IAFP 2002 ABSTRACTS

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Journal Editorial Office

International Association for Food Protection, 6200 Aurora Avenue, Suite 200W, Des Moines, Iowa 50322-2864, USA; Phone 515.276.3344; Fax 515.276.8655; E-mail: bcorron@foodprotection.org

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Poster Abstracts

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P001 Monitoring the Effectiveness of Cleaning in Food Processing Plants

GINNY MOORE and Chris Griffith, University of Wales Institute-Cardiff, Food Research and Consultancy Unit, Colchester Ave., Cardiff, Wales, CF23 9XR, UK

The food production environment is an important factor in determining the safety and quality of the product. Dirty surfaces can harbor both pathogens and food spoilage organisms; thus, the inadequate cleaning and disinfection of these surfaces represents a significant risk factor for contamination. Food companies, therefore, require a simple and rapid method for assessing the effectiveness of their sanitation procedures. Although microbiological testing is considered the "gold standard," no one ideal method exists to determine surface cleanliness. This can lead to difficulties and errors in selecting the most appropriate method for use within different food companies. Visual assessment, ATP bioluminescence, protein detection and conventional microbiology were assessed as a means to evaluate the cleanliness of food contact surfaces in four different high-risk food processing environments. Forty-five different surfaces were sampled using all three hygiene monitoring systems before and after normal cleaning procedures. After cleaning, 90% of the surfaces sampled within the cheese production unit appeared visually clean; however, the use of traditional microbiology revealed that 60% of these surfaces were contaminated with bacteria at levels >2.5 CFU/cm^2. Similarly, despite all appearing visually clean, 67% of the surfaces within the bakery and 80% of the surfaces within the frozen ready-meal plant were deemed unacceptable for food production by use of protein detection and ATP bioluminescence, respectively. The implications of these findings will be discussed in relation to inadequacies within the cleaning process. Additionally, the results will be used to demonstrate how, after consideration of the types of food produced and the nature of the food residues present, visual, non-microbiological and microbiological methods can be used in combination to form an integrated cleaning monitoring strategy.

P002 Comparison of Methods to Improve Sensitivity in a Multiplex PCR Reaction for Detection of *Escherichia coli* O157:H7 in Fresh Produce

MICHAEL A. GRANT, FDA, 22201 23rd Drive SE, Bothell, WA 98021, USA

Current US Food and Drug Administration procedures for detection of *E. coli* O157:H7 in fresh produce involve initial preparation of a 1:1 rinsate using Butterfield's diluent. Components of the produce as well as adhering soil particles may interfere with subsequent tests which use the rinsate as a template for PCR reactions. A study was conducted to determine whether preliminary preparatory procedures, or addition of selected compounds, could minimize such interference. The PCR reaction examined was a multiplex analysis for the universal *E. coli* genes *gad A/B* and two genes coding for production of shiga toxin, stx1 and stx2. Rinsates from 15 varieties of produce were treated by a preliminary centrifugation/clarification step, or by adding Nonfat Dry Milk (NFDM), polyvinylpolypyrrolidone (PVPP), or InstaGene™ to PCR reaction mixtures. Initial centrifugation/clarification and addition of either NFDM or PVPP provided improved detection of diagnostic gel bands with all 15 produce types, when spiked at 10^7/ml. InstaGene-treated preparations yielded the strongest bands after electrophoresis with 11 of the 15 rinsates, but all three diagnostic bands were lost completely in the other 4 rinsates, even after spiking at 10^8/ml. Initial preparation of produce by rinsing was also shown to be superior to homogenization. The multiplex PCR procedure for detection of *E. coli* O157:H7 in produce was an effective method and sensitivity was increased 10 to 100 fold by use of selected reagents to block compounds interfering with the PCR reactions.

P003 Evaluation of COMPASS L. mono, a New Chromogenic Medium for Highly Specific Isolation of *Listeria monocytogenes*

CHRISTOPHE QUIRING, David Miller, and Pierre-Yves Marquet, Biokar Diagnostics-Solabia, 29, rue Delizy, Pantin cedex, 93698, France

A new culture medium, based on a phosphatidylinositol substrate and the chromogen X-glucoside, has been developed for the isolation and enumeration of *Listeria monocytogenes*. Sensitivity and specificity are achieved through detection of β-glucosidase common to the genus and phosphatidylinositol specific phospholipase C (PI-PLC) of pathogenic *Listeria*. An innovative antibiotic mixture inhibits contaminating flora, particularly *Bacillus* spp. and *L. ivanovii*, often associated with false positives using other *Listeria* isolation media. In pure culture testing, 38/38 strains of *L. monocytogenes* produced blue colonies surrounded by an opaque halo within 24 h, while all other *Listeria* strains formed only blue colonies with *L. ivanovii* inhibited. Among 82 non-*Listeria* strains only 4 (3 Staphylococci, 1 *Enterococcus*) grew weakly, producing respectively white and

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blue colonies. Within the context of ISO 11290-1, using PALCAM, Oxford and another commercialized chromogenic agar, COMPASS L. mono detected 100% of the naturally contaminated samples with no false-positives in 42 food samples tested. Additionally, direct recognition of *L. monocytogenes* colonies was possible whereas picking suspected colonies from the aforementioned media often led to unnecessary identification of non-monocytogenes colonies, including *Bacillus cereus*, using the second chromogenic medium. No similar problem was observed using COMPASS L. mono. In conclusion, the use of a judiciously selected mixture of chromogenic substrates and selective agents in COMPASS L. mono allowed detection of *L. monocytogenes* with a remarkably high level of precision, thus reducing time, labor, and overall cost of analysis. These advantages are particularly pertinent when potentially large numbers of unnecessary false-positive examinations can be eliminated.

P004 The Effect of pH and Agitation on the Growth of *Listeria monocytogenes* in Brain Heart Infusion (BHI) Broth Containing Combined Potassium Lactate and Sodium Diacetate Stored at 4°C and 10°C

RUTH A. BARRATT, Ki. S. Yoon, and Richard C. Whiting, University of Maryland Eastern Shore, Dept. of Human Ecology, Backbone Road, Princess Anne, MD 21853, USA

Conditions under which broth media are incubated may affect microbial growth to the extent that growth in broth is a poor predictor of microbial behavior in food, especially after addition of antimicrobial factors. The objective of this study was to compare the effect of pH on the growth kinetics of *Listeria monocytogenes* in static versus agitated broths stored at 4°C and 10°C with or without Optiform 4™ (combination of 1.85% potassium lactate and 0.13% sodium diacetate). The pH of BHI broth containing 0% or 3.3% of Optiform 4™ was adjusted to 5.5, 6.5, and 7.5. 100 *L. monocytogenes* Scott A (ATCC 49594) were inoculated into pH-adjusted broth and stored at 4°C and 10°C with or without agitation. At appropriate times, diluted aliquots of samples from flask were enumerated by spiral plating on tryptose agar, incubating at 37°C for 24 h and counting with an automated colony counter. In both pH 6.5 and 7.5 broths stored at 4°C, agitation decreased lag time and increased the growth rate of *L. monocytogenes*, regardless of Optiform 4™ addition. A similar but less obvious trend was observed in broths stc-ed at 10°C. However, at pH 5.5, a listeriostatic effect of Optiform 4™ was observed at both 4°C and 10°C, despite agitation. These results indicate that pH of the broth media along with agitation strongly affects Optiform 4™ effectiveness on *L. monocytogenes* growth. Future studies will investigate the effect of pH and Optiform 4™ on the behavior of *L. monocytogenes* in ready-to-eat seafood and whether the resulting growth fits more closely to agitated or non-agitated growth curves.

