final merged data set contained 53 unique ribotypes. In a separate ongoing study, more than 3,000 *Campylobacter* isolates from ten broiler production farms in Holland were also typed with PstI. This led to the definition of 116 ribotypes. Using the RiboPrinter® system's networking capabilities, representative pattern data generated from both studies were examined. Approximately 39% of the observed ribotypes were found to be present in both countries. This indicates that many strains within these species are geographically limited in their distribution. Automated ribotyping has proven to be an efficient high-throughput molecular typing method for *C. jejuni* and *C. coli*. In addition, the system's process standardization and network capabilities make it a valuable tool for other epidemiological studies.

T25 INACTIVATION OF BACTERIAL FOOD-BORNE PATHOGENS ON FRESH PRODUCE BY LOW-DOSE GAMMA IRRADIATION

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Low-dose gamma irradiation was evaluated as a potential treatment for reducing the foodborne pathogen load of fresh produce. Fresh products

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(carrots, strawberries, tomatoes, cantaloupes, cucumbers, lettuce, and apples) were inoculated with *E. coli* O157:H7, *Salmonella* serotypes, or *Listeria monocytogenes* and treated with 60Co gamma radiation. Irradiation doses were 0, 0.12, 0.25, 0.38, 0.50, 0.62, or 0.75 kGy, and irradiation was done aerobically at 23°C. For comparison, pure cultures of the test bacteria suspended in 0.1 M phosphate buffer were also irradiated under the same conditions. Following irradiation, survivor curves were generated by plotting populations of survivors vs radiation dose. There was a clear linear response; therefore, D-values were determined by calculating the negative inverse of the slope of the best fit line. Irradiation was effective in reducing pathogen populations on inoculated produce and in pure culture. On produce, D-values were 0.13, 0.15, and 0.33 kGy for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, respectively. In pure culture, D-values were 0.10, 0.16, and 0.20 kGy for the three respective pathogens. While irradiation shows promise as an effective antimicrobial treatment for fresh or minimally processed produce, further work is ongoing to determine effects of irradiation on sensory and physiological properties of produce.

T26 EFFECT OF IRRADIATION TEMPERATURE ON INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *STAPHYLOCOCCUS AUREUS*

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The resistance of *Escherichia coli* O157:H7 and *Staphylococcus aureus* in ground beef to gamma radiation was significantly higher at sub-freezing temperatures. Because most commercial radiation sources have no means to refrigerate the product during treatment, dry ice may be used when needed to keep the products frozen. Ground beef was inoculated (ca. 2×10^8 CFU/g) with five isolates of either *E. coli* O157:H7 or *S. aureus* and subdivided into 25 g samples, vacuum packaged in barrier pouches, and tempered to 20, 12, 4, 0, -4, -12, -20, -30, -40, or -76°C before irradiation. All samples were gamma irradiated within a temperature controlled radiation source (Cs^{137}) to absorbed doses of 0, 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 kGy at a dose rate of 0.10 kGy/min. The studies were repeated twice. D-values were calculated from the inactivation curves. The D-values for both of these pathogens increased significantly at sub-freezing temperatures, reaching maxima at approximately -20°C. The D-values for *E. coli* O157:H7 at 4° and -20°C were 0.39 ± 0.04 and 0.98 ± 0.23 kGy, respectively. The D-values for *S. aureus* at 0° and -20°C were 0.51 ± 0.02 and 0.88 ± 0.05 kGy, respectively. The decreased sensitivity of the pathogen at sub-freezing temperatures must be considered by the processor.

T27 NON-THERMAL PROCESSING ALTERNATIVES FOR THE EFFECTIVE ELIMINATION OF *ESCHERICHIA COLI* O157:H7 IN APPLE CIDER

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Escherichia coli O157:H7 has been identified as the causative agent in numerous foodborne illness outbreaks associated with the consumption of fresh apple cider. In an attempt to provide alternatives to pasteurization, sulfite, ultraviolet light and Velcorin™ were investigated for their efficacy in reducing *E. coli* O157:H7 in different apple ciders.

Apple cider was prepared from eight different varieties of apples and inoculated with 3 different strains of *E. coli* O157:H7 at 10^8 CFU/ml. The apple cider was then subjected to three different treatments that included 25, 50, 75 & 100 ppm of sulfite, 125 and 250 ppm Velcorin™ and ultraviolet light irradiation using a CiderSure 3500 UV unit. For sulfite and Velcorin™ treatments, the inoculated apple ciders were sampled after 2, 4, 6 and 24 h of incubation and enumerated for surviving *E. coli* O157:H7. The time required to achieve a 5-log reduction was extrapolated from the collected data. *E. coli* O157:H7 inoculated apple cider was exposed to ultraviolet light and the microwatt exposure to achieve a 5-log reduction of the pathogen was calculated. Each of the treatments was performed in triplicate. The results of this study provides small and large apple cider producers non-thermal processing alternatives that are effective in reducing contaminating *E. coli* O157:H7 in apple cider to improve the safety of their product.

T28 INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* ON APPLES AND IN FRESH APPLE CIDER USING SONICATION AND COPPER ION WATER

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This study examined the efficacy of sonication (600 watt ultrasonic processor, Sonics and Materials, Inc.) and copper ion generated in water (Superior Aqua Systems, Superior Aqua Enterprises, Inc.) for reducing populations of *L. monocytogenes* and *E. coli* O157:H7 in an aqueous model system, inoculated whole apples and apple cider. Inoculated apple cider (or sterile water for controls) containing ca. 10^6 CFU/ml of either pathogen was sonicated at 20 kHz

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for 3 min. and appropriately plated to determine D-values. Alternatively, whole apples were inoculated by dipping to contain ca. 10^5 *L. monocytogenes* or *E. coli* O157:H7 CFU/g and then dried overnight. Inoculated apples that were untreated were examined for numbers of *L. monocytogenes* and *E. coli* O157:H7 after pressing. A second set of inoculated apples was treated with 0.7 ppm copper ion water for 10 min and then plated to determine D-values. D-values for sonication were 30 and 35 s for the model system and apple cider, respectively. In the model system and cider, numbers of both pathogens decreased >3 logs following 3 min of sonication. Populations of *E. coli* O157:H7 and *L. monocytogenes* were 2 logs lower in cider after untreated inoculated apples were pressed. When inoculated apples were treated with 0.7 ppm copper ion water, both pathogens yielded D-values of 80-82 s with both organisms decreasing >5 logs after 10 min of exposure. Consequently, the combination of copper ion water and sonication may prove useful in obtaining the required 5-log reduction for these pathogens in fresh apple cider.

T29 INFLUENCE OF ENVIRONMENTAL STRESSES ON BIOCIDE SUSCEPTIBILITY OF *ESCHERICHIA COLI* O157:H7

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Bacteria on food contact surfaces experience a range of environmental stresses including temperature, pH, osmotic conditions, variation in nutrient availability, and exposure to cleaning chemicals, which could induce a cross-protection response leading to subsequent resistance to other stresses. This research aims to investigate the relationship between pre-exposure to environmental stresses on subsequent disinfectant susceptibility, to provide data to improve the overall effectiveness of the cleaning and disinfection regime, through the optimization of any synergistic effects of the cleaning phase on the subsequent disinfection phase.

Bioluminescent strains were produced by the transformation of the gene constructs lux AB and lux CDABE into a non-toxigenic auxotrophic mutant of *Escherichia coli* O157:H7. These strains were shown to exhibit a similar susceptibility to stress as the wild type. Pre-exposure to both organic and inorganic acids (citric, lactic, nitric and hydrochloric) at pH 4.5 and commercial detergents (non-ionic and anionic surfactants) at their working concentrations, for 20 min and 4 h, on the susceptibility of *E. coli* O157:H7 to biocides was investigated. Exposure to the acids and detergents did not affect total viable counts; however, bioluminescence readings showed a marked reduction within 1 h for lactic acid, indicating cell stress. The effect of pre-exposure to these stresses resulted in a 1 to 2 log_{10} increase in biocide resistance of *E. coli* O157:H7. The non-ionic surfactant, however, showed a 1 log_{10} decrease in biocide resistance. Results are also presented that show the effect of these pre-treatments on the biocide resistance of *E. coli* O157:H7 when surface-attached.

T30 INHIBITION OF *LISTERIA MONOCYTOGENES, SALMONELLA* TYPHIMURIUM DT104 AND *ESCHERICHIA COLI* O157:H7 ON BOLOGNA AND SUMMER SAUSAGE USING WHEY PROTEIN ISOLATE-BASED EDIBLE FILMS CONTAINING ANTIMICROBIALS

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Whey protein isolate (WPI) films (pH 5.2) containing p-aminobenzoic acid (PABA) or sorbic acid (SA) were assessed for antimicrobial activity and mechanical properties while in direct contact with sliced bologna and summer sausage. WPI film solutions containing WPI, glycerol, $CaCl_2$, and water were adjusted to pH 5.2 using 1 N lactic acid. After incorporating 0.5:0.5, 0.75, and 1% (w/v) SA or PABA, films were cast and placed between slices of bologna or summer sausage that were previously inoculated to contain ~10^6 CFU/g *L. monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium DT104. *Listeria*, *Salmonella*, *E. coli*, mesophilic aerobic bacteria (MAB), lactic acid bacteria (LAB), and yeast/mold were quantitated using standard plating methods following 0, 4, 7, 10, 14, and 21 days of storage at 4°C. Film tensile strength (TS) and % elongation (%E) were determined after 24 and 48 h. Using WPI films containing 0.75% SA, and 1.0% SA and PABA, populations of *E. coli* O157:H7 and *S.* Typhimurium DT104 decreased ~3-3.5 log on both products after 10 days of storage at 4°C, with *L. monocytogenes* decreasing only ~1.5 log. PABA and SA (0.5:0.5) yielded greater reductions for *Listeria*, *E. coli*, and *S.* Typhimurium on summer sausage (3, 3.6, and 3.9 log) than on bologna (2.8, 3.1, and 3.1 log). Growth of LAB, MAB, and yeast/mold on summer sausage and bologna was inhibited compared to controls. Antimicrobial-free WPI films were noninhibitory. Film TS decreased while %E remained unchanged following 48 h of direct contact with the slices.

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T31 DISINFECTION OF BACTERIAL PATHOGENS AND SELECTED VIRUSES ON FRESH ROMAINE LETTUCE

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The effectiveness of chlorine solutions and a commercial vegetable rinse against two pathogenic bacteria and selected viruses seeded onto the surface of romaine lettuce was examined. The survival of rifampicin-resistant strains of *Salmonella montevideo* and *Escherichia coli* O157:H7 along with the bacteriophages MS2, Phi-X, and PRD-1 were measured after treatment with chlorine solutions (50,100,200,300 ppm free chlorine) and a commercial vegetable rinse product, Carnebon 200® containing aqueous stabilized chlorine dioxide (100ppm). A tap water treatment was used as a control to provide initial counts of inoculated test organisms. Treatments were applied using an immersion combined with physical manipulation for two minutes at 45°C, followed by an immersion in a neutralizing solution. The effectiveness of the chlorine solutions did increase with the concentrations, with all treatments above 50ppm demonstrating significant reductions in both bacteria and viruses ($P < 0.05$). 300ppm chlorine solutions demonstrated 84% and 93% reduction in the *E. coli* and *S. montevideo* respectively. The viruses exhibited some differences in their response to the chlorine treatments, with Phi-X having an 83% reduction, followed by PRD1 at 74% at 300ppm free chlorine while MS2 was only reduced 69%. The Carnebon 200® demonstrated a lower percent reduction in the test bacteria, but did however exhibit a greater reduction of MS2 (77%) than did the highest concentration of chlorine evaluated. While statistically significant decreases were obtained with both chlorine and chlorine dioxide treatments, bacteria and viruses were still recovered from all treated samples, indicating a need for the use of more effective treatments.