P005 A Comparison of the Microbact System with the Conventional ISO Method and the API Gallery for Identification of *Listeria* Isolates

Marie-Laure Sorin, Sandrine Rougier and PATRICE ARBAULT, Diffchamb SA, 8 rue Saint Jean de Dieu, Lyon, 69007, France

The Microbact 12L *Listeria* identification system relies on conventional tests such as sugar fermentation and esculin hydrolysis, and on micro-haemolysis reaction enabling differentiation of *Listeria monocytogenes* and *Listeria innocua*, without requiring additional CAMP test. This system (24 h incubation) was compared with the conventional ISO 11290 method and the API *Listeria* system for the identification of 50 *Listeria* and 10 non-*Listeria* isolates. Each strain was studied blindly in duplicate. Among the 100 *Listeria* identifications (2 × 50 isolates), the ISO and Microbact methods classified correctly 99 isolates, when the API *Listeria* showed 94 correct results. These misidentifications were noticed with *L. seeligeri* (n=3), *Listeria ivanovii* (n=1) and *L. grayi/murrayi* (n=1) and were due to the absence of sugar fermentation such as arabitol and methyl-D-glucoside. One isolate was contaminated by coccoidal bacteria and so gave a biochemical pattern unknown by the 3 methods. For the 10 non-*Listeria* isolates, the 3 methods showed unknown biochemical and haemolytic patterns, different from *Listeria* species. Furthermore, when incubated only 6 h, the Microbact *Listeria* system gave 93 correct identifications. An extra incubation step of 18 h allowed 98 correct identifications. The study confirmed that the Microbact *Listeria* system gives reliable identification results and offers quick results in most of the analyses.

P006 A Rapid Antibody Specific Method for the Detection of Food Pathogens from Environmental Surfaces Using the RBD2100

KRISTI R. HARKINS, Kelley Harrigan, Lillian M. Erdahl, and Jan M. Tippett, Advanced Analytical Technologies, Inc., 2901 S. Loop Drive, Ames, IA 50010, USA

With a zero tolerance level in place for most food pathogens, a period of 24 to 48 h of enrichment is typically required prior to the use of most rapid technologies available today. This enrichment period allows the target organism to increase to over 100,000 CFU/ml and facilitates recovery of potentially stressed organisms. Polyclonal antibody shown to bind to *Salmonella* spp. was conjugated to .^LEXA 647 (Molecular Probes) and diluted 1:50 in phosphate buffer (PB). Serial dilutions of an overnight *Salmonella* Typhimurium culture were made in Lactose Broth yielding 10^3 to 10^6 CFU/ml. A sample of each dilution was plated for count comparisons. 50 µl of each dilution was mixed with 50 µl of diluted antibody and incubated for 30 min. A negative control of uninoculated medium and antibody solution was also prepared. After incubation, 1 ml of PB was added and the sample analyzed for enumeration of labeled

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salmonellae with the RBD2100. RBD2100 counts ranged from 100-10,000/ml and correlated well with plate counts ($r^2 > 0.99$). These data translate to a lower detection limit of approximately 100 salmonellae in 50 µl of media or approximately 2000 bacteria/ml. The use of fluorochrome-conjugated antibodies for specific pathogen labeling, in conjunction with the laser-based detection and enumeration capabilities of the RBD2100, provides a novel and rapid means of detecting food pathogens in near real-time following brief enrichment. Work will also be presented using this new rapid tagging method to detect *Salmonella* isolated at low levels (1 to 10 CFU/ml) from environmental surfaces (swabs).

P007 Detection of Salmonellae from Poultry by Real-Time PCR

AYSEGUL EYIGOR, Kamil Tayfun Carli, and Can Bora Unal, Uludag University, Faculty of Veterinary Medicine, Dept. of Food Hygiene and Tech., Gorukle Kampusu, Bursa, 16384, Turkey

Detection of *Salmonella* by bacteriological methods is time consuming and may lead to false-negative results. Therefore, we have developed a real-time probe-specific PCR (Light-Cycler, Roche Diagnostics, Mannheim, Germany) to rapidly detect *Salmonella invA* gene based PCR products from chicken feces and carcasses by a fluorescence resonance energy transfer assay. The sensitivity and the specificity of this system was determined as 3 CFU/ml and 100%, respectively. Overnight tetrathionate broth (TTB) enrichment cultures of chicken feces and carcass samples were used in template preparation for PCR. Also, we performed standard bacteriology (NPIP-USDA) tests for confirmation. Sixty-five cloacal swab, 90 intestine, and 20 whole fresh carcass samples were examined. Fourteen and 10 of the intestinal and carcass samples were found to harbor *Salmonella* both by PCR and bacteriology. *Salmonella* PCR product specificity was determined after the analysis of the probes' melting curves, by the use of LightCycler software. Results indicate that this assay has the potential for use in routine monitoring and determination of *Salmonella* in infected flocks and carcasses.

P008 Inactivation of Refrigerator Biofilm Bacteria for Application in the Food Service Environment

BARRY MICHAELS, Troy Ayers, Marlene Celis and Vidhya Gangar, Georgia-Pacific Corporation, P.O. Box 919, Palatka, FL 32178-0919, USA

In food processing, food service and the domestic environment, biofilms have been identified as a problem when growth occurs on refrigerator surfaces. Refrigerator biofilm formation can cause food spoilage and result in foodborne illness outbreaks. Foodborne pathogens such as *Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni, Escherichia coli* O157:H7 and *Salmonella* spp. have been seen to persist on food contact surfaces due to biofilm formation. *L. monocytogenes* has been commonly found on refrigerator surfaces in all 3 food venues. Refrigerator surfaces offer challenges for clean-up and sanitization, while methods for their removal are not commonly understood by food safety managers or the general public. In this study, biofilm cultures of mixed wild strains were started using ground beef and vegetable scraps, suspended in TSB and grown on polycarbonate plastic refrigerator trays. Biofilms were allowed to reach densities of approximately 10^9 CFU per tray test area. Species identified in these cultures included *Pseudomonas putida, Sphingobacterium multivorum, Citrobacter freundii* and *Proteus vulgaris*. A series of different treatment interventions were trialed in an attempt to elucidate the dynamics of biofilm clean-up in refrigerator environments. Results obtained from treatments ranged from a less than 1 $\log_{10}$ reduction for light duty cleaning operations to over a 5 $\log_{10}$ reduction involving more complex treatment methods. The most successful treatments involved removing loose organic soil with paper towels, followed by a 10 to 15 min exposure to either hot soapy water or a high pH (12.0) cleaner, with scrubbing and rinsing followed by acetic acid exposure and drying with paper towels.

P009 Prediction of Raw Produce Surface Area from Weight Measurement

JOSEPH EIFERT, Gabriel Sanglay and Dah-Jye Lee, Virginia Tech., Dept. of Food Science and Tech., Mail Code 0418, Blacksburg, VA 24061, USA

Raw or minimally processed fruits and vegetables are increasingly implicated as a source of microbial pathogens. Often, these microorganisms are found in higher numbers on outer surfaces rather than the interior regions. Microbial populations of intact produce may be more accurately characterized if the entire product surface was sampled, rather than an excised and weighed portion. Furthermore, microbial concentrations could be reported on a per sampled area basis. The objective of this study was to determine a statistical relationship between sample unit surface area and raw weight for selected fruit or vegetable crops. A computer vision technique was developed to achieve this task for objects with irregular shape. An imaging system acquires and stores images of multiple projections of the object. Surface fitting and approximation of a 3-D wire-frame model was used to calculate object surface area. For cantaloupes (n=80, mean weight 923g, S.D. 116), mean surface area measurement was 51,713 sq. mm. (S.D. 580). Measurements of the surface area of cantaloupes were used to develop an equation to predict cantaloupe surface area from a weight measurement. The relationship ($r^2 = 0.76$) between surface area and weight was expressed by: SURFACE AREA (sq. mm.) = 19,702 + [34.7 × WEIGHT (g)]. With this equation, analytical microbiologists could gravimetrically sample whole cantaloupes and enumerate microorganisms per unit surface area

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rather than per sample weight or per rinse volume. This project will lead to improvements in the estimates of pathogen concentrations on produce and consumer exposure to pathogens from ready-to-eat produce consumption.