T32 THE ANTIMICROBIAL EFFICACY OF HERBS IN MARINATED CHICKEN

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The antimicrobial properties of many herbs have been investigated by in-vitro means, but few studies have been done using "in vivo" applications. Our objective was to create an herbal chicken marinade, which might inhibit growth of spoilage and pathogenic microorganisms. Fresh chicken breasts were inoculated (~ log 4 CFU/g) with *C. jejuni*, *S.* Typhimurium, *L. monocytogenes*, and *E. coli* O157: H7. The following marinade treatments (base of water, salt, sodium phosphate, and citric acid; pH 4.5) were evaluated using fresh inoculated chicken breasts (20% marinade by weight): control (no herbs), grapefruit seed extract (GSE-0.3%), oregano essential oil (ORG-0.3%), thyme essential oil (THY-0.3%), combination of GSE, ORG, and THY (0.3% of each herb), and dried oregano leaves (1%). APC and survival of pathogenic bacteria during storage at 4°C were determined at days 0, 3, 6, 9, 12, and 15. Dried oregano leaf marinade was the least effective treatment in inhibiting all microorganisms. The GSE marinade reduced the APC and growth of *C. jejuni*, (<2 log CFU/g) but did not significantly inhibit *S.* Typhimurium, *E. coli* O157:H7, or *L. monocytogenes* ($P > 0.05$). THY, ORG, and the combination treatment significantly reduced microbial counts ($P < 0.05$). The combination treatment was the most effective ($P < 0.05$) in reducing APC and *E. coli* O157:H7, and was highly lethal (~ log 4 CFU/g reduction) to *S.* Typhimurium, *C. jejuni*, and *L. monocytogenes*. These data suggest that selected herbs have powerful antimicrobial potency in a chicken marinade and may be used to increase the shelf life and safety of poultry.

T33 EFFECT OF FAT CONTENT, EVAPORATIVE COOLING AND FOOD TYPE ON PATHOGEN SURVIVAL DURING MICROWAVE HEATING

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Pathogen survival during microwave reheating and cooking has emerged as a growing point of concern for consumers, the government, and the food industry. This study concentrates on the effect of fat content, food type, and evaporative cooling on microwave treated foods. A Luxtron was used to determine heating curves and F-values of wrapped (using Saran Wrap) and unwrapped products heated in a microwave oven (Sharp% 1000W) according to their recommended cooking times. Products tested in triplicate included raw ground beef patties (fat levels of 30%, 15% and 7%), chicken tenders (fat free and regular) and fresh broccoli. Significantly ($P > .05$) greater f-values were observed in higher fat products. Covering products with Saran Wrap to prevent evaporative cooling significantly ($P > 0.05$) increased f- values. Inoculated pack studies were performed in triplicate on each food, using multi-strain cocktails of *Listeria monocytogenes* (4 strains), *Salmonella* (*Salmonella enteritidis*, *S. arizonae*, and two *S.* Typhimurium strains) and *Escherichia coli* O157:H7 (4 strains). Survival was determined by testing for presence

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or absence of growth of each pathogen after non-selective and selective enrichment, isolation and confirmation of organisms. Inoculated pack studies confirmed f-value determinations. Greater survival was observed in lower fat products, just opposite of what is expected for conventional heat products, where fat acts to protect the cells. Pathogen survival was greater than expected in some products, indicating that other factors, such shape, added ingredients (breading, etc.) may affect survival.

T34 MICROBIOLOGICAL EVALUATION AND MANUFACTURING PRACTICES OF SPROUTS IN CANADA

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In 1996, 1997, and 1999, foodborne illness outbreaks associated with the consumption of raw sprouts were reported in Canada. A nationwide inspection and sampling project was undertaken to assess the sprout industry manufacturing practices and to determine the microbiological profile of sprouts produced in Canada. Canadian Food Inspection Agency (CFIA) inspectors collected samples of sprouts, including alfalfa, radish, mustard, clover, and sunflower, from 43 different manufacturers across the country between July and December 1999. At each manufacturers, 5 sample units (200 g each) were selected for microbial testing. Laboratory results showed total confirmed coliforms (TC) and faecal coliforms (FC) ranging from <1.8 to 2.4×10^8 and from <1.8 to 1.6×10^7 CFU/g, respectively. *Listeria monocytogenes* was detected in only one subsample. *Salmonella* spp. and *E. coli* O157:H7 were not detected, although an outbreak of *Salmonella* Java occurred in western Canada during the time of the survey. Sprouts were also evaluated for the presence of *Klebsiella pneumoniae*. The inspection of sprout manufacturing plants in Canada showed that not all sprout manufacturers followed Good Manufacturing Practices (GMP). Response to questions about seed storage, water treatment, plant sanitation, temperature during product distribution, Hazard Analysis Critical Control Point (HACCP) implementation, and record keeping showed a lack of consistency in sprout manufacturing. The absence of a consistent seed treatment application was common practice among the industry. The findings from this survey are being used in the development and implementation of a Code of Practice, based on GMP and principles of HACCP, that will be introduced to sprout manufacturers in order to reduce the risk of sprout-associated illness.

T35 EFFECT OF BLANCHING CUCUMBERS ON THE MICROFLORA OF NON-ACIDIFIED REFRIGERATED PICKLES

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Minimally processed vegetable products may present a health risk due to contamination with pathogenic microorganisms. Washing procedures generally result in a one or two log reduction in microbial cell counts, which can exceed 10^6 CFU/g on fresh fruits and vegetables. Our objective was to develop blanching procedures for preparing non-acidified refrigerated pickles (NARP) without compromising quality or safety due to spore outgrowth and/or growth of pathogens. Blanching for 15 s at 80°C resulted in approximately a 3-log reduction in cucumber microflora without obvious changes in the sensory properties of the fruit. Blanched cucumbers were brined (2% NaCl, equilibrated) in 1.4 liter jars. The natural lactic acid bacteria on fresh cucumbers, typically 10^3 CFU/g, were reduced to between 10 and 10^2 CFU/g after blanching, as determined by homogenizing cucumbers, concentration of microorganisms by filtration, and plating on MRS agar containing 0.02% sodium azide. Cucumbers were blanched for 15 s at temperatures of 60 to 100°C; NARPs were prepared and stored at 5, 10, and 25°C. For all blanching treatments, a heterolactic fermentation resulted when brine turbidity (spoilage) developed, as determined by HPLC analysis of organic acids. With an 80°C for 15 s blanch and storage at 5°C, the time until turbidity ($>10^7$ CFU/ml aerobic plate count) was typically 30 days or greater, compared to 20 days or less for unblanched cucumbers, and the pH was 4.0 to 4.5. Optimum blanching procedures may be an effective strategy for increasing the shelf life of NARPs, without compromising the safety.

T36 EFFECTS OF WATER WASHING AND RINSING TEMPERATURE ON HANDWASHING EFFICACY

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For many years, sanitarians have specified that hands should be washed and rinsed in warm water to reduce the risks of cross cantamination and disease transmission. Various authors have indicated that water temperatures should be between 100°F and 120°F. However, it also has been suggested that handwashing with warm water may contribute to skin damage when frequent handwashing is necessitated (e.g., in health care and food service). To study

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the effects of water temperature on handwashing efficacy, the hands of four experimental subjects were soiled with sterile or contaminated substances (tryptone soya broth (TSB) and hamburger meat) and then washed and rinsed with water at various temperatures. The hands were exposed to sterile carriers to study the effects of treatment temperatures on the reduction of normal microflora, and they were exposed to *Serratia marcescens*-inoculated samples to study treatment effects on the reduction of transient contamination. Using the glove juice method, one hand on each subject was sampled after being soiled (as a control), while the other was sampled after washing and rinsing. Five water temperatures were used: 40°F, 55°F, 70F, 95°F and 120°F. After hands were soiled, they were moistened with water at each test temperature, washed for 15 s with bland soap, and rinsed for ten s with the same temperature water. Results of this study show that no significant differences in the reduction of normal or transient hand flora can be attributed to handwashing/rinsing water temperatures when bland soap is used.

T37 CONTINUOUS ON-LINE PROCESSING OF FECAL AND FOOD CONTAMINATED POULTRY CARCASSES

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Using a USDA approved study design, five commercial plant evaluations were conducted to demonstrate the efficacy of an acidified sodium chlorite pre-chill antimicrobial intervention on fecal or food contaminated post-evisceration poultry carcasses. The performance of the ASC spray application in combination with carcass wash systems (Continuous On-line Processing – COP) was monitored and the performance compared with individual plant off-line reprocessing practices.

Analysis of the accumulated data gave average contamination levels on carcasses pre-final wash of 2.87 $\log_{10}$ (*Escherichia coli*). *Salmonella* spp. incidence averaged 37.3%. For *E. coli*, the ASC-COP process averaged 2.28 $\log_{10}$ reduction. *Salmonella* incidence was reduced to 10%. Post-chill microbial levels were comparable to those seen, post-ASC treatment. In comparison, normal plant, off-line reprocessing practices had marginal effects on microbial contamination. These were identical to the effect of carcass wash systems alone. *E. coli* was reduced by only 0.05 $\log_{10}$ and *Salmonella* spp. incidence was lowered to 31.6%. Both systems (ASC-COP and off-line reprocessing) were consistently able to sustain compliance to the Zero Fecal Tolerance Rule.

Properly configured carcass wash systems combined with an ASC pre chill rinse consistently generated a highly significant improvement in carcass microbial and organoleptic quality. All plants experienced significant improvements in yield, efficiency of operation and were compliant with USDA microbiological and fecal tolerance standards as a result of the operation of the ASC-COP system.

T38 EFFICACY OF ELECTROLYZED WATER IN INACTIVATING *LISTERIA MONOCYTOGENES* AND *SALMONELLA ENTERITIDIS* ON SHELL EGGS

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The efficacy of acidic electrolyzed (EO) water produced at three levels of free chlorine (16, 41, and 77 ppm) and chlorinated water (45 and 200 ppm) for inactivating *Listeria monocytogenes* and *Salmonella enteritidis* was investigated. Shell eggs were inoculated with a five strain mixture each of *Listeria* and *Salmonella* (7 and 5.5 $\log_{10}$ CFU/shell egg; respectively). The reduction of *Listeria* and *Salmonella* using acidic EO water increased with increasing chlorine concentration and treatment time, resulting in a maximal reduction of 4 and 3.5 $\log_{10}$ CFU/shell egg, respectively. There was no significant difference in antibacterial activities against *Salmonella* and *Listeria* at the same treatment time between chlorinated water (45 ppm) and acidic EO water (41 ppm chlorine). Chlorinated water at 200 ppm was the most effective in reducing the mean populations of *Listeria* and *Salmonella* (4.9 and 3.8 $\log_{10}$ CFU/shell egg, respectively). Alkaline EO water (pH=11.2) is a companion solution to acidic EO water produced during EO water production. A combination of alkaline EO water wash for 1 min followed by another minute of acidic EO water wash (41 ppm chlorine) achieved similar reduction level on *Salmonella* and *Listeria* compared to 200 ppm chlorinated water wash. This study indicates that a combination of alkaline and acidic EO water wash can be an effective means for shell eggs disinfection.

T39 EFFECT OF PRE-CHILL SKINNING ON THE LEVEL OF *CAMPYLOBACTER* RECOVERED FROM BROILER PARTS

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Broiler skin carries a high number of bacteria. Nevertheless, it has been reported that, with retail product, neither skinless parts nor aseptically

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skinned parts carry less bacteria than paired parts with skin. This study was undertaken to determine if aseptic removal of skin prior to the carcass entering the chill tank has an effect on the microbiological quality of pre-chill broiler parts. Carcasses were removed from the shackles immediately before entering the chill tank in a commercial processing plant. Each carcass was aseptically cut into parts: legs, thighs and boneless breast. One of each part from each carcass was skinned and the other was left with the skin on for comparison. Each part was rinsed in 50 ml PBS; skin that was removed was stomached. Dilutions were plated for enumeration of *Campylobacter*, coliforms, *E. coli* and total aerobic bacteria. Five replications were completed, using 2 of each part with and without skin per replication. Significantly less *Campylobacter* was recovered from product that had been skinned: 1.2 $\log_{10}$ CFU/100g reduction for breast, 1.4 $\log_{10}$ CFU/100g reduction for thigh and 0.5 $\log_{10}$ CFU/100g reduction for legs. Skin that had been removed from these parts was found to have *Campylobacter* levels ranging from $\log_{10}$ 3.6 to 3.9 CFU per piece. Coliform counts and total counts were also lower on parts that were skinned. Skinning product prior to chilling could lead to less *Campylobacter* and other bacteria on the final product.

T40 ABILITY OF OLEIC ACID TO REDUCE THE NUMBER OF BACTERIA ON POULTRY SKIN AND IN RINSATES OF POULTRY SKIN

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Many bacteria that colonize the skin of live poultry remain attached to the carcass throughout processing operations. Salts of fatty acids are bactericidal surfactants that aid in the removal of microorganisms from surfaces. The purpose of the present study was to determine if washing poultry skin in solutions of the potassium salt of oleic acid would reduce the number of bacteria on the skin and in rinsates of the skin. Skin from processed broiler carcasses was washed twice in various concentrations oleic acid, then rinsed in peptone water. The final rinsate was decanted, and bacteria were enumerated. Significantly fewer *Campylobacter* and *enterococci* were recovered from rinsates of skin washed in 2% oleic acid; significantly fewer total aerobes were recovered from rinsates of skin washed in 6% oleic acid; and significantly fewer *Enterobacteriaceae* were recovered from rinsates of skin washed 10% oleic acid than from the control samples. Attached flora of poultry skin subjected to 2 consecutive washes in 10% oleic acid and a rinse in peptone water were also compared to the flora of untreated skin and skin subjected to 3 consecutive rinses in peptone water. Untreated and treated skin was blended and subjected to microbial analysis. Significantly fewer total aerobes, *Enterobacteriaceae*, *Campylobacter*, and *enterococci* were recovered from skin washed in oleic acid than from skin rinsed in peptone water or from untreated skin. Findings indicate that washing poultry skin in oleic acid reduces the bacterial population of the skin and rinsates of the skin because of the bactericidal activity of the fatty acid.

T41 COMPARISON OF THREE COMMERCIAL COMPETITIVE EXCLUSION PRODUCTS ON REDUCING *SALMONELLA* IN BROILERS

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The efficacy of three commercial competitive exclusion (CE) products against *Salmonella kedougou* (SK) NCTC 112173 Nal® in broiler chicks in five treatments with five replicates in each treatment was evaluated. The five treatment groups were T1) Non treated and non challenged; T2) SK challenged only; T3) Treated with Product A and challenged with SK; T4) Treated with Product B and challenged SK; and T5) Treated with Product C and challenged SK. The treated birds were gavaged with 0.5 ml of CE product by feeder tube. 1.3×10^4 CFU of SK was gavaged to each challenged bird 24 h after CE treatment. The ceca were examined for SK five days after challenge by using XLT4 with Nalidixic acid with confirmation by PCR. T1 was negative for SK; T2 was positive on 46.29% ceca which had 1.7 $\log_{10}$ CFU/g of SK in cecal contents; T4 was positive on 18.18% ceca which had 0.6 $\log_{10}$ CFU/g of SK in cecal contents; T5 was positive on 63.64% which had 2.3 $\log_{10}$ CFU/g of SK in cecal contents. The Protection Factor (PF) for T3 was 10.62, PF of T4 was 4.31, and PF of T5 was 0.45. This study showed that T3 (MSC) resulted in the highest PF value for reducing *Salmonella kedougou* colonization in broiler chicks.

T42 EFFECTIVENESS OF POTASSIUM LACTATE AND LACTIC ACID AGAINST *CAMPYLOBACTER* AND PSYCHROTROPHIC BACTERIA ON CHICKEN BREASTS

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This study examined the efficacy of potassium lactate and lactic acid to control naturally occurring *Campylobacter* sp. and psychrotrophic bacteria.

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Boneless, skinless chicken breasts were injected with two levels of potassium lactate (0%, 2%), in conjunction with two levels of lactic acid. The lactic acid was injected (0%, 0.1%) as well as applied directly to the surface (0.1%). The samples were stored at 4°C for 32 days, with sampling every eight days. Two percent potassium lactate in conjunction with the 0.1% lactic acid-surface application demonstrated the greatest inhibition (2 log reduction) of *Campylobacter* sp. and psychrotrophic bacteria. Shelf life was extended 2.5 days for chicken breasts receiving these two treatments compared to untreated control samples. The 2% potassium lactate and 0.1% lactic acid-surface treatment was significantly different ($P<0.05\%$) than all other treatments containing 0% potassium lactate and any level of injected lactic acid. These two treatments did not significantly affect color of the chicken breasts compared to untreated controls. Treated chicken breasts had lower L-values. Results of this study indicate that potassium lactate and surface application treatment of lactic acid can be used to decrease the growth and/or recovery of *Campylobacter* sp. and psychrotrophic bacteria on boneless chicken breasts.

T43 APPLICATION OF NATURAL ANTIMICROBIAL SYSTEMS FOR CONTROL OF *LISTERIA MONOCYTOGENES* IN FOODS

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The objective of this study was to develop a natural antimicrobial system for reducing the survival of *Listeria monocytogenes* in cooked meat products. Based on *in vitro* demonstrations of synergistic interactions between nisin, lysozyme and natural flavor extracts, a novel food grade antimicrobial system was developed for cooked ham.

Efficacy of this system was evaluated by a cooked ham *Listeria* challenge study. The cooked ham was inoculated with a three-strain cocktail of *L. monocytogenes*, and a suspension of the antimicrobial system was sprayed onto the surface of the ham. Each piece was then vacuum packed and stored at 4°C for periodic sampling.

Significant bactericidal effect against *L. monocytogenes* was observed from this study. Data demonstrated that inoculated *L. monocytogenes* cells (>2 log initial load) were rapidly killed after topical treatment with the system. Treated hams also remained *Listeria*-negative after enrichment throughout the remainder of the 60-day storage period, while the same inoculum in a control group increased over original levels by > 4 logs.

Data presented in this study indicate that natural antimicrobial systems may constitute an effective, multiple hurdle strategy for control of *L. monocytogenes* growth in cooked meat products.

T44 COMPARATIVE STUDY OF SEMISYNTHETIC DERIVATIVE OF NATAMYCIN AND THE PARENT ANTIBIOTIC ON THE SPOILAGE OF SHREDDED CHEDDAR CHEESE

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The polyene macrolide antibiotic natamycin (Antibiotic A-5283) is used to retard the growth of surface molds on various cheese varieties. It is applied to the surface of cheese by dipping or spraying, using an aqueous dispersion containing 200 to 300 ppm of the additive. The large molecular weight of natamycin, 666 g/mol, and conjugated double bond structure causes it to be extremely insoluble in water and most food grade solvents. The inability to apply natamycin in true solution creates void non-treated areas on the food surface. These non-treated areas may allow the growth of fungal organisms.

A water soluble *N*-alkyl semisynthetic derivative of natamycin was synthesized by the Michael addition reaction of the parent with a *N*-substituted malemide. A comparative study investigated the effectiveness of the semisynthetic derivative of natamycin and the parent antibiotic in suppressing mold growth on shredded Cheddar cheese stored under modified atmosphere (MA). The effects of 0, 10, and 20 ppm antimycotic treatments were examined. A 20 ppm natamycin treatment effectively suppressed visible mold growth ($<10^4$ CFU/g) in MAP samples for up to 30 days after opening. The 20 ppm semisynthetic derivative performed similarly to the 10 ppm natamycin treatment. Visible mold growth did not occur for these treatments in MAP samples until 20 days after opening. Analysis of storage conditions revealed that an outgrowth of mold in shredded cheese occurred in MAP packages stored longer than 15 days. This bloom in mold growth was attributed to the degradation of natamycin and the derivative throughout storage.

T45 Co-60 IRRADIATION FOR INACTIVATION OF *GIARDIA LAMBLIA* CYSTS IN WATER AND ON TOMATOES

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Cysts of *Giardia lamblia* contaminate many environments and can cause gastrointestinal disease in humans and animals. Many means of inactivation

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of the resistant cyst stage have been investigated, because cysts are usually associated with water that can contact food. The effects of irradiation on a human isolate of cysts were studied. Low dose ionizing irradiation using a Co-60 source was evaluated at 0, 0.6, 0.12, 0.25, 0.465, 1.0, and 2.0 kGy using cysts in water. After treatment, cysts (50,000/os) were orally inoculated into gerbils. 7-8 days post-inoculation (DPI), fecal samples were analyzed for cysts. 13 DPI, duodenal scrapings were analyzed for trophozoites at necropsy. Doses of 0.25 kGy and higher inactivated cysts, in that no animals were infected. Cysts irradiated at lower doses caused infection in all animals. Tomatoes that had been dipped in water containing *G. lamblia* cysts were irradiated at various doses: 0.12, 0.25, and 0.5 kGy. Cysts were evaluated using the gerbil bioassay. All animals that received cysts irradiated at 0.12 kGy became infected; no animals that received cysts irradiated with 0.5 kGy became infected. Only 6 out of 36 animals that received cysts irradiated at 0.25 kGy became infected. Samples of irradiated cysts were also treated with propidium iodide. Supposedly, this chemical can enter and stain only dead cysts. Staining with propidium iodide indicated that viability of irradiated cysts was equal to that of non-irradiated cysts. This implies that irradiation does not damage the cyst wall or cell membrane and that inactivation must occur by some other cellular disruption.

T46 INHIBITORY EFFECT OF GAMMA IRRADIATION ON THE GROWTH OF *FUSARIUM MONILIFORME* AND FUMONISIN PRODUCTION

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This study was investigated to determine inhibitory effect of gamma irradiation on the growth of *Fusarium moniliforme* and fumonisin B1, B2 production. The corn inoculated with initial inoculum (1.1×10^3 CFU/g) of *Fusarium moniliforme* was gamma irradiated and determined the inhibition of growth and fumonisin production after storage at 25°C for 7 week. Five kGy gamma irradiation showed 2 log reduction and *Fusarium moniliforme* was completely inactivated at 10kGy treatment compared to controls immediately after gamma irradiation. *Fusarium moniliforme* was grown to 4×10^4 CFU/g at 20kGy treatment at normal conditions after 7 weeks incubation, but 1×10^9 CFU/g was observed at optimal condition. However, the mold was completely inactivated at 30kGy treatment irrespective of culture conditions. Five kGy gamma irradiation significantly reduced fumonisin production and FB1 and FB2 (1,5 μg/g, 0.8μg/g) was almost completely inactivated at 20kGy treatment, respectively, compared to control (FB1 1,5μg/g, FB2 0.8μg/g) at normal conditions. However, at the optimal conditions, FB1 and FB2 was 23 times, 6.5 times at 5 kGy treatment and 30 times, 3 times more increased than those of normal condition, respectively.

T47 RESISTANCE OF POLIOVIRUS TO INACTIVATION BY HIGH HYDROSTATIC PRESSURES

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Modern consumer preferences for fresh and minimally treated foods have encouraged the development of novel processing and preservation techniques, such as high hydrostatic pressure, which avoid the use of heat. High pressure has been shown to have considerable effect against various microorganisms, but there have been few studies on response of viruses, and no detailed studies have been reported on any effect of high pressure against a foodborne virus type. This study was performed to investigate whether poliovirus, which has often been used as a model of enteric viral pathogenic types, is affected by pressure regimes similar to those used in food processing. Adenovirus, a dissimilar virus type, was included in the study for comparison.

Suspensions of poliovirus were subjected to high hydrostatic pressures up to 600 MPa for 1 h. Suspensions of adenovirus were treated likewise. Virus numbers were determined before and after treatment by quantal cell culture. Whereas adenoviruses were inactivated at pressures of 400 MPa and above, poliovirus completely resisted hydrostatic pressure up to 600 MPa for 1 h. This difference in response was probably due to morphological differences between the virus types. These results may have implications for the use of high pressure in food processing, as poliovirus is morphologically similar to significant foodborne pathogenic agents.