P010 A Practical Solution to the Problems Associated with Rapid Pathogen Detection

ADRIAN PARTON and Roy Betts, Matrix Microscience Ltd., Lynx Business Park Fordham Road, Newmarket, Cambridge, CB8 7NY, UK

Conventional microbiology, and indeed many so called "rapid" methods, suffer from the delays associated with the need to have pre-enrichment and selective enrichment steps in order to generate sufficient numbers of target organisms before detection takes place. None of the methods currently available utilize all of the sample and typically volumes as low as 1 ml or 0.1 ml are used from sample sizes of 250 ml. Using a very novel high volume Immunomagnetic system (IMS) called Pathatrix, the growth step is reduced to approximately 3 h and removes the need for classical pre-enrichment and selective enrichment steps. The target organisms are simply purified, then either plated directly onto the suitable agar with results being obtained first thing next day, i.e. within 20 h from point of sample, or examined by a computer-controlled fluorescent microscope system that identifies the organism using immunofluorescent viability reagents, so giving a confirmed result within 4.5 h from sample receipt. Studies for *E. coli* O157 and *Salmonella* spp. have shown that detection of 1 CFU/25g is achieved in a variety of food types, e.g. ground beef, chicken meat, dairy products, vegetable and fruit juices. The test method used gave rapid results using practical standard laboratory microbiological techniques, resulting in the production of viable colonies. This system allows food producers the option to progress to a real time positive release system.

P011 DSC Detection of Pathogenic *Yersinia enterocolitica* in Drinking Water and Vegetables by a Mutiplex PCR

T. S. LEE, B. K. Park, and D. H. Oh, Kangwon National University, Div. of Food and BioTech., Chunchon, Kangwon, Korea

The study was conducted to develop a rapid method for the detection of *Yersinia enterocolitica* in drinking water and vegetables via a multiplex polymerase chain reaction (PCR) technique using *ail*, *yst*, *virF* and *Y16S* as markers for pathogenicity. Specificity and sensitivity of multiplex PCR and application of *yst* and *Y16S* primer, which showed best band pattern, for the detection of *Y. enterocolitica* from drinking water and vegetables were investigated. *Ail*, *yst* and *Y16S* primer gave a single 356, 134 and 200 bp specific band for *Yersinia enterocolitica*, but *virF* primer did not show any band. Also, other *Yersinia* spp. and other bacteria did not show any bands with *ail* and *yst* primer except for *Y16S*, which is genus specific primer. In the meantime, only 1 strain showed virulence with *yst* primer among 18 *Y. enterocolitica* isolates from vegetables and 1 strain with ail primer among 32 *Y. enterocolitica* isolates from drinking water. Sensitivity was significantly improved by the further second PCR after first PCR amplication. Drinking water, cabbage and mushroom samples were inoculated with *Y. enterocolitica* at concentrations ranging from 0 to 10^7 CFU/ml or g to determine the most sensitivity of multiplex PCR using *yst* and *Y16S* primer for the rapid detection of *Y. enterocolitica*. As few as 70 CFU/ml for drinking water and 7 CFU/g for lettuce and mushroom were detected in the presence of 2.5 to 7×10^5 other bacteria. This multiplex PCR assay provides a sensitive assay for the detection of virulent *Y. enterocolitica* in vegetables and drinking.

P012 Viability and Morphology Assessment of *Bacillus cereus* Following Exposure to Sanitizers

M. E. Peta, D. Lindsay, V. S. Brözel, and A. VON HOLY, University of the Witwatersrand, School of Molecular and Cell Biology, Private Bag 3 WITS, 2050 Johannesburg, South Africa

Scanning electron microscopy (SEM) and confocal scanning laser microscopy (CSLM) in conjunction with viability staining were used to assess surface morphology and viability of a dairy-associated *Bacillus* (β) *cereus* (DL5) isolate following exposure to five selected commercial sanitizers. Sanitizers containing chlorine dioxide (CD), a twin-chain quaternary ammonium compound (QAC) blend, a peroxyacetic/peroxyoctanoic acid (PPA) combination, a peroxyacetic acid/hydrogen peroxide (PAH) combination and a nitric acid/iodine (NAI) combination were evaluated at double the manufacturers' recommended concentrations against 24-h old planktonic cells. Cells were exposed for 10 min, and CSLM and SEM observations compared. Results with CSLM and SEM showed that control cells not exposed to sanitizers stained predominantly green (live) and showed no morphological irregularities, respectively. SEM revealed that cells exposed to CD and QAC generally exhibited morphological changes such as surface roughness, indentations or elongation. CSLM images also showed that cells treated with CD or QAC stained predominantly red (dead) indicating loss of metabolic activity. Combinations of green (live), yellow-orange (injured) and red (dead) cells were observed by CSLM after exposure to the acid-based sanitizers. Following exposure to PPA, cells stained predominantly yellow-orange in CSLM images. This suggested that PPA, more than other sanitizers, induced injury of bacterial cells. This finding corresponded to less severe morphological damage observed by SEM following exposure to PPA. SEM and CSLM observations suggested that CD and QAC were more effective than PPA, PAH or NAI against planktonic *B. cereus* DL5 cells. The microscopic

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observations of this study broadly corresponded to kill percentages based on plate counts.

P013 *Bacillus cereus* Cell Size Decreases When Exposed to Alkaline pH

D. Lindsay, M. C. Oosthuizen, V. S. Brözel, and A. VON HOLY, University of the Witwatersrand, School of Molecular and Cell Biology, Private Bag 3 WITS, 2050 Johannesburg, South Africa

In a previous study, *Bacillus* strains were isolated from alkaline wash solutions in South African dairies. Upon investigation of their alkaline tolerance using a representative isolate, *B. cereus* DL5, apparent cell size decreases occurred when cells were exposed to alkaline pH. This study investigated changes in cell size of *B. cereus* DL5 as a result of exposure to alkaline pH (7–12) quantitatively by measuring length and width of individual cells followed by volume calculations, scanning electron microscopy (SEM) and confocal scanning laser microscopy (CSLM) coupled to the dead/live Baclight Bacterial Viability Kit TM. Volume calculations showed that cell sizes steadily decreased when *B. cereus* DL5 was exposed to increasingly alkaline pH up to 10. The smallest cell volumes were recorded at pH 10 and were significantly smaller ($P < 0.05$) than those calculated at any other pH. Volumes of cells exposed to pH 10.5 increased and then decreased gradually up to pH 12. CSLM and SEM images of *B. cereus* DL5 confirmed the results of cell volume calculations and smaller cells were observed at pH 10 compared to pH 7, 10.5 or 12. Cells exhibited noticable stress symptoms when exposed to pH 10 (ca. 50% stained red, i.e., dead) than at neutral pH or pH above 10 (stained green – viable, or yellow/orange-injured). Previous studies observed cell elongation for *Bacillus* cells exposed to sub-lethal acid pH. This study suggested that decreases in cell size may have facilitated alkaline tolerance in the neutrophile *B. cereus* DL5.

P014 Improving the Sensitivity of Detecting Bacterial Foodborne Pathogens in Fresh Produce by PCR

CHING-HSING LIAO and Lisa M. Shollenberger, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

We have recently shown that the presence of alfalfa seed components in pre-enrichment broth (BPW) reduces the sensitivity of PCR for detection of *Salmonella* by 100- to 1,000-fold. In this study, we examined the presence of potential PCR inhibitors in fresh produce. Four commercial PCR detection kits, including the BAX® systems for screening *Salmonella*, *E. coli* O157:H7 and *Listeria monocytogenes* and the D2 *Salmonella* PCRSCREEN™ system, were tested. The sensitivity of detecting *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* by these PCR kits was markedly reduced in BPW containing tissue homogenate of alfalfa seed, strawberry, cantaloupe, or Romaine lettuce. The sensitivity was only slightly reduced in the presence of parsley or alfalfa sprout homogenate. The PCR-inhibitory activity of alfalfa seed homogenate was partially eliminated by heating (100°C, 10 min) or dialysis (M.W. cut-off 12,000 Daltons) but completely removed by the use of Dynal, immunomagnetic capturing and separation techniques. The minimal concentration of *Salmonella* in BPW detectable by PCR was estimated to be 10^5 to 10^6 CFU/ml and 10^3 to 10^4 CFU/ml for samples containing or lacking alfalfa seed homogenate, respectively. With the aid of immunomagnetic beads, the minimal concentration of *Salmonella* in BPW detectable by PCR was reduced to 10^1 to 10^2 CFU/ml even in the presence of seed homogenate. Direct application of immunomagnetic beads thus effectively eliminated the PCR-inhibitory activity of produce homogenates and did not appear to interfere with the PCR assays. Several known PCR facilitators (PVPP, skim milk, and ficoll) showed very little or no effect on neutralizing the inhibitory activity.

P015 Comparison of Two Methods for the Detection of *Salmonella* Enteritidis in Shell Eggs

I. E.VALENTÍN-BON, K. H. Seo, R. E. Brackett, T. S. Hammack, and W. Andrews, FDA-CFSAN, 200 C St. SW, HFS-300, Washington, D.C. 20204, USA

The effectiveness of 2 methods for the recovery of *Salmonella* Enteritidis (SE; phage types 4, 8, 13, 13A) from jumbo shell eggs was evaluated. The first method used in the comparison consisted of a pre-enrichment of the sample (1993 Poultry Science 72:1611-1614) and the second was developed by the Animal and Plant Health Inspection Service (APHIS). Three bulk samples of eggs containing 220 liquid whole eggs each were artificially inoculated with high (1×10^3 CFU/ml), medium (100 CFU/ml) and low (10 CFU/ml) populations of SE cells. Twenty subsamples containing approximately 10 eggs each were withdrawn from each of the inoculated bulk samples and incubated for 4 days at room temperature (23°C). For the APHIS method, each pool was cultured by direct plating onto brilliant green (BG) and xylose-lysine desoxycholate (XLD) agars. For the pre-enrichment method, 25g portions, from each pool, were enriched in modified tryptic soy broth with $FeSO_4$, selectively enriched in tetrathionate and Rappaport-Vassiliadis broths and streaked to BG, bismuth sulfite, and XLD plates. SE isolates were confirmed using biochemical and serological tests. In all of the experiments, the pre-enrichment method recovered significantly more SE isolates ($P < 0.05$), from all the phage types, than the APHIS method. From a total of 478 test portions, 332 were SE-positive by the pre-enrichment method and 160 were positive by the APHIS method. The pre-enrichment method provides greater sensitivity for the isolation of SE in contaminated egg slurries.

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P016 Comparison of Four Selective Agar Media for *Campylobacter* Detection from Poultry Samples

Marius Van Eck, Esther Broekmaat, and FLORENCE GORSE, bioMerieux, R and D Industry Laboratory, 69280 Marcy l'Etoile, France

The recovery of thermotolerant *Campylobacter* on agar plate media is one of the critical steps for their detection in food samples and particularly in the presence of an abundant competitive flora. The BAM procedure recommends the use of mCCDA agar while the ISO procedure recommends the use of Karmali agar and a second medium of choice. In this study we evaluated the performance of four selective media for the confirmation of *Campylobacter* from poultry samples. First we compared Karmali agar to Virion agar. The latter medium, which is more selective, allowed a higher recovery of positive samples. Second we compared Campylosel agar (bioMerieux) to mCCDA and Virion agar. Fifty-five samples were positive with at least one confirmation medium, 44 with Campylosel (sensitivity = 80%), 47 with mCCDA (sensitivity = 85.5%), and 40 with Virion agar (sensitivity = 72.7%). The combination of 2 confirmation media increased the recovery of positive results slightly, and the association of Campylosel agar + mCCDA agar is the most effective and Campylosel agar the most complementary medium: Campylosel + mCCDA, 54 positive results (sensitivity = 98.2%); Campylosel + Virion, 53 positive results (sensitivity = 96.4%); and mCCDA + Virion, 51 positive results (sensitivity = 92.7%).

P017 Evaluation of a New Alternative Method for *Campylobacter* Detection in Food Samples

Marius Van Eck, Esther Broekmaat, and FLORENCE GORSE, bioMerieux, R and D Industry Laboratory, 69280 Marcy l'Etoile, France

As *Campylobacter* is now recognized as the most common pathogen responsible for foodborne illness, new simple and rapid methods have to be proposed for its detection as an alternative to the long and fastidious cultural method. In this respect, we have evaluated Vidas *Campylobacter*, a rapid, automated immunoassay method, in comparison with the ISO method. This new method consists of an enrichment for 48h at 42°C in Bolton broth without blood in a Stomacher® bag (Combibag) specially modified to support a microaerobic generator, followed by an immunoenzymatic detection oı, the Vidas instrument. Positive results are confirmed after isolation on two selective media, Campylosel agar (blood agar) and mCCDA (charcoal + deoxycholate agar). One hundred poultry samples (fresh meat, organs and chicken skin) were analyzed by both methods. Enrichment in Bolton broth and incubation under microaerobic conditions developed in the Combibag system allowed a better recovery of *Campylobacter* than enrichment in Preston broth (55 versus 42 positive results). Compared to the ISO method, the complete Vidas method showed a higher sensitivity (84% versus 70%). In addition, the release of negative products is obtained within 48 h versus 3 to 7 days for the standard technique. The use of the Combibag system and simultaneous screening of up to 30 samples per h on the instrument allow a high productivity compared to the traditional method.

P018 DSC Development of Fluorescence Polarization Immunoassay (FPIA) for the Rapid and Quantitative Determination of Herbicide, 2,4-dichlorophenoxyacetic Acid

JI-HUN KIM, Jung-Hyun Park, Yoon-Jung Kim, Sung-Jo Kang, and Duck-Hwa Chung, Gyeongsang National University, 900 Gajwadong, Jinju, Gyeongnam, 660-701, Korea

We have developed a direct competitive homogeneous fluorescence polarization immunoassay (FPIA) for the quantitative determination of herbicide, 2,4-dichorophenoxyacetic acid (2,4-D). For this study, we used four kinds of anti-2,4-D monoclonal antibodies (MAb 2,4-D-121-57, 2,4-D-121-46, 2,4-D-286, 2,4-D-121-69) produced by immunization with 2,4-D-hapten conjugated via carboxyl group to keyhole limpet hemocyanin (KLH) and fluorescence-labeled 2,4-D tracers. For increasing sensitivity and selectivity, several kinds of tracers were used. The fluorescein was modified by introducing an amino function (ethylenediamine fluorescence; EDF) to couple with 2,4-D or several kinds of analogs, and 2,4-D was chemically modified by introducing an amino function to enable coupling with fluorescein isothiocyanate. 2,4-D competitively inhibited the binding of the developed MAbs with synthesized 2,4-D-tracers, such as 2,4-D-EDF, 2,4,5-T-EDF 3,4-D-EDF, 3,4-DPA-EDF, 2,4-DCA-SA-EDF, and 2,4-DCA-FITC, causing a decrease in polarization. FPIA requires only the addition of the fluorescent probe to the antibody, followed by an incubation and measurement of polarization with TDx (Abbot Co.) photo-check mode. 3,4-DPA-EDF and 2,4,5-T-EDF tracers were more sensitive than other conjugates. MAb 2, 4-D-121-57 and 121-46 reacted specifically with 2,4-D but cross reacted somewhat with its analogs instead of methylester 2,4-D (68%) and 4-chloro-o-tolyoxyl acetic acid (65%). When 2,4-D was spiked in water at different concentrations, the average recovery ratio was 100+2%. The best dose-response curve presented in this study was almost linear in the concentration range 50 to 1,000 ng/ml. This immunoassay is a potentially valuable analytical tool for the rapid and sensitive determination of 2,4-D from various samples.

P019 Automated Measurements of AntiListerial Activities of Lactate and Diacetate in Ready-to-eat Meat

EVELYNE MBANDI and Leora A. Shelef, Wayne State University, Dept. of Nutrition and Food Science, Detroit, MI 48202, USA

Standard procedures to enumerate *Listeria* spp. rely on plating food samples, using selective agar media. The procedures are labor-intensive. Moreover,

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because of their limited sensitivity, a pre-enrichment step is required for the detection of low numbers of the pathogen. In the present study, an automated rapid optic procedure using the BioSys instrument (MicroSys Inc., Ann Arbor, MI) and the standard procedure were used simultaneously to determine the behavior of the pathogen in ready-to-eat meat and to test the effect of antiListerial agents. *Listeria monocytogenes* strain Scott A or a six-strain mixture of *Listeria* was studied using lactate (2.5%), diacetate (0.2%), and their combination in beef bologna and in sterile beef emulsion. Samples were tested at time intervals during storage at 5 and 10°C for up to 60 days. Numbers of *Listeria* increased from 3 to over 7 log CFU/g after 45 days at 5°C and after 20 days at 10°C. Each of the salts caused a delay in growth of the pathogen, and the salt combination was most effective, causing listeriostatic effects and decline in growth of the pathogen at 5°C. High negative correlation (r), ranging from 0.92 to 0.99, was obtained between detection time (DT) recorded by the optic procedure and cell numbers determined by the plate count procedure. The rapid (< 24h) optic procedure was reliable in assessing the efficacy of the antimicrobials, and in rapid detection of low levels of Listeriae that were undetectable by the direct plating procedure.