T48 THE EFFECT OF THERMAL PROCESSING SCHEDULES AND UNIT OPERATIONS ON THE QUALITY OF BLUE CRAB (*CALLINECTES SAPIDUS*) MEAT

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The effects of initial thermal processing, plant sanitation, and employee habits on the microbiological quality of blue crab (*Callinectes sapidus*) meat were determined in a commercial processing

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facility. Thermal processing of crabs was evaluated after 5, 7, and 8 min of processing at 250°F for the destruction of microorganisms, with specific reference to total aerobes, anaerobs, coliforms, fecal coliforms, *Escherichia coli*, and *Listeria monocytogenes* using the Bacteriological Analytical Manual. Calculated F-values indicated a sufficient reduction of *L. monocytogenes* at each processing time. Crabs from the 8 min cook, the current industry practice, were profiled through cooling, overnight refrigerated storage, and storage on the picking table during the following day. Coliforms, fecal coliforms, and *E. coli* were eliminated during the 7 and 8 min cooks, while present at low levels after the 5 min cook. Reductions in total microbial populations were found at each cooking time. A minimum of 5 min provided a sufficient reduction of *L. monocytogenes* and other microorganisms to meet regulatory action levels. Fresh picked crab meat (8 min cook) was evaluated for microbial levels when exposed to ambient temperatures (67 - 72°F) over four h. Neither air temperature nor meat temperature had a significant effect on microbial counts except during the four hour test period. Plant sanitation was evaluated based on levels of adenosine triphosphate (ATP) and microbial counts. Areas having high ATP levels typically had low microbial counts (<10 CFU/sq cm), suggesting that crab meat residuals were the problem. *Listeria* species were found under picking tables and on employees' hands and aprons, cooler doors, refrigerator evaporator coils, gloves of workers handling cooked crabs, and sinks.

T49 RISK ASSESSMENT OF *SALMONELLA ENTERITIDIS* IN CANADIAN SHELL EGGS

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This paper describes an assessment of the risk of *Salmonella* infection from shell eggs for Canadian consumers. While salmonellosis outbreaks attributable to eggs have been common in the US and the UK, Canada does not appear to have experienced outbreaks or sporadic illnesses to the same extent, despite similar consumption patterns. The assessment considered prevalence of contaminated eggs, and handling conditions from the layer house to preparation. Key results include the egg contamination rate (9 per million; CI: 5-15), and the proportion of eggs which can be expected to be highly contaminated just prior to preparation (0.5%; CI: 0.1-1.5%). The results of exposure assessment are highly sensitive to (i) experimental findings regarding the integrity of the vitelline membrane, (ii) assumptions regarding the temperature handling of eggs at key stages of distribution and storage, (iii) the extent of egg pooling in foodservice and in the home and (iv) the proportion of consumers who consume eggs raw (typically as uncooked ingredients in recipes). The expected number of illnesses in Canada is approximately 2,500 with very wide uncertainty bounds (CI:500-25,000). The risk characterization is dominated by significant uncertainty regarding the dose-response curve (particularly with regard to the treatment of susceptible individuals). The paper also describes some differences in Canadian conditions (as compared to the US) that may contribute to a lower risk among Canadian consumers.

T50 A RISK ASSESSMENT MODEL FOR *SALMONELLA* SPP., *CAMPYLOBACTER JEJUNI*, AND CHICKEN

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A risk assessment model for *Salmonella* spp., *Campylobacter jejuni*, and chicken was created in an Excel notebook and was simulated using @Risk, a spreadsheet add-in program. Model design and settings were based on published data. The model simulates the change in *Salmonella* spp. and *C. jejuni* load (i.e., exposure assessment) of 100,000 servings of chicken as they move from packaging at the processing plant to consumption. Incidence and extent of events that change pathogen load during cold storage, distribution, cooking, and cooling were modeled using a combination of discrete and pert distributions. Pert distributions for infection dose and a linear model were used to simulate response of consumers to pathogen exposure (i.e., dose-response assessment). Dose-response results and epidemiological data were used in risk characterization to determine the cases of adverse health outcomes from infection to death. In addition, cases of each adverse health outcome were multiplied by arbitrary weight factors to determine the severity of pathogen infections per 100,000 servings. Simulation results from a baseline and three test scenarios demonstrated the importance of considering multiple pathogens and the interaction among pathogen type and chicken handling practices and infection dose in the distribution channel when evaluating the microbiological safety of chicken. The model was incorporated into version 2.0 of the USDA, ARS, Poultry Food Assess Risk Model (i.e., Poultry FARM) where it provides a framework for a food safety program based on the principles of risk assessment.

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T51 RISK ASSESSMENT FOR HARMFUL ALGAL BLOOMS — CAN *VIBRIO VULNIFICUS* BE A MODEL FOR THESE AGENTS?

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A risk assessment is a process of determining the probability of occurrence of adverse health effects resulting from exposure to a hazard. There are four steps: (1) hazard identification; (2) hazard characterization; (3) exposure assessment, including a dose-response assessment; and (4) risk characterization. Ideally, for harmful algal blooms (HAB), exposure assessments should begin with toxigenic plankton in the sea and end with a probability of illness after ingestion of a seafood containing toxin. Microbiological risk assessments have been developed with models from an animal host through to the consumer, where there is limited information to connect the reservoir with the hazard in the food. A microbiological risk assessment for *Vibrio vulnificus*, a bacterium that grows well in warm seawater, is suggested as a model to build upon for HABs. Oysters in the Gulf of Mexico which concentrate this organism through filter feeding have been implicated in illnesses. The assessment considers information from the *Vibrio* in the sea to the ingestion of an oyster meal. A model was developed to consider the prevalence, numbers and seasonality of *V. vulnificus* in oysters, and the influence of meal sizes. Assumptions have been made based on existing knowledge and an allowance has been made for uncertainty. The bacterial model would have to be adapted for specific HAB situations. Both are seasonal blooms, but the development of HABs is more complex and contact with shellfish does not always occur. Heat is not a barrier for either Vibrios or toxins, since many people prefer to eat shellfish uncooked or lightly cooked.

T52 *CYCLOSPORA OOCYSTS* ON RASPBERRIES FROM GUATEMALA — A QUALITATIVE RISK ASSESSMENT

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In recent years there have been a number of foodborne outbreaks of cyclosporiasis in North America. Most of these outbreaks were epidemiologically associated with the consumption of fresh Guatemalan raspberries. In an effort to identify research needs and to facilitate risk management decisions regarding this issue, Health Canada and the Canadian Food Inspection Agency developed a qualitative risk assessment on *Cyclospora* contamination on Guatemalan raspberries. This risk assessment follows CODEX principles and guidelines. The general format of the risk assessment is as follows: (1) Hazard Identification includes the general biology of *Cyclospora cayetanensis*, modes of transmission, information regarding recent outbreaks of cyclosporiasis in North America, and prevalence studies; (2) Exposure Assessment includes a discussion of the possible sources of contamination of raspberries in Guatemala, the volume and distribution of raspberries in Canada, and potential control methods; (3) Hazard Characterization includes information regarding the infectious dose, symptoms and pathogenicity of cyclosporiasis, susceptibility factors, and diagnosis and treatment; (4) Risk Characterization includes conclusions pertaining to the contamination of produce, transmission of infection, epidemiology, information gaps, and risk to consumers. Even with the limited information available regarding *Cyclospora* contamination on raspberries, the risk to consumers in Canada was shown to be unacceptably high. Contaminated water and worker hygiene were considered as important factors in contamination of raspberries. Contamination appears to be seasonal as the majority of outbreaks have occurred in the late spring. Future research should be directed towards detection methodology and effectiveness of various control measures.

T53 SAFETY AND QUALITY EVALUATION OF THAI FERMENTED SAUSAGE (NHAM)

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Nham is a traditional Thai fermented pork sausage. It is usually served and consumed raw after 3-4 days of uncontrolled fermentation. Nowadays, the production of nham still depends on natural microorganisms. This results in products with inconsistent quality that are sometimes unsafe. The objective of this study was to survey the microbiological properties of nham products in Thailand as related to chemical properties. Microbiological and chemical characteristics of nham, commercially produced in Thailand, were evaluated for quality and safety. Sixty samples of nham, with and without irradiation, were collected from retail markets in Bangkok and nearby provinces of Thailand. They were examined for water activity, pH, nitrite residual, and microbiological properties. The pH values, water activity, and nitrite content of nham samples were in the range of 5.62 to 4.23, 0.93 to 0.78, and 72 ppm to 1 ppm, respectively. Mean aerobic plate counts and lactic acid bacteria counts

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of irradiated nham were about 2 to 3 log CFU less than those of non-irradiated ones. Of the nham samples tested, 16%, 15%, and 12% were found to be contaminated with *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes*, respectively. However, none of the nham was found to contain *Escherichia coli* O157: H7 and *Yersinia enterocolitica*. No pathogenic bacteria were detected in nham with pH below 4.6 and subjected to irradiation. This study illustrates the relationship between chemical properties and microbiological characteristics of nham and provides useful guideline information for controlling quality and safety of nham products.

T54 THE USE OF HOUSEHOLD SHOPPING PATTERNS TO IDENTIFY SOURCES OF FOODBORNE DISEASE

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Strategies for the control of foodborne disease depend upon accurate and up-to-date epidemiological data. Epidemiological data are collected in England through retrospective questioning, supported by food and faecal analyses where available. However, data such as food histories, used to establish casual agents and contributing factors, are generally unreliable and inaccurate. The investigation of a US multi-state outbreak of *Salmonella agona* infection from ready-to-eat savory snacks used household shopping patterns in affected and control groups as an alternative to gathering information by food histories.

This UK study aims to determine whether data from such household shopping patterns could be incorporated into a routine surveillance system to identify food vehicles and sources.

Information on household shopping patterns for eggs and poultry were collected by telephone interview following confirmation of each case of *Salmonella* spp. within a health authority district. Analysis of the data obtained highlighted novel trends in sources of *Salmonella* infections which would not be recognized by conventional surveillance.

This approach has the potential to enhance the effectiveness of routine surveillance systems.

T55 QUANTIFICATION AND VARIABILITY ANALYSIS OF BACTERIAL CROSS-CONTAMINATION RATES IN THE KITCHEN

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This study investigated bacterial transfer rates between the hand and other common surfaces involved in food preparation in the kitchen. Nalidixic acid-resistant *E. aerogenes* B199A was used as an indicator microorganism to follow the cross contamination events. Samples from at least 30 different participants were collected to determine the distribution of each cross contamination rate, and to quantify the natural variability associated with the rate expected under most circumstances. The transfer rates between hands, foods, and the kitchen surfaces were highly variable, being as low as 0.0005% and as high as 100%. A normal distribution described fairly well the rate distributions. The mean and standard deviation of the normal distributions were, in % transfer rate, chicken-to-hand (8.7, 4.8), cutting board-to-lettuce (7.9, 3.9), spigot-to-hand (2.3, 7.9), hand-to-lettuce (0.76, 11.7), hand pre wash-to-hand post wash, i.e., handwashing efficiency, (0.63, 26.3), and hand-to-spigot (0.16, 12.3). Quantifying the risk associated with various steps of the food preparation process may provide a scientific base for rational risk management efforts to control cross contamination in the kitchen.

T56 THE USE OF NOTATIONAL ANALYSIS TO ASSESS CROSS CONTAMINATION DURING DOMESTIC FOOD PREPARATION

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Cross contamination is increasingly reported as a contributory risk factor in food poisoning, with one recent UK study implicating it in 39% of general outbreaks. However, while many cases of food poisoning are probably contracted in the home, relatively little is known about the extent of cross contamination during domestic food preparation.

A technique known as notational analysis, a means for objectively and systematically recording and studying actions, was adapted to study cross contamination. The time taken and the number of actions required by 92 participants to prepare one of three meals varied considerably (42 min to 184 min). Across all participants a total of 9,268 actions with the potential for cross contamination were notated, representing a mean of 101 actions per participant (range 15-208). Raw meat or poultry was touched by participants an average of 3.7 times per preparation, yet only 6% washed their hands properly after handling raw food. Kitchen surfaces

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most likely to be touched by contaminated hands were fridge and tap handles, which were touched an average of 13.8 and 7.7 times per preparation, respectively. An average of four kitchen surfaces came into contact with raw food or packaging. 83% of participants allowed ready to eat food to come into contact with a contaminated object, which was most likely to be the hands. Sanitizers were seldom used even after raw food had been handled.