P020 A Comparison of Vidas *Listeria monocytogenes* II with the EN ISO 11290-1 Method for the Detection of *Listeria monocytogenes* in Food Samples

Stéphanie Souchon, Carole Ragot, Christine Cullafroz, and JEAN-MICHEL PRADEL, bioMerieux, R and D Industry Laboratory, 69280 Marcy l'Etoile, France

Listeria monocytogenes is the only *Listeria* species involved in human foodborne outbreaks and there is a need for a rapid and direct method for its detection. The purpose of this study was to evaluate the performance of a new automated immunoassay method, Vidas *Listeria monocytogenes*, developed to address this need. The method comprises a pre-enrichment in half Fraser broth for 24 h at 30°C followed by an enrichment in Fraser broth for 24 h at 30°C, before Vidas testing. The immunoassay showed no cross reactivity when tested against 54 strains from other *Listeria* species or other bacterial genera. The detection limit was estimated to be between 5×10^4 and 5×10^5 CFU/ml. A total of 203 foods samples (meat, dairy, fish and seafood, and vegetables) were analyzed both with the ISO method and the new method. Viable *L. monocytogenes* were recovered from 45 samples with the automated method and from 36 samples with the traditional method. Sensitivity was 100% and 80%, respectively. The percentage of agreement between the 2 methods was 95.6% and the specificity of the immunoassay was 98.1%. In conclusion, the new method gave sensitive and accurate results. Compared to the ISO culture method, it gave a negative result and a presumptive positive result at least 1 day earlier. The ready-to-use unitary reagent strips as well as simultaneous processing of up to 30 samples per h make this method well suited both for unit testing needs and high throughput needs.

P021 DSC Characterization of *Staphylococcus aureus* Isolated from Stock Farms in Korea Using the Polymerase Chain Reaction and Random Amplification Polymorphic DNA Analysis

KWANG-SOO HA, Seon-Ja Park, Ann F. Draughon, and Duck-Hwa Chung, Gyeongsang National University, 900 Gajwadong, jinju, Gyeongnam 660-701, Korea

To screen the incidence of exotoxigenic *Staphylococcus aureus* strains, which pose a significant threat to human and animal health and dairy product safety, a polymerase chain reaction (PCR) assay and random amplification polymorphic DNA (RAPD) analysis were performed. Ninety-six samples were collected from three stock farms in Gyeongnam Province of Korea in 2001. *S. aureus* was detected using the FDA isolation method. Isolated strains were confirmed as to predominant gene type by PCR with oligonucleotide primers, which were encoding of the enterotoxin B gene (*seb*), exfoliative toxin gene (*eta* and *etb*) and the toxic shock syndrome toxin-1gene (*tst*). The results showed that 63 samples (66%) were contaminated by *S. aureus*. Each primer pair successfully amplified its target gene in DNA from well-characterized positive controls but did not generate nonspecific amplification of DNA from a nontoxigenic *S. aureus*. Sixty-three isolated *S. aureus* possessed gene sequences coding for *seb* (49.2%), *eta* (41.2%), *etb* (82.5%) or *tst* (15.8%) genes. Among them, 3 strains had no toxigenic genes, 19 strains had one toxigenic gene and the other strains were shown to have two or four kinds of toxigenic genes simultaneously. In the RAPD assay, the combination of primer 23 and 25 had a homology of 36% and the lowest clusters. The results suggest that PCR may be an effective screening method for the rapid, sensitive, specific detection in the genetic typing of *S. aureus* isolates.

P022 Rapid Detection of *Campylobacter jejuni* on Chicken Carcasses Using PCR-based Fluorescent Method

HONG WANG, Yanbin Li, and Michael F. Slavik, University of Arkansas, POSC O-320, Fayetteville, AR 72701, USA

Campylobacter jejuni is reported to be the leading cause of bacterial foodborne diarrheal disease in the United States. Eating or handling contaminated poultry products are important risk factors associated with human infection. A DNA binding fluorescence method based on polymerase chain reaction (PCR) products was evaluated for rapid detection of *C. jejuni* in poultry products. Wash water samples of chicken carcasses and ground turkey were inoculated with *C. jejuni* to obtain final concentrations of 5 to 50,000,000 million CFU/ml. One ml of each sample was used to get the DNA template and 5 ml of the sample template was added to 25 ml of SYBR Green PCR Master Mix and two specific *C. jejuni* gene

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primers (portion of Cj0414 gene). The reaction was carried out in a thermocycler. Finally, the fluorescence signal of each PCR product was measured using a fluorometer. Results indicate that this test could detect as few as 50 CFU/ml of *C. jejuni*. In addition, the PCR-based fluorescence method could detect the target bacteria in min after PCR amplification compared to hours by gel electrophoresis and also could be done at an earlier time during PCR amplification. The detection limit of this method for *C. jejuni* in the poultry samples was 50 CFU/ml without any enrichment. The method is in the process of being evaluated using chicken carcasses naturally contaminated with *C. jejuni*.

P023 Detection of Verocytotoxigenic *Escherichia coli* by Use of a PCR/DNA Probe Membrane-based Colorimetric Detection Assay

JUSTINE FITZMAURICE, Geraldine Duffy, Maura Glennon, Terry Smith, Cyril Carroll, Majella Maher, National Diagnostics Centre, BioResearch Ireland, National University of Ireland, Galway, Ireland

A number of enrichment broths and growth conditions for *E. coli* O157:H7 were investigated, namely growth in brain heart infusion broth, *E. coli* broth + novobiocin (0.02g/L) and modified tryptone soya broth + novobiocin (0.02 g/L) at 37°C and 41.5°C and for enrichment periods of 16, 18, 20 and 24 h. *E. coli* broth with novobiocin and an incubation temperature of 41.5°C for 16 h was chosen for the isolation of *E. coli* O157:H7 in minced beef. Two methods for the capture of bacteria from enrichment broths for DNA extraction, immunomagnetic separation, and a membrane attachment technique were evaluated, and the DNA was analyzed in a multiplex PCR with post PCR/DNA probe colorimetric membrane detection of PCR products. The multiplex PCR (Paton and Paton, 1998) was performed, using biotinylated PCR primers to amplify regions of *Vt* 1, *Vt* 2, *Eae*, *Hly* A and O157 genes. Specific DNA probes were designed for each of these genes and were incorporated into a colorimetric reverse hybridisation membrane based detection format. The colorimetric assay technology offers an alternative to gel electrophoresis and has the advantage of being able to differentiate PCR products of similar size and simultaneously identify the presence of a number of genes. The mean detection limit of the PCR/DNA probe colorimetric membrane based assay for DNA extracted following IMS capture was $\log_{10}$ 5.4 CFU/g, while a detection limit of $\log_{10}$ 6.5 CFU/g was achieved with DNA extracted following capture of the bacteria on a specialized membrane.

P024 Evaluation of MIST Alert™ in Paralytic Shellfish Poison Testing of Clams and Molluscs

B. H. HIMELBLOOM, University of Alaska-Fairbanks, Fishery Industrial Tech. Center, 118 Trident Way, Kodiak, AK 99615-7401, USA

Incidences of paralytic shellfish poison (PSP) are associated with harmful algal blooms occurring in the waters offshore of many countries. Shellfish testing for saxitoxin requires the standard mouse bioassay (SMB) or sophisticated techniques such as high performance liquid chromatography and tissue culture assays. The objective was to conduct a comparative study between the new antibody-based Maritime In-Vitro Shellfish Toxin (MIST) Alert™ and the SMB for clams and molluscs collected at recreational harvesting sites. Ninety-seven samples of several types of molluscan shellfish were collected from five locations in the Kodiak Archipelago during the summer of 2000. Shucked samples were ground, divided and prepared for testing using the MIST Alert. The remaining sample pairs were frozen and sent to the state's testing lab for the SMB. The MIST Alert detected toxin in 72 samples, and the relative levels of toxicity paralleled a trend of increasing saxitoxin levels using the SMB. The 25 samples that contained none to very low levels of saxitoxin corresponded to non-toxic levels (<40 μg/100 g of shellfish tissue) by the SMB. All sampling sites had toxin-positive shellfish, with butter clams and blue mussels comprising the highest percentage of toxin-containing species. The MIST Alert detected a sharp increase in toxin contamination, confirmed quantitatively using the SMB. This event occurred after a heavy rainfall following a sunny dry period during August. The MIST Alert could serve as an early warning field test indicating that unsafe levels of PSP are present in molluscan shellfish.

P025 DSC Efficacy of a Unique Quaternary/Peroxide Foaming Sanitizer against Spoilage and Pathogenic Foodborne Microorganisms

J. M. BIEKER, H. Thippareddi, R. K. Phebus, C. L. Kastner, and A. L. Reicks, Kansas State University, 139 Call Hall, Manhattan, KS 66505, USA

The efficacy of a unique quaternary/peroxide foaming sanitizer developed by Sandia National Laboratories against suspensions of spoilage and pathogenic foodborne microorganisms was evaluated. Five-strain cocktails of *Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157:H7, and *Staphylococcus aureus* were used in a tube-dilution method. Three replicates were performed, each using a control (no contact with the foaming sanitizer), a zero-min contact time (immediately analyzed), and a 15 min contact time with the sanitizer for each of the strain mixtures. An 8-log (complete) reduction for both the zero-min and 15 min treatments for five-strain cocktails of *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus* was achieved. In the second part of the study, Minimal Bactericidal Concentration (MBC) for individual strains of *Salmonella* spp., *L. monocytogenes*, *E. coli* O157H7, *S. aureus*, and three strains of *Pseudomonas* spp. were determined using a microtiter plate method. Serial dilutions of the foaming sanitizer were prepared in tryptic soy broth and inoculated with each strain, separately. The foaming sanitizer was bactericidal at levels of 0.16% for two and three strains of *Salmonella* spp. and *Pseudomonas* spp., respectively. The foaming sanitizer was bactericidal at levels of 0.032% for two

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strains of *Salmonella* spp. and one strain of *S. aureus*. The foaming sanitizer was bactericidal at levels of 0.0064% for the remainder of the microorganisms tested. The foaming sanitizer is effective against common foodborne pathogens and spoilage organisms and therefore will be a useful sanitizer for the food industry.

P026 Rapid Detection of Microorganisms in Aseptic Products Using an ATP Bioluminescent System

TOSHINORI IGARASHI and Seiji Murakami, Kikkoman Corporation Research and Development Div., 399 Noda, Noda, Chiba Pref. 278-0037, Japan

In food production plants, such as plants that produce milk-based products, the ability to achieve aseptic conditions is desired for high quality products and for food safety. Present methods used to detect bacteria include traditional culture methods that take over 48 h. Attempts have been made to use ATP assays but sensitivity and reproducibility were not satisfactory. We have made many advances in ATP technology, including several types of mutant firefly luciferase (heat-resistant and surfactant tolerant) and an ATP degradation system that reduces extracellular ATP. Now we have combined certain of these advances to yield a rapid method to monitor aseptic conditions in milk-based products. We used three bacteria strains of *Pseudomonas fluorescens*, *Bacillus mycoides* and *Klebsiella pneumoniae*, which were isolated from non-sterile products. Each of these bacteria was cultured in dairy creams or cocoa drink for 24 h at 37°C and then an aliquot of each cultured sample was assayed using our new ATP bioluminescent kit and the traditional plating method. Using our new ATP kit, the detection limits of *P. fluorescens*, *B. mycoides* and *K. pneumoniae* were 2×10^4, 1×10^3, 5×10^3 CFU/ml, respectively. This means that an ATP assay can be used as a rapid measure of aseptic conditions, shortening the sterility test by 2 to 3 days, because our new ATP assay kit eliminates almost all non-bacterial ATP and achieves higher sensitivity than current ATP kits.

P027 Rapid Detection of Coliforms Using a Sensitive Bioluminescence Assay

HIROKI TATSUMI and Satoshi Fukuda, Kikkoman Corporation Research and Development Div., 399 Noda, Noda, Chiba Pref. 278-0037, Japan

Coliforms are the indicator bacteria to monitor cleanliness in food and food production plants. Traditional plate methods to detect coliforms take over 18 h. More rapid methods are desired to distribute fresh products or to save storage costs. We have made many advances in ATP technology, including several types of mutant firefly luciferases (heat resistant, surfactant tolerant). We have combined the advances with a synthetic luminescent substrate, D-luciferin-O-beta-galactopyranoside, to yield a rapid detection method for coliforms. To evaluate the rapidness of this new assay system (CheckLite™ CT), we conducted an inoculation test. Five to ten CFU of *Escherichia coli* IAM12119, *Enterobacter cloacae* IAM12349, *Klebsiella pneumoniae* NISL4308 and *Citrobacter freundii* JCM1657 were each inoculated into 50 ml of water. Each sample, after mixing with Pro-media XM-50 (sterile dehydrated medium for coliforms, Elmex, Tokyo), was incubated at 37°C. One ml of each culture was collected after 6, 7, 8 and 9 h, followed by the detection procedure for CheckLite™ CT. *E. coli* and *E. cloacae* were detected in 6 h, while *K. pneumoniae* and *C. freundii* were detected in 7 and 8 h, respectively. Above results demonstrate that our new system, CheckLite™ CT, is one of the most rapid methods for coliform detection.

P028 Evaluation of a Rapid Detection Method for *Listeria* Species in Meat Products Following the USDA/FSIS Enrichment Protocol

CHARLES CARVER and Jean Michel Pradel, rtech laboratories, P.O. Box 64101, St. Paul, MN 55164-0101, USA

The current USDA/FSIS Microbiology Laboratory Guidebook specifies that rapid methods for the detection of *Listeria* species utilize a primary enrichment in UVM broth, followed by a secondary enrichment in Fraser broth (FB), to be considered an acceptable alternative to the USDA/FSIS reference method. The purpose of this study was to determine the effectiveness of the bioMérieux VIDAS LIS *Listeria* assay following the USDA/FSIS enrichment protocol when compared to the USDA/FSIS reference method. The study consisted of naturally contaminated and inoculated product testing. Products tested for natural contamination included raw and cooked beef products, poultry and sausage-type products. Sixty product lots were tested. The inoculated products were cooked roast beef and raw ground turkey. Seventeen replicates of each were tested, of which 15 were inoculated at a target of 0.04 to 0.2 CFU/g and two remained uninoculated. All samples were enriched in UVM enrichment broth and then transferred to FB without ferric ammonium citrate to be tested via the LIS assay. For raw poultry products, the transfer amount was 1.0 ml. For all other products, the transfer amount was 0.1 ml. UVM-enriched samples were also tested via the USDA/FSIS reference method. All presumptive positive samples were confirmed by the USDA/FSIS method. Method agreement was calculated to be 93% over all products tested. Sensitivity of the assay was determined to be 90%, and specificity was calculated to be 96%. These results suggest the UVM / FB enrichment protocol is an acceptable enrichment scheme for this assay.