A small minority of participants did prepare meals with little risk of cross contamination but the majority gave it little consideration.

T57 CONTAMINATION OF KITCHEN SURFACES AFTER DOMESTIC FOOD PREPARATION

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A number of laboratory studies have indicated the potential for microorganisms to spread within kitchens during food preparation although little data is available on the speed or ease with which this spread happens in practice.

A study of contamination levels in a domestic kitchen was undertaken following food preparation by 92 participants. Prior to the start of food preparation the kitchen was cleaned using a validated cleaning protocol leaving known residual levels of ATP and microbial contamination (measured as aerobic colony counts and enterobacterial counts). During and after food preparation, participants were asked to follow or implement their normal cleaning practices. Overall 1,351 kitchen sites were tested using ATP and 1,572 using microbiological methods. The vast majority (76%) were in excess of previously determined target values and critical limits obtained following "realistic cleaning". Only 10% were within the ATP and 19% within microbiological critical limits. For 6 particular locations over 95% of results where in excess of critical limits. Hand contact sites, including tap handles, drying cloths, etc., showed particularly high levels of contamination. Thirteen percent of sites exceeded enterobacterial critical limits with chopping boards (21%) and cleaning cloths (87%) of particular concern. Cumulatively these data, which may be an underestimate, indicate how quickly microbial contamination can build up on kitchen surfaces during food preparation and give rise to both intra and inter meal cross contamination.

T58 THE SIGNIFICANCE OF HAND DRYING AFTER HANDWASHING

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The effective removal of microorganisms from hands during the handwashing process has been termed "hygienic efficiency". Removal of bacteria during handwashing is the result of mechanical friction (scrubbing), the lifting action of soap and the physical effects of thorough rinsing, followed by drying. While various aspects of this hygiene process have been studied, hand drying for the most part has been overlooked. In the handwashing experiments described here, the hands of test subjects were contaminated artificially with *Serratia marcescens*, which, along with resident flora counts, was used to identify the effectiveness of the handwashing and drying processes. Using an experimental procedure calling for a preliminary moistening rinse of hands, a 98% reduction of transient and 35% reduction in resident flora was shown when hands were washed with E1 antimicrobial soap and dried with paper towels. In experiments using the same preliminary moistening rinse, but then paper towel drying only, a 90% reduction in transient and 35% reduction in resident flora was seen. This confirms earlier work quantifying the effectiveness of paper towels in reducing bacterial counts on hands. In addition, these data, in combination with results from other similarly performed handwashing and drying experiments, allow the construction of a hygiene model that identifies the cleansing contribution provided by each phase of the handwashing process.

T59 CHANGES OF AFLATOXINS DURING THE RIPENING AND STORAGE OF KOREAN SOY SAUCE AND SOYBEAN PASTE AND THE CHARACTERISTICS OF THE CHANGES

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The changes of aflatoxins of traditional Korean soy sauce (kan-jang/kahn-jahng) and soybean paste (doen-jang/dwen-jahng) during ripening and storage for 12 months and the characteristics of the changes were investigated. Food components and aflatoxins were analyzed at 0, 3, 6, 9, and 12 months. At the initial stage aflatoxins were detected at a trace

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level, 7.2 ppb, 2.8 ppb, and 3.9 ppb in soybeans, meju cakes (soybean cakes), soy sauce and soybean paste, respectively. We suspect that the process of making and fermenting meju cakes was mainly responsible for the aflatoxin contamination in the soy sauce and soybean paste. Part of the toxins in the soybean paste were degraded during ripening and storage and showed only a trace level in 6 months. Although the aflatoxin content of soy sauce increased over 1 year of storage time, it was still far below the 10 ppb safety limit of Korea; we could expect destruction of parts of toxin in the soy sauce during cooking. It was strongly suspected that these changes in the soy sauce and soybean paste were due to the difference in their crude fat and fatty acid content.

T60 MIGRATION OF *PENCILLIUM SPINULOSUM* FROM PAPERBOARD PACKAGING TO EXTENDED SHELF-LIFE MILK

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Growth and survival of the psychrotroph *Penicillium spinulosum* in paperboard was studied, along with the wicking characteristics of ultra-pasteurized milk, to understand sporadic fungal contamination of ultrapasteurized, extended shelf-life milk products. Paperboard is a potential source for fungal contamination. Condia were inoculated into sterilized paperboard squares (57.2 by 57.2 mm) made from ultra-pasteurized milk cartons. Test-squares were sealed on three edges and inoculated at 3.2, 6.4, 9.5, and 12.7 mm from the unskived edge and placed in ultra-pasteurized milk for a 60-day shelf life. The surrounding milk was tested for the fungus. *Penicillium spinulosum* was detected in 84% of samples at 3.2, 72% at 6.4, 50% at 9.5, and 28% at 12.7 mm. Survival in paperboard was investigated in sealed paperboard test-squares incubated in ultra-pasteurized skim milk at 7°C every 10 days up to 60 days. The fungus survived in the interior of paperboard for the entire incubation period. Wicking characteristics of ultra-pasteurized skim or whole milk were measured in four types of paperboard held at 7°C. Wicking distances were measured every 10 days up to 60 days. A significant interaction was seen between the types of paperboard and milk. Most likely the fungus had access to milk as a source of nutrition by day 40 in the migration study. To minimize migration of fungi from paperboard packaging and thereby reduce spoilage of the product, it is important to either limit the access of liquid to the microorganisms or limit the number of fungi in the packaging.

SYMPOSIA

S01 *LISTERIA MONOCYTOGENES*: CURRENT ISSUES AND CONCERNS—SESSION I: PATHOLOGY, VIRULENCE, AND RISK ASSESSMENT OF *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes continues to be a serious concern to the food industry and to regulatory agencies. Foodborne outbreaks caused by this pathogen have had severe public health and economic consequences. The infective dose in humans is believed to be high, but is still unknown. Evidence suggests that not all *L. monocytogenes* strains are equally likely to be implicated in human illness. The wide distribution and unique ecology of this pathogen in the food chain creates difficult challenges for intervention strategies and control. This two-session symposium presents an update on current progress and developments to address these formidable issues.

Presentations in the first session will describe the application of animal models to investigate the virulence and to determine the infective dose of *L. monocytogenes*. The relationship between virulence factors among different genotypes of *L. monocytogenes* will also be discussed. Risk assessments of *L. monocytogenes* in the commercial food supply and in home-prepared foods will be presented along with an update on the status of the Food and Drug Administration's *Listeria* risk assessment.

Detection methods and intervention strategies for *L. monocytogenes* at production will be the focus of the second session. A review of rapid, genetic-based methods for the detection of *L. monocytogenes* will be presented. The session will also include presentations on the ecology of *L. monocytogenes* in primary production and various intervention strategies like irradiation, pasteurization, and control measures in high-risk production areas. A panel discussion with speakers from both sessions will conclude this symposium.

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S02 SAFER PRODUCTION OF SPROUTS FROM SEEDS

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Between 1995 and 1999, in the US 15 outbreaks of foodborne disease were associated with consumption of sprouts grown from seed. In 13 of these, *Salmonella* spp. were implicated; in two *Escherichia coli* O157:H7. Internationally, at least six other sprout-associated outbreaks have been reported.

In 1999, the NACMCF prepared a white paper addressing production practices affecting the production of safer sprouts. Also that year, the US FDA prepared and published two guidance documents relating to the production of safer sprouts and microbial monitoring of the product.

This symposium will present an update of industrial, academic, and regulatory activities to address this issue. Topics considered include an overview of the epidemiology of sprout-associated disease and regulatory policy, and studies on the growth of organisms of concern during production, as well as testing of seeds, sprouts, and spent irrigation water, with respect to guidance in the FDA documents. Assessment and evaluation of chemical treatments applied to seeds and sprouts, and the effects of irradiation, will be discussed. Finally, there will be a summary of activity in this area with particular reference to future research direction and the potential benefits to related industries.

S03 COOK-CHILL/SOUS VIDE TECHNOLOGY

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Today, there is an increasing consumer demand for fresh-like food with a minimum of preservatives. "Cook-chill/sous vide (under vacuum) technology" meets this need. It can be applied to virtually the entire range of food that is cooked/pasteurized or washed (as with fruits and vegetables) so that when purchased by the consumer or used in a food establishment, the food item is perceived as being very fresh and convenient.

One key feature of this food is that it is refrigerated and not frozen. In the case of meat, fish, and poultry dishes, there is no significant difference in quality between a correctly frozen product and a chilled product. However, the chilled product is much easier to reconstitute and heat for service. Frozen food allows for easy distribution. Chilled foods probably should be limited to a 250-mile distribution in order to assure freshness. The less processed a food is, the shorter the shelf life, but the "fresher" it is.

In Europe, this technology has undergone continual evolution for the last 20 years. It began with the introduction of sous vide technology in France, under the auspices of the French government. Today, under the guidance of the European Commission, there is an ongoing research program to understand the food safety and spoilage variables associated with the food.

In America, the large deli roasts of corned beef, beef, turkey, chicken, etc. have been marketed by food processors for years. Actually, this is a chilled, sous vide product. In Europe, a sous vide product is usually a product that is approximately 3/4 inch thick, weighing about one pound (e.g., lamb chop, chicken breast, etc.). In the US, we tend to produce 10- to 12-pound meat, fish, and poultry roasts. Our kettle-cooked and pumped, chilled food is usually packaged in a casing, while in Europe, it is in a form-fill pouch.

We have been unable to introduce sous vide and vacuum-packed technology to the retail food sector in the US, because FDA believes that sous vide is a high-hazard process and has specified that there must be at least two barriers to the possible outgrowth of *Clostridium botulinum*. One barrier could be temperature, but the second barrier must be such that if the food sits at room temperature, *C. botulinum* will not grow. In retail operations, putting in additives to accomplish this is unacceptable to the consumer, who wants as fresh a product as possible, with the least possible additives.

The purpose of this symposium is to explain the types of products that are available and how they are manufactured, and then, discuss the safety technology of this food under refrigeration.

In order for this to be successful, the retail chef must be allowed the same processing options that USDA wholesale food processors and Europeans have. Under the USDA, if one can write a HACCP and do temperature control, then additives are not required. USDA process standards rely solely on temperature as the food safety control.

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This symposium will provide the information necessary to show the advantages of this technology and that there is no reason why this technology should not be used in retail foodservice operations in the US, based on its success and long history of safety in Europe and in USDA-inspected operations in the US.

S04 THE ROLE OF MOLECULAR TECHNIQUES FOR VIBRIOS AND VIRUSES IN MAKING RISK MANAGEMENT DECISIONS

Ken Moore, Interstate Shellfish Sanitation Conference, 115 Atrium Way, Suite 117, Columbia, SC 29223, USA; Asim K. Bej, University of Alabama Birmingham, Dept. of Biology, Birmingham, AL 35294-1170, USA; Gary P. Richards, USDA, Delaware State University, Dover, DE 19901, USA; Marianne Miliotis, US FDA, Office of Seafood, 200 C St., SW, Washington, D.C. 20204, USA; Chris Nelson, Bon Secour Fisheries Inc., County Road 49 South, Bon Secour, AL 36511, USA

Quality of life has improved because of advances in science and engineering. Molecular biochemical techniques have significantly impacted agriculture and aquaculture, enhancing food production. Molecular techniques are being developed so that there is a significant reduction in risk associated with food safety. Currently, the United States Food and Drug Administration permits the use of molecular techniques for presumptive screening of foods for pathogenic microorganisms, a negative result stands but a positive result must be confirmed using standard methods. Advances in molecular biology will ultimately replace conventional assay methods with contemporary molecular techniques. However, when contemporary molecular techniques are used to replace conventional assay methods, it is necessary that results of both methods provide reliable information. Contemporary molecular techniques are being increasingly used as a tool in epidemiological evidence. These methods provide a rapid result of the cause of the disease associated with the symptoms. When disease is manifested, the microorganism that caused the disease has all genetic codes associated with disease intact. Thus detecting the presence of the viable organism after a disease state, through conventional and/or contemporary methods, would be a matter of choice. However, when molecular techniques are extrapolated to other areas as a preventive tool for food safety it is important that the methods must be based on quantitative risk assessment rather than qualitative risk assessment. Further, there are several cases of avirulent pathogenic microbes that can multiply and yet not produce disease symptoms. This symposium will use the oyster as a model and reflect the benefits and limitation of molecular techniques when applied to Vibrios and viruses. The regulatory agency and the industry shall share their perspective and concerns on the molecular techniques.