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P029 The Effectiveness of Sanitizers to *Escherichia coli* O157:H7 Biofilms with *Micrococcus* Species

D. K. JEONG and J. S. Lee, Kosin University, Dept. of Food and Nutrition, Busan 606-701, Republic of Korea

The effectiveness of the following four sanitizers, peroxyoctanoic acid mixture (PAM), peroxyacetic acid (PA), alkyldimethyl benzyl ammonium chloride (ABAC), and sodium hypochlorite (SH), was studied in mixed culture biofilms of *Escherichia coli* O157:H7 and *Micrococcus* species on stainless steel coupons. *Micrococcus* spp. was isolated from the environment of a meat plant and used for this study. *E. coli* O157:H7 and *Micrococcus* spp. were inoculated into 0.2% and 1% tryptic soy broth with coupons and incubated at 21°C. Stainless steel coupons were transferred to fresh media every 48 h to produce biofilm until day 6. Coupons were removed after day 6 and immersed in solution of PAM, PA, ABAC and/or SH with concentration of 0, 0.1, 0.2, 0.4, and 0.8% for 1 or 5 min. Biofilm with a single culture of *E. coli* was also studied as a control group. Surviving cells in the biofilm coupon were detached with a sterile teflon scraper and enumerated using tryptic soy agar for total counts and violet red bile agar for *E. coli* counts. Microorganisms in mixed culture biofilm showed more resistance than those in single culture biofilm at most sanitizer treatments. PAM was most effective against *E. coli* among four treated sanitizers. *E. coli* in a single culture biofilm was totally destroyed at 0.4% and 0.8% PAM in 1 min and at 0.2, 0.4, and 0.8% PAM in 5 min. However, *E. coli* in a mixed culture biofilm was not totally destroyed until PAM concentration reached 0.8% at both 1 and 5 min.

P030 Independent Laboratory Evaluation of a New Rapid Method for the Detection of *Listeria monocytogenes* in Various Meats

CHARLES CARVER and Jean Michel Pradel, rtech laboratories, P.O. Box 64101, St. Paul, MN 55164-0101, USA

The current USDA/FSIS Microbiology Laboratory Guidebook specifies that rapid methods for the detection of *Listeria monocytogenes* must follow an enrichment scheme consisting of primary enrichment in UVM broth, followed by secondary enrichment in Fraser broth (FB), to be considered an acceptable alternative to the USDA/FSIS reference method. The primary purpose of this study was to determine the effectiveness of the bioMérieux VIDAS *Listeria monocytogenes* 2nd generation (LMO2) assay following the USDA/FSIS enrichment protocol. The study was divided into naturally contaminated product testing and inoculated product testing. In the naturally contaminated product portion of the study, 60 samples were tested over a wide variety of meats including beef, sausage-type meats, and raw poultry. The products were tested via three enrichment schemes including UVM and demi-Fraser broth primary enrichments and volume transfers of 0.1 and 1.0 ml to a secondary enrichment of FB minus ferric ammonium citrate. Cooked roast beef and raw ground turkey were tested in the inoculated portion of the study. Samples were divided into 17 replicates, of which 15 were inoculated at a target of 0.04 to 0.2 CFU/g and 2 remained uninoculated. All samples were enriched as listed above, and all presumptive positive samples were confirmed by the reference method. The data shows overall 94% method agreement, 94% sensitivity, and 100% specificity for inoculated samples. Naturally contaminated samples show 97% method agreement, 87% sensitivity, and 96% specificity for the assay. These results suggest that the UVM/FB enrichment protocol is an acceptable enrichment protocol for this assay.

P031 Independent Laboratory Evaluation of a New Rapid Method for the Detection of *Listeria monocytogenes* in Vegetables and Seafood

CHARLES CARVER and Jean Michel Pradel, rtech laboratories, P.O. Box 64101, St. Paul, MN 55164-0101, USA

The purpose of this study was to provide an independent performance evaluation of the bioMérieux VIDAS *Listeria monocytogenes* 2nd generation (LMO2) assay in terms of sensitivity and specificity when compared to the FDA-BAM reference method for vegetables and seafood. The study was divided into naturally contaminated product testing and inoculated product testing. In the naturally contaminated product portion of the study, 40 samples were tested over a variety of seafood and vegetable products. The samples were tested via demi-Fraser broth primary enrichment followed by a volume transfer of 1.0 mL to a secondary Fraser broth (FB) enrichment. All presumptive positive samples were confirmed via the FDA-BAM method. The inoculated product portion of the study focused on raw peeled shrimp and cauliflower. Samples were divided into 34 replicates, of which 30 were inoculated with *Listeria monocytogenes* at a target of 0.04 to 0.2 CFU/g and 4 remained uninoculated. 17 samples were initially enriched in demi-Fraser broth and transferred to a secondary enrichment of FB minus ferric ammonium citrate before being tested by the assay. The other 17 samples were tested via the FDA-BAM method. All presumptive positive samples were confirmed via the FDA-BAM method. The data showed overall 81% method agreement, 100% sensitivity, and 90% specificity for inoculated samples. Naturally contaminated sample data showed 90% method agreement for the assay. This data suggests that the assay is comparable to the FDA-BAM reference method for detecting *Listeria monocytogenes*.

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P032 Protein Profile Changes in *Listeria monocytogenes* after Sub-lethal High Pressure Processing

NICOLE MAKS, Sadhana Ravishankar, Claudia Rodriguez, and Peter J. Slade, Illinois Institute of Technology, National Center for Food Safety and Tech., 6502 S. Archer Road, Summit-Argo, IL 60501, USA

Sub-lethal high pressure processing (HPP) has been used as a model "minimal" stress process. Differences were observed in the 2-D gel protein profiles of treated and non-treated cells in a model food system. *Listeria monocytogenes*, strains Scott A and V7, were grown in tryptic soy broth (TSB) under the following conditions: pH 6.0 at 37°C, pH 7.0 at 37°C, pH 6.0 at 7°C, and pH 7.0 at 7°C. Cultures were centrifuged and the pellets swabbed onto the surfaces of commercial hot dogs. Hot dogs were bagged, sealed and processed by high-pressure at 27 kpsi for 30 min at ambient temperature using a QFP-6 High Pressure Food Processor (ABB Autoclave Systems, Inc.). After processing, the bacteria were rinsed off the hot dogs and centrifuged, and the pellet was washed twice. The pellet was resuspended in an extraction buffer and sonicated to extract protein. Two-dimensional PAGE was performed. Two control treatments, inoculated unprocessed hot dogs and culture only, were also examined for each strain and growth condition. All 2-D PAGE profiles of proteins from the high pressure processed *Listeria* and controls were compared by software analysis. There were no differences in the protein profiles from the two controls. There are distinct strain differences under each growth condition. Moreover, variations in protein patterns were detected under different growth conditions within the same strain. The differences described above indicate that changes in protein constituents are taking place in the bacteria as a result of a response to the lower growth temperature and high pressure processing.

P033 3M™ Petrifilm™ Staph Express Count Plate for the Rapid Enumeration of *Staphyloccocus aureus* in Foods

BARBARA HORTER and Muriel Moreau, 3M Microbiology Products, 3M Center, 260-6B-01, St. Paul, MN 55144-1000, USA

Accurate and rapid detection of *Staphylococcus aureus* is of significant interest to the food industry. Enumeration of this bacterium is often used as an indication of food quality and safety. The 3M™, Petrifilm™ Staph Express Count plate has been developed in order to provide a quantitative *S. aureus* result from food within 22 to 29 h. The performance of the method was demonstrated by comparing the counts from 120 foods using both the Petrifilm plate and the reference method of Baird-Parker agar followed by a tube coagulase test of selected colonies. Analysis of variance showed that there was not a statistical difference between the two methods (P value = 0.3). These results suggest that the Petrifilm plate method gives similar quantitative results in approximately one-third the time of the Baird-Parker agar method using rabbit plasma coagulase.

P034 DSC Analysis of mRNA as a Marker for Viability of *Campylobacter* spp. by RT-PCR

KIDON SUNG, Kelli L. Hiett, and Norman J. Stern, University of Georgia, Dept. of Food Science and Tech., Athens, GA 30602, USA

Many methods have been developed for the detection of foodborne pathogens by targeting conserved DNA sequences for amplification. However, these assays can result in false-positive results because of the amplification of DNA from nonviable cells. Therefore, an alternative assay for detection of bacterial cells that differentiates between living and dead cells is needed. Most messenger RNA (mRNA) of bacteria has a very short half-life and its presence would indicate the presence of a recently viable cell. A reverse transcriptase polymerase chain reaction (RT-PCR) technique was developed for the detection of mRNA from *fla*, *tkt*, and *momp* genes in *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* cells killed by heat. Total RNA was purified by using RNeasy Mini kit (Qiagen Inc. Valencia, CA), and following RNase-free DNase I treatment, the RNA was amplified by both RT-PCR and PCR with primers specific for the three genes. mRNA from all three genes was amplified immediately after the cells had been inactivated by heating at 72°C for 5 min but showed a negative signal after 4 h when the cells were held at 37°C. These results show an amplification of mRNA by RT-PCR method has potential as an indicator of viability in *Campylobacter* spp. cells. Cell death under all heat treatment conditions was confirmed by a failure to grow in Bolton's enrichment broth during incubation for 48 h at 42°C.