S05 APPROACHES TO CONTROL PATHOGENS IN THE NEXT MILLENNIUM

Christine Bruhn, University of California-Davis, Center for Consumer Research, 1 Shields Ave., Davis, CA 95616-8598, USA; Jim Dickson, Iowa State University, Dept. of Microbiology, 207 Science I, Ames, IA 50011, USA; Kathleen T. Rajkowski, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; W. J. Stadelman, Purdue University, Dept. of Food Science, W. Lafayette, IN 47907-1160, USA; J. Stan Bailey, USDA-ARS-RRC, P.O. Box 5677, Athens, GA 30613-5677, USA; Gary Acuff, Texas A&M University, Dept. of Animal Science, College Station, TX 77843-2471, USA; G. V. Barbosa-Canovas, Washington State University, Dept. of Bio Systems Engr., 207 Smith Agri. Bldg., Pullman, WA 99164, USA; Susan S. Sumner, Virginia Tech., Food Science and Technology (0418), Blacksburg, VA 24060, USA; Chuck Sizer, National Center for Food Safety and Technology, 6502 S. Archer Ave., Summit Argo, IL 60501-1933, USA; David Golden, University of Tennessee, Dept. of Food Science and Technology, 2605 River Road, P.O. Box 1071, Knoxville, TN 37901-1071, USA

Advances in food safety to control foodborne pathogens has expanded. This poster symposium will explain several technologies along with data to support their use to control pathogens in foods. Some of the technologies presented include the use of electron beam irradiation, gamma beam irradiation, competitive exclusion, high intensity pulsed electric fields, high hydrostatic pressure, microwave cooking, steam vacuuming, organic acid washes and in-shell egg pasteurization. In addition, the aspect of consumer acceptance of new technologies will be presented.

S06 *LISTERIA MONOCYTOGENES*: CURRENT ISSUES AND CONCERNS — SESSION II: DETECTION, ENUMERATION, AND INTERVENTION STRATEGIES FOR *L. MONOCYTOGENES*

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Listeria monocytogenes continues to be a serious concern to the food industry and to regulatory

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agencies. Foodborne outbreaks caused by this pathogen have had severe public health and economic consequences. The infective dose in humans is believed to be high, but is still unknown. Evidence suggests that not all *L. monocytogenes* strains are equally likely to be implicated in human illness. The wide distribution and unique ecology of this pathogen in the food chain creates difficult challenges for intervention strategies and control. This two-session symposium presents an update on current progress and developments to address these formidable issues.

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S07 CURRENT INTERNATIONAL ISSUES IN PRODUCE SAFETY

Linda J. Harris, University of California-Davis, Dept. of Food Science and Technology, One Shields Ave., Davis, CA 95616-8598, USA; Art Miller, FDA-CFSAN, HFS-32, 200 C St. SW, Washington, D.C. 20204, USA; Marie-Claude Thibault, Canadian Produce Marketing Assn, and Canadian Horticultural Council, 310-1101 Prince of Wales Dr., Ottawa, Ontario K1C 5J6, Canada; Alejandro Castillo, University of Guadalajara, Monte Alban 1347, Guadalajara, Jal. 44340, Mexico; Bob Gravani, Cornell University, Dept. of Food Science, 11 Stocking Hall, Ithaca, NY 14853, USA; Christine Bruhn, University of California-Davis, Center for Consumer Research, One Shields Ave., Davis, CA 95616-8598, USA

The consumption of produce continues to increase as do foodborne illness outbreaks associated with these items. To date, domestic produce has been responsible for the majority of foodborne illness outbreaks in the US. However, imported produce has received considerable attention in association with larger multi-state outbreaks of foodborne illness.

Meeting consumer demands for fresh produce year round is successfully achieved by relying on the international market for the importation of off-season items. With a multitude of different countries of origin, there are virtually equal numbers of differing farming, field sanitation, and post-harvest handling practices. It is now clearly recognized that sanitary deficiencies in any of these practices could provide a potential for compromising the safety of domestic and imported produce.

Intervention and preventative measures should be applied to produce from production to consumption. The development of good agricultural practices, implementation of good manufacturing practices in packing houses, effective use of produce disinfectant products and consumer education are essential in enhancing the safety of domestic and imported produce.

This symposium will discuss current international issues in produce safety that include domestic and foreign traceback, foreign and domestic programs in good agricultural practices and consumer education of produce safety.

S08 RELEVANCE OF TESTING TO REDUCE RISK

Russell Flowers, Silliker Labs, Inc., 900 Maple Road, Homewood, IL 60430, USA; Lee-Ann Jaykus, North Carolina State University, Food Science Dept., Box 7624, Raleigh, NC 27695-7624, USA; Todd McAloon, Cargill, Inc., P.O. Box 9300-Rb, Minneapolis, MN 55440, USA; Donald Schaffner, Rutgers University, Food Science, 65 Dudley Road, New Brunswick, NJ 08901-8520, USA

Most of the food industry uses some type of testing to help assure product safety. Testing methods range in complexity from simple assays to assure adequate equipment sanitation; to general raw/finished product analyses (such as standard plate count); to identification of microbiological indicators (such as fecal coliforms and Enterococci); to organism-specific assays (*Salmonella*, *L. monocytogenes*, *E. coli* O157:H7). Significant work has been done in the past to assure that sampling plans are optimized with respect to maximizing the likelihood of finding a positive sample (if present) while performing a realistic number of tests. However, the current food safety climate has necessitated another look at sampling for the detection of foodborne pathogens. For instance, we are increasingly reminded about the intermittent nature of contamination within a lot of food. Some of the "emerging" foodborne pathogens cause disease at particularly low doses and their presence in raw

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food products is of concern. For example, the institution of the USDA-FSIS 1996 HACCP rule has necessitated a degree of pathogen testing in meat and poultry processing. Detection methods themselves are evolving to decrease testing time and improve detection sensitivity such that online detection may become a reality in the near future. The purpose of this symposium is to explore the relevance of testing to reduce the risk associated with foodborne pathogens. Specifically, speakers will provide an overview of statistical sampling methods; discuss emerging detection methods that may increase speed and improve detection limits for foodborne pathogens; outline the legal and regulatory implications of testing; and present the potential impact of sampling strategies on risk analysis and risk mitigation. By following the presentations with counterpoint discussion, the audience will be engaged to provide their own views regarding the usefulness of testing to reduce risk. This symposium should provide a forum upon which professionals from diverse backgrounds can converse about an increasingly important food safety issue.

S09 HACCP-BASED STRATEGIES FOR COOKED READY-TO-EAT SEAFOODS BASED ON QUANTITATIVE RISK ASSESSMENT

Robert Tauxe, CDC, 1600 Clifton Road, CDC, A38, Atlanta, GA 30333, USA; Mike Moody, Louisiana State University, Knapp Hall, Baton Rouge, LA 70803, USA; George J. Flick, Virginia Tech., Dept. of Food Science and Technology, 25 Duck Pond Drive, Blacksburg, VA 24060, USA; Brian Perkins, Auburn University, Mobile, AL 36615, USA

Following the implementation of the mandatory seafood HACCP regulations on Dec. 18, 1997 it has become evident that a few food safety concerns need to be addressed. An identified concern is time-temperature control for post-thermal handling (include unit operations of cooling through packaging) of cooked ready-to-eat fresh seafood products such as crabmeat. There are two major methods used for crab processing. Generally, along the Atlantic coast crabs are retorted and refrigerated in cooler overnight before crabmeat is debacked, picked and packed. Along the Gulf Coast, crabs are boiled and subsequently debacked and held refrigerated. On the next day, crabmeat is picked and packed. Due to differences in post-cooking handling operations, the FDA's current regulatory requirement requires processors to follow defined time-temperature controls. The time-temperature controls due to difference in processing approaches are outlined in the FDA's seafood Encore HACCP course. This symposia will provide a description for the differences in post-thermal handing time temperature controls for cooked ready-to-eat products using crab products as a model. Data from Center for Disease Control and Prevention will provide information on seafood illness and death associated with consumption of fresh cooked crabmeat products. The FDA will provide information on the regulatory policy for cooked ready-to-eat fresh products with a focus on crab products. Research data will also be presented that reflects the dynamic microbial changes of the crab products following post-thermal handling and changes in microflora during storage. Optional processing strategies will be suggested and economic data for Blue crab HACCP will be presented.

S10 *CAMPYLOBACTER* PERFORMANCE STANDARDS: IMPLEMENTATION AND CONTROL

Geraldine Ranson, USDA Food Safety and Inspection Service, Rm. 3175, Franklin Court, 1400 Independence Ave. SW, Washington, D.C. 20250-3700, USA; Eric Line, USDA-ARS-RRC, P.O. Box 5677, Athens, GA 30604, USA; James S. Dickson, Iowa State University, Dept. of Microbiology, 207 Science I, Ames, IA 50011, USA; F. J. (Eric) Bolton, Public Health Laboratory Service, Central Public Health Laboratory, 61 Collindale Ave., London, NWA9 5HT, UK; Norman J. Stern, USDA-ARS-RRC, Poultry Microbiological Safety Research Unit, P.O. Box 5677, Athens, GA 30604-5667, USA

Although the United States food supply is among the world's safest, significant food safety problems, such as posed by *Campylobacter* spp., can cause either human illness or economic losses and threaten the international competitiveness of American agriculture. Concerns over disease transmission of *Campylobacter* spp. through foods of animal origin have only recently been elevated in status. All sectors of the food industry, regulatory agencies, and research organizations are mobilized. This symposium serves to address the current status and action being taken by these three sectors to reduce public exposure and risk presented by this etiologic agent. Specifically, the Food Safety Inspection Service (FSIS) is undertaking studies to determine the existing levels of the pathogen in the United States foods of animal origin and, are assessing methods that will be useful for future sampling. It is likely that these methods will be implemented by the food industry for self-monitoring studies. Within the poultry industry recognition that control of *Campylobacter* spp. was lacking and thus, specific interventions are being developed. An important development in control has been studied and incorporates litter amendments to reduce the

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transmission of the organism from infected to non-infected broiler chickens. Within the United States, pork is routinely a carrier of *Campylobacter coli* and this association potentially has implication in human disease. In Northern Europe, where appropriate isolations methods are used by clinical laboratories, substantial numbers of *Campylobacter coli* is isolated. Studies are required to provide precise, reliable and rapid testing to detect the organism throughout the farm to fork continuum. We must develop effective, reliable, and cost effective methods to control or eliminate *Campylobacter* spp. in food animal operations. We need to gather data using the most appropriate samples to protect the public health and develop scientifically based regulatory statutes.

S11 GENETIC METHODS TO TRACK MICRO-ORGANISMS IN FOOD PRODUCTION AND PROCESSING

Martin Wiedmann, Cornell University, Dept. of Food Science and Technology, 114 Stocking Hall, Ithaca, NY 14583-7201, USA; Tim Barrett, CDC, 1600 Clifton Road, Mail Stop C03, Atlanta, GA 30333, USA; Jack Shere, University of Wisconsin, Food Research Institute, 1925 Willow Drive, Madison, WI 53706, USA; Kelli Hiett, USDA-ARS-RRC, P.O. Box 5677, Athens, GA 30604-5677, USA; Joseph Meyer, Kraft Foods, 801 Waukeegan Road, Glenview, IL 60025, USA; Mike Barney, Miller Brewing Company, 3939 W. Highland Blvd., Milwaukee, WI 53208-2866, USA

It is no longer sufficient to know that hamburger may be contaminated with *E. coli* O157:H7 or that the chicken we buy in the grocery store has *Campylobacter* on it. Preventing the pathogens from getting to the food product in the first place is the key to a successful food production or processing system. The best way to understand the ecology and epidemiology of microbial pathogens in food production systems is to utilize genetic typing assays that enable the trace back of specific clonal types of a pathogen from the final product to its original source. Likewise, when microbes are used in the production of foods, maintenance of the strain of the microbe used to produce the food is critical to protect product identity. There are several different techniques that can be used to genetically characterize microbial pathogens. Each of these techniques has advantages and disadvantages. For the non-molecular microbiologist it is important to know how to interpret the results from the different tests and to understand their strengths and weaknesses. Understanding the different genetic techniques and practical field studies using them will be highlighted in this symposium.

S12 ISSUES FACING TODAY'S LARGE DAIRY PRODUCERS

Ron St. John, 4951 NW 170th St., Trenton, FL 32693, USA; John Worley, University of Georgia, Driftmier Engineering Center, Athens, GA 30602, USA; Bill Bickert, Michigan State University, Dairy Faculty Ag Engineering, 120 Farrall Hall, East Lansing, MI 48824, USA; William Thomas, University of Georgia Extension Service, College of Ag., Athens, GA 30602, USA; Carissa Itle, National Milk Producers Federation, 2101 Wilson Blvd., Suite 400, Arlington, VA 22201, USA

The dairy industry is no different than many other industries in the world today. It is definitely going through dramatic change. We're aware of the many mergers and buy-outs taking place with handlers and processors. Also the size of producers is changing very rapidly. Every year the number of active dairy farms decreases yet production increases in many parts of the country. Along with increasing the size of a dairy farm comes many issues that must be addressed. In this symposium, we have people with years of experience and much expertise to address some of these issues. We have attempted to get the viewpoints of various individuals involved in day to day, hands-on experiences to shed light on the expanding dairies. There should be some excellent information for all involved in the dairy industry.

S13 APPROACHES TO FOOD SAFETY IN LATIN AMERICA AND CARIBBEAN COUNTRIES

James Estupinan, Pan America Health Organization/WHO,Talcahuano 1660, 1640 Martinez, Buenos Aires, Argentina; Jairo Romero, Associacion Colombiana de Ciencias y, Tecnologia de Alimentos, A. Aéreo 4371, Bogata, Colombia; Maritza Collon Pulano, FDA, 5600 Fishers Lane, Rockville, MD 20857, USA; Ronald Gordon, CARICOM Secretariat, Bank of Guyana Bldg., Georgetown, Guyana; Pablo Guillermo Galli, Analista Tecnico Especialista en Normas Multilaterales, Servicio Nacional de Sanidad y Calidad Agroalimentaria, Av Paseo Colon 367, Piso 5, Buenos Aires, Argentina; Jaime Almonte, Asesor Secretaria deAgricultura y Ganaderia de Mexico, Mexico, D.F.

Foodborne disease has been recognized in these countries as contributing to the morbidity and mortality of the population with high socio-economic impacts, as when cholera struck Peru in 1990 with an estimated cost of US$700 million, most of which were for lost food sales and tourism. Focal points for a regional surveillance system, coordinated by INPPAZ/PAHO, and improvement at the national level with active surveillance have

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been established to detect the main foodborne pathogens. Information gathered from 1995 to July 1999 from 25 countries by the Regional Surveillance system documented 3411 outbreaks, with 107,146 cases and 205 deaths. The most important pathogens identified were *Salmonella enteritidis* and *Staphylococcus aureus*. High levels of hemolytic uremic syndrome are reported in the region but have not been particularly related to foodborne outbreaks. Central American countries have had trouble exporting produce to North America because of potential problems with pathogens. Surveillance and appropriate control, therefore, are all the more important with expanding free trade and an increasing demand for produce in North America continues. The topics below cover the main aspects of food safety surveillance in these countries leading to practical recommendations for safer food in the Americas.

S14 BIOTERRORISM AND FOOD PROTECTION

Richard Lee, State University of New York, Division of Obstetrics and Geographic Medicine, 7664 E. Quaker Road, Orchard Park, NY 14127, USA; Raymond Harbison, University of South Florida, College of Public Health, Tampa, FL, 33612, USA; Dale Hancock, Washington State University, ADBF 2041, Pullman, WA 99164-6610, USA; Jeremy Sobel, CDC, 1600 Clifton Rd., Atlanta, GA, 30333, USA; Gary Hurst, U.S. Army, 3100 Ricketts Point Road, APG-EA, MD 21050-5400, USA; Ann Draughon, University of Tennessee, Agric. Exper. Station, Dept. Food Science and Technology, Knoxville, TN 37901-1071, USA

Food safety professionals must not only meet the challenge of improving safety of today's food supply but must also anticipate future events which may impact food safety and the supply of wholesome food. Release of disease-causing organisms into foods by individuals or groups with a personal or political agenda is not only a possibility, but has already occurred. At least 750 cases of salmonellosis from a salad bar were caused by the Rajneeshee religious cult in Oregon in 1997 in a plan to influence county elections. Unfortunately, little training is required to cultivate many common foodborne pathogens and the costs associated with their production are minimal. Genetic manipulation of foodborne pathogens may create ever more virulent strains than those currently implicated in foods. The goal of terrorists is to create terror and few things create terror more quickly than disease. Even if death is not the goal, illness and panic associated with bioterrorism may seriously impact the structure of society and the national economy. Education and training in bioterrorism associated with the food supply and its potential consequences must become national priorities.

S15 FOOD BIOTECHNOLOGY: PERSPECTIVES, CHALLENGES AND OPPORTUNITIES

Michael Phillips, Biotechnology Industry Organization, 1625 K St., NW, Suite 1100, Washington, D.C. 20006, USA; Sylvia Rowe, International Food Information Council, 1100 Connecticut Ave., NW, Suite 430, Washington, D.C. 20036, USA; Martina McGloughlin, University of California-Davis, 355 Briggs Hall, Davis, CA 95616-3260, USA; Janet Andersen, US Environmental Protection Agency, 7511C USEPA Headquarters, Ariel Rios Building, 1200 Pennsylvania Ave., NW, Washington, D.C. 20460, USA; Adrianne Massey, Massey and Associates, 6823 Falconbridge Road, Chapel Hill, NC 27514, USA; Donald Kendall, USDA-GIPSA, Technical Division, 10383 N. Executive Hills Blvd., Kansas City, MO 64153, USA

For centuries, humans have been selecting, sowing and harvesting seeds that produce food products that will sustain them. Global food demand has increased the need for improved crops. Biotechnology has offered the needed technology to produce more nutritious and better tasting foods, higher crop yields and plants that are naturally protected from disease and insects.

Crops produced through biotechnology are becoming significant components of the US harvest. These include soybeans, corn, canola, tomatoes, squash and potatoes that are improved versions of the traditional, with added beneficial traits.

Consumer acceptance is critical to the future of food biotechnology, but consumer views vary dramatically from country to country. Two out of three consumers support foods produced through biotechnology and have confidence in the FDA policy for labeling biotech foods. These were among the findings of a national survey of 1,002 US adults, conducted by Wirthlin Worldwide from October 8-12, 1999 for the International Food Information Council (IFIC). The vast majority of American consumers still place a great deal of confidence in the benefits of, and the regulatory climate for agricultural biotechnology. A three-fold increase in media coverage of food biotechnology and confusion in the international marketplace have raised questions with some consumers. But most people remain positive and look forward to the benefits of biotechnology.

While reports of arguments against biotechnology have impacted consumer attitudes, the new data emphasize the need for experts to clearly articulate the benefits of biotechnology. Nearly four in ten

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Americans reported that they are aware that there are products in the supermarket produced through biotechnology. Consumers tended to be aware of produce examples, such as tomatoes and fruit, although many more Americans than in past surveys realized corn and soybeans have been improved through biotechnology.

This symposium will address the past, present and future of biotechnology, highlight new information on consumer perceptions of biotechnology, review environmental impacts, food product enhancement and detection of biotechnologically derived food ingredients.

S16 BIOSENSORS AND REAL-TIME DETECTION SYSTEMS

Robert Brackett, FDA, HFS-300, 200 C St. SW, Washington, D.C. 20204-1000, USA; Peter David, Dtek, 26982 Beaver Lane, Los Altos Hills, CA 94022-1964, USA; David S. Gottfried, Georgia Tech Research Institute, Electro-Optics, Environment, and Material Lab, 925 Dalney St., Baker 225, Atlanta, GA 30332-0825, USA; Gerry Crawford, USDA-REE-ARS-NAA-ERRC-MB&BR, Room 0123, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA; Eric Johnson, University of Wisconsin, Food Research Institute, 1925 Willow Dr., Madison, WI 53706, USA; Donald Conner, Auburn University, 245 Animal Science, Auburn, AL 36849, USA

Biosensors are analytical devices incorporating biologically derived material or biomimic with a physiochemical transducer or transducing microsystem. Biosensors are being developed for rapid time direct or indirect detection of foodborne microorganisms, toxins, or undesirable metabolites or compounds. These systems have a potential application in real-time validation of critical control points. Sensitive, specific and rapid processes have been developed that require minimal culture enrichment and utilize immuno-based biosensors, such as imunomagnetic-electrochemiluminescence, to detect pathogenic microorganisms in food systems. Immuno-based biosensors to detect low levels of *E. coli* O157 and *Salmonella* within 2-8 h are currently being field-tested in meat and poultry plants. Flow cytometry provides a precise means for the rapid detection and characterization of individual cells from mixed populations. Cells are individually illuminated by an intense light source and data are collected and analyzed via computer. When combined with an appropriate fluorescent reporter molecule (e.g. labeled nucleic acid probes or antibodies), low numbers of specific pathogens can be detected against a high background of competing microflora. New technologies such as acoustic wave biosensors and radio frequency identification (RFID) sensor tags promise to greatly improve food safety. Research to develop a single computer chip that will automatically inventory and assess food safety at any point from source to consumption is ongoing. A presentation will focus on integration of radio frequency communication, sensor, data acquisition, processing, storage, and power management systems into a single functional unit. This symposium will present the fundamentals of biosensors and real-time detection systems and provide recent developments in the field.

S17 TRANSPORTATION OF RAW MILK AND FINISHED DAIRY PRODUCTS

Mike Culpepper, Georgia Dept. of Ag., 19 Martin Luther King, Capitol Square, Atlanta, GA 30334, USA; Dan Erickson, Minnesota Dept. of Ag., 90 W. Plato Blvd., St. Paul, MN 55107, USA; Patrick Boyle, Readington Farms, Inc., 12 Mill Road, P.O. Box 164, Whitehouse, NJ 08888, USA; Rick Barefoot, H. Fred Barefoot Trucking, Inc., P.O. Box 25, Alum Bank, PA 15521, USA; Ruth Fuqua, Quality Chekd Dairies Inc., 7236 Lebanon Road, Mt. Juliet, TN 37122-7201, USA

It's been said that the weak link in the system is hauling. The NCIMS took some steps to correct that at the conference in May 1999. A hauling committee was formed and many of the proposals submitted by the committee were passed by the delegates and are in affect today. With this symposium, we are addressing some of the issues facing our haulers and producers in the real world. The attempt is to cover regulations spelled out in the P.M.O. as well as everyday labor issues that owners/operators face in their business. We have tapped many available resources to address the topics facing today's industry. This symposium should offer something for anyone involved in the dairy industry.