P035 Microwave vs. Dry Ash Digestion as Used as a Precursor in the Mineral Analysis by Inductively Coupled Plasma Emission Spectroscopy of Infant Formula

Wai Yip, EUGENE P. WOLKOW, Michael Iorsh, and Mohammed R. Islam, FDA, 158-15 Liberty Ave., Jamaica, NY 11433, USA

Comparisons were made of two methods of digesting baby formula to determine its mineral composition. One was the more traditional Dry Ashing Method (DA) and the other the Microwave Digestion Method (MD). Six formulas were taken for analysis: (1) powder with milk or (2) soy base, (3) concentrate with milk or (4) soy base and (5)"Ready to Feed" liquid with milk or (6) soy base. All samples were analyzed for the minerals Ca, P, Mg, Fe, Zn, Cu, Na and K. All samples were spiked with these minerals and their recoveries, after digestion, were noted. Standard Reference Materials (SRM) were run along with these formulae and their recoveries were noted. Calculated values of the mineral compositions

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of these infant formulae, in parts per million (ppm), were made on the basis of physical data supplied by the companies and the listings on their labels. The predigestion step in MD was modified by running an open cell digestion program followed by a more traditional closed cell program for digestion. Inductively Coupled Plasma Emission Spectroscopy (ICP) was the method of choice for determination of the minerals because of its extended linear range, which permitted a large variance of concentrations without dilutions. Results were compared between the DA and MD methods. Both methods gave analytically acceptable results with some differences noted. The MD procedure required about seven steps and a total of 3 h. The DA procedure took 10 steps and a total of 3 days. MD gives slightly higher values of minerals than DA. The sodium value of powdered soy in the labeling seems too high, as confirmed both by MD and DA. The percent relative standard deviation (%RSD) was lower for MD than DA in the case of the mineral compositions, SRMs and spiking determinations.

P036 Detection of Naturally Occurring *Campylobacter* in Poultry Rinses by Capacitance Monitoring

ERIC LINE, USDA-ARS-RRC, Poultry Microbiological Safety Research Unit, P. O. Box 5677, Athens, GA 30604-5677, USA

A conductimetric method utilizing a new selective enrichment broth, Campy-Line Broth + arginine (CLB+ arg), was evaluated for detection and quantification of *Campylobacter* spp. from poultry carcass rinses. Fresh poultry carcasses (n = 8, post-chill, post-drip) were obtained for each of 6 separate trials and rinsed in buffered peptone water. Module wells containing CLB+arg (2× concentrated) were inoculated with rinse samples for detection of *Campylobacter* presence by the Bactometer system; each sample was also directly plated onto Campy-Line agar (CLA) for enumeration of *Campylobacter*. By monitoring changes in the capacitance signal from the wells, *Campylobacter* was detected in 100% of the naturally contaminated carcass rinse samples. Samples with higher *Campylobacter* populations exhibited shorter detection times (DT); thus, a negative correlation was observed between (DT) and number of *Campylobacter* enumerated by plate count. DTs ranged from about 45 h down to about 15 h for rinses found to contain between about 2 and 1448 *Campylobacter* CFU/ml, respectively. The correlation coefficient between DT and plate count results was significant; however, some variability was observed. In samples removed from module wells and examined by phase contrast microscopy, only low numbers of contaminants were present. CLA plates prepared from samples removed from each module well (after DTs registered) indicated *Campylobacter* was the predominant organism causing the significant conductimetric changes. CLB+arg may be useful for rapid detection of *Campylobacter* in poultry rinse samples; however, the variability in DTs observed would make accurate enumeration by this conductimetric method difficult.

P037 Determination of *Listeria* Attachment Using a Polystyrene Culture Tube Method

NURDAN A. KOCAOGLU-VURMA and Hua Wang, The Ohio State University, Parker Food Science and Tech. Bldg., 2015 Fyffe Road, Columbus, OH 43210, USA

To compare attachment abilities of *Listeria monocytogenes* strains to abiotic surfaces, we established a rapid method using polystyrene culture tubes with crystal violet (T-CV) staining. Two milliliters of cultures were used in the T-CV method to minimize handling variables. Three *L. monocytogenes* strains, namely California, V7, and Scott A, were used in the study and a direct plate count (DPC) method was used to establish the feasibility of the T-CV method. One percent of overnight cultures were inoculated into TSB and incubated at 37°C for 1 and 3 days. For the T-CV method, bacterial cells were stained with 0.1% of CV followed by washing with water. The stains retained by the attached cells were quantified by dissolving in ethanol followed by measuring absorbance at 600 nm. The attachment was measured indirectly by the absorbance reading. The absorbance differences for the 1-day cultures among the three strains were minimum. For the 3-day cultures, the absorbance was significantly higher for V7 and Scott A than for California, suggesting a better attachment of V7 and Scott A than California. For the DPC method, attached cells were vortexed with glass beads in peptone water, and cell numbers were determined on TSA plates. In agreement with the T-CV method, the differences on attachment for 1-day cultures were minimum; for 3-day cultures, attachments of V7 and Scott A by the DPC method were also higher than that of California. Using the T-CV method, the influence of bacterial media on Listerial attachment was also compared. All *Listeria* strains have better attachment in TSB than in MRS.

P038 *Campylobacter jejuni* Transformation Frequency Declines during Log Phase in Liquid Culture

DAVID WILSON, Julia Bell, Linda Mansfield, and John Linz, Michigan State University, National Food Safety and Toxicology Center, East Lansing, MI 48824, USA

Campylobacter jejuni is the leading cause of foodborne bacterial gastroenteritis in the United States. The species is characterized by a high degree of antigenic diversity and intraspecies genetic recombination. *C. jejuni* is naturally competent for genetic transformation, offering one mechanism for generating the organism's observed diversity. Natural transformation is a potential mechanism for acquiring antimicrobial resistance, virulence factors, and a variety of antigenic determinants that may impact

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vaccine efficacy. However, the process is poorly understood in *C. jejuni* with regard to efficiency, specificity and molecular mechanisms. To better understand the process of natural transformation, our laboratory has generated ciprofloxacin resistant mutants of *C. jejuni* and used the chromosomal DNA of these mutants to assess the competence of susceptible isolates. *C. jejuni* cultures were incubated in liquid medium with shaking under microaerophilic conditions; cell density increased approximately 10,000 fold. At several time points during growth, aliquots of culture were mixed with chromosomal DNA encoding ciprofloxacin resistance, and after allowing time for DNA uptake and expression, the transformation mixtures were plated onto selective medium. In general our data indicate that transformation frequency is relatively high (1×10^{-3} to 1×10^{-4}) at the early time points of growth but declines over time. Transformation frequency in *C. jejuni* 33560 decreases approximately 10,000 fold in our growth system while the declines in *C. jejuni* 81176 (approximately 1,000 fold) and *C. jejuni* 3130 (approximately 100 fold) also appear to be substantial. Dilution of mid-log culture into fresh medium effectively reverses this trend. The results suggest competence in *C. jejuni* may be cell density and/or stress related.

P039 Membrane Filtration as Part of Sample Treatment for Improved Pathogen Detection

TONG-JEN FU and Olif M. VanPelt, FDA, National Center for Food Safety and Tech., 6502 South Archer Road, Summit-Argo, IL 60501, USA

Water rinsing has been shown to be effective for the recovery of pathogens from contaminated produce. Existing rapid tests are capable of detecting low levels of pathogens in rinse water. One advantage of using water rinsing for pathogen recovery is that further concentration of pathogens can be easily achieved. Membrane filtration (MF) has been routinely used for the concentration of microorganisms. Integration of MF as part of sample treatment for rapid tests would allow large volumes of rinse water to be analyzed with a single test and thus reduce the cost of testing. This study examined the use of MF for improved