S18 SIGNIFICANCE OF MYCOTOXINS IN THE GLOBAL FOOD SUPPLY

J. David Miller, Carleton University, Institute of Biochemistry, Dept. of Chemistry, 228 Steacie Bldg., Ottawa, Ontario K1S, Canada; Thomas E. Massey, Queen's University, Dept. of Pharmacology and Toxicology, Kingston, Ontario K7L 3N6, Canada; William P. Norred, USDA-RRC, Toxicology and Mycotoxin Research Unit, 950 College Station Road, P.O. Box 5677, Athens, GA 30605, USA; James J. Pestka, Michigan State University, Dept. of Food Science and Human Nutrition, 234 GM Trout Bldg., East Lansing, MI 48824-1224, USA; Angelo Visconti, National Research Council, Institute of Toxins and Mycotoxins, Vaile Einaudi, 51, Bari, 70127, Italy

Efforts to limit mycotoxins in human food and animal feeds are based on concerns over the adverse

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effects of direct exposure to mycotoxin-contaminated food and feed on human or animal health, and potential mycotoxin residues in foods of animal origin. Food producers and processors are increasingly challenged to understand mechanisms of mycotoxin formation and to develop control and prevention measures. This symposium is designed to provide an overview of the most important mycotoxins worldwide including evidence for the cause and effect of mycotoxins on human disease. Several speakers will address factors influencing the occurrence of several important mycotoxins (aflatoxins, fumonisins, and deoxynivalenol), their toxicology, and related human and animal health issues. Analytical methods and international food safety standards and industry efforts to control the occurrence of mycotoxins in the food supply will also be discussed.

S19 THE ROLE OF NORWALK-LIKE VIRUSES (NLVS) IN FOODBORNE DISEASE

Stephan S. Monroe, CDC, Division of Viral and Rickettsial Diseases, Mailstop G04, 1600 Clifton Road, NE, Atlanta, GA 30333, USA; John D. Cheesbrough, Public Health Laboratory, PHLS Northwest, Royal Preston Hospital, P.O. Box 202, Sharoe G, Preston, PR2 9HG, UK; Doris D. D'Souza, North Carolina State University, Food Science Dept., Box 7624, Raleigh, NC 27695-7624, USA; Christine Moe, University of North Carolina, Dept. of Epidemiology, School of Public Health, CB 7400, Chapel Hill, NC 27599-7400, USA; Daniel J. Maxson, Clark Co. Health District, P.O. Box 3902, Las Vegas, NV 89127-0902, USA

Human enteric viruses are significant human pathogens ranking within the top ten causes of foodborne disease in the US. Recently, the substantial role that the Norwalk-like viruses (NLVs) may play in foodborne disease outbreaks has been estimated, although the true scope and significance of these "emerging" foodborne pathogens is unknown. These viruses present unique challenges to food microbiologists because they are environmentally persistent and resistant, are frequently associated with poor personal hygiene of infected food handlers, and cause disease at extremely low doses, providing immunity that is frequently short-lived. Recent scientific advances, most notably, increased epidemiological surveillance and advances in molecular biology, have significantly improved our ability to detect these previously understudied agents of foodborne disease. The purpose of this symposium is to provide an overview of the most current knowledge about the NLVs with respect to epidemiology, detection, characterization and control. Specifically, speakers will address the significance of NLVs to total foodborne disease; detection of NLVs in clinical and food specimens; genetic characterization of this diverse virus group; dose-response relationships as evaluated by human challenge studies; and the role of environmental contamination in the propagation of primary foodborne NLV outbreaks. A major theme of the symposium will be the identification of ways in which food and medical microbiologists, epidemiologists, and other public health and industry representatives can collaborate to effectively increase our knowledge about the NLVs. Ultimately, the scientific community and food industries must work together to find feasible methods to reduce the incidence of foodborne disease associated with the NLV group. This symposium will provide a forum upon which these discussions can begin.

S20 INTERNATIONAL TRENDS IN ON-FARM FOOD SAFETY

Phillip Corrigan, Australian Embassy, 1601 Massachusetts Ave. NW, Washington, D.C. 20036, USA; Thomas Quigley, Food Safety Authority of Ireland, Abbey Court, Lr. Abbey St., Dublin, 1, Ireland; Albert Chambers, Canadian On-farm Safety Program, 19 Elm St., Ottawa, Ontario K1R 6M9, Canada; Dave Pyburn, National Pork Producers Council, P.O. Box 10383, Des Moines, IA 50306, USA; Ivone Delazari, SADIA, Concordia, Brazil; Richard Baines, Royal Agricultural College, Management Systems for Food Safety & Environment, Cirencester, GL7 6JS, UK; Bonnie Buntain, USDA-FSIS-OPHS, 1400 Independence Ave., SW., Washington, D.C. 20250, USA

The symposium will provide an opportunity for speakers from Europe, North America, Latin America and Australasia to outline the on-farm food safety initiatives in their respective countries or regions. Case studies of particular on-farm initiatives as well as comparative research will be presented. As well, the emerging international "standards" (e.g. Codex Guidelines on Quality Assurance Systems, etc.) will be reviewed.

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S21 THE EARTH IS CURVED (AND SO ARE KINETIC DATA)

Frank Busta, University of Minnesota, Dept. of Food Science and Nutrition, 1334 Eckles Ave., St. Paul, MN 55018, USA; Micha Peleg, University of Massachusetts, Dept. of Food Science, Chenoweth Laboratory, Box 31410, Amherst, MA 01003, USA; Peter McClure, Unilever Research, Colworth House, Sharnbrooke, Bedford MK44 1LQ, UK; Karen Mattick, PHLS Food Microbiology Research Unit, Church Lane, Heavitree, Exeter, Devon EX2 5AD, UK; Martin Cole, Food Safety and Quality, Food Science Australia, 16 Julius Ave., Riverside Corporate Park, Delhi Road, P.O. Box 52, North Ryde, 1670, Australia

In 1810, Nicholas Appert was granted a patent for preservation of food by canning, but it was not until the 1920s that Esty and Meyers published the first systematic study on thermal resistance of toxigenic spores, leading to the "bot cook" or commercial sterilization process. Since then, microbiologists have been analyzing thermal inactivation data using the linear D- and z-value models, even though visual inspection of the data when plotted often showed curvature. Process engineers have been using the results of these analyses to establish safe food processes ever since. This traditional approach to inactivation kinetics is based on the mechanistic theory which assumes that microorganisms or their spores die exponentially following first order kinetics. It also assumes that all cells or spores have identical heat resistance. In no other area of biology are either of these two assumptions made. This approach does allow for simple, straightforward calculations and comparison of thermal process equivalencies to be made. However, throughout the past 80 years, deviations in log-linear models have been repeatedly noted and the "phenomenon" of shoulders and tails has been hotly debated.

When semi-logarithmic survival curves are scrutinized, it can be seen that in fact they are not linear, but slightly curved. The vitalistic theory of inactivation explains these observations by viewing shoulders and tails as being due to underlying physiological reactions of the cells/spores to lethal conditions rather than as "artifacts" of experimentation. This theory states that the individual microorganisms in a population do not have identical resistances to inactivation, that these differences are permanent, and that there is a distribution of microbial sensitivity to heat or any other means of inactivation. This theory is more in line with the other areas of biological study.

The technology available today for handling experimental data has advanced considerably since the 1920s with software capable of non-linear curve fitting readily and cheaply available. Therefore, there is no longer a reason to use only linear regression when other methods may give a better description of the data generated. We have made great strides in other areas of microbiology including culture techniques, microscopy and other laboratory equipment over the past 80 years, yet we still analyze our data as we did in the 1920s. Why? A relatively small group of scientists has actively debated our approach to modeling inactivation data for the past 20 years, however the debate has not reached the microbiological community as a whole. The objective of this symposium is to present information on how and why non-linear models for analyzing inactivation data should be used, generate discussion on this topic and persuade you to at least consider collecting and analyzing your inactivation data with a different approach in mind. Nearly 40 years ago Stumbo wrote "Should it finally be determined that the order of death of bacteria is not strictly logarithmic and that destruction curves could be described better mathematically than is now possible by considering death to be logarithmic, the procedures for evaluating the processes should of course be revised accordingly." The time to follow his advice and make this revision has arrived. Anyone who collects or uses microbial inactivation data, establishes safe food processing regimes, teaches microbial inactivation kinetics or has any other interest in microbial inactivation should attend this symposium and become a participant in this historical debate.

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Abstract Book Addendum

as of July 31, 2000

MONDAY MORNING — AUGUST 7, 2000

(P7) **Withdrawn — Influence of Processing Flow Velocity on Attachment Rates of *Pseudomonas fluorescens* Isolated from the Egg Industry.**

(P14) **Title Change — Survival and Growth of *Escherichia coli* O157:H7 Inoculated onto Cut Lettuce Before or After Heating in Chlorinated Water, Followed by Storage at 5°C or 15°C.** Authors: Yue Li, Robert E. Brackett, Jinru Chen, and Larry R. Beuchat.

(P32) **Efficiency of Sanitation Procedures against *Listeria monocytogenes:* Application to Cold-smoked Fish Industry in France** — Anne Bouttefroy will replace Fabrice Bourion — Authors are as follows: M. Gay, Fabrice Bourion, and Anne Bouttefroy.

(T4) **Withdrawn — Growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 is Enhanced in Ready-to-eat Lettuce Washed in Warm Water.**

MONDAY AFTERNOON — AUGUST 7, 2000

(P53) **Impact of Heating Stress on the Behavior of Two *Listeria monocytogenes* Strains in a Broth which Mimics the Camembert Cheese Composition** — Anita Metivier will replace Emmanuelle Helloin — Authors are as follows: Emmanuelle Helloin, Sandrine Marchau, Anne Bouttefroy, Anita Metivier, and Marielle Gay.

(S8) **Relevance of Testing to Reduce Risk** — Gary Acuff, Texas A&M University, College Station, TX, USA will speak on **Legal and Regulatory Implications of Testing — A Company's Perspective.**

TUESDAY MORNING — AUGUST 8, 2000

(P78) **Food Handlers' Beliefs about Food Safety Procedures and Risks** — Chris Griffith will replace Debbie Clayton.

(P96) **Prevalence of *Pseudomonas* spp. in Process Water, Recycled Water and Dairy Products** — Reginald Bennett will replace Jill Gebler.

(S10) ***Campylobacter* Performance Standards: Implementation and Control**—Victor Cook will replace Geraldine Ransom.

(T34) **Microbiological Evaluation and Manufacturing Practices of Sprouts in Canada** — Francois Veillette will replace Maria Nazarowec-White.

TUESDAY AFTERNOON — AUGUST 8, 2000

LATE-BREAKING SESSION

12:15 p.m. –1:15 p.m.

Grand Salon E

Multi-State Foodborne Outbreak Investigations

Convenor: Jack Guzewich, Food and Drug Administration, Washington, D.C.

The Food and Drug Administration (FDA), in collaboration with the Centers for Disease Control and Prevention (CDC), the US Department of Agriculture (USDA), and the Environmental Protection Agency (EPA), are working with state and local agencies on the National Food Safety System (NFSS) to improve coordination, cooperation and communication among local, state and federal agencies with respect to multi-state foodborne outbreak investigations. The working group established to coordinate multi-state foodborne disease outbreaks, the Outbreak Coordination Workgroup, has developed a draft document, *Multi-State Foodborne Outbreak Investigations: Guidelines for Improving Coordination and Communication.* This group would like comments and suggestions on the draft document from multiple organizations including IAFP Members. Please attend this session and share your thoughts.

Complimentary copies of the Draft Document *Multi-State Foodborne Outbreak Investigations: Guidelines for Improving Coordination and Communication* are available at the IAFP Registration Desk.

WEDNESDAY MORNING — AUGUST 9, 2000

(S16) **Biosensors and Real-Time Detection Systems—** Chandi Wijey will replace Gerry Crawford.

(S18) **Significance of Mycotoxins in the Global Food Supply** — Tracie Sheehan, Kellogg Company, Battle Creek, MI, USA will speak — **Control of Mycotoxins in the Food Supply: A Food Industry Perspective.**

WEDNESDAY AFTERNOON — AUGUST 9, 2000

(S20) **International Trends in On-Farm Food Safety —** Thomas Quigley and Richard Baines will not be able to attend.

IAFP WISHES TO THANK THE FOLLOWING:

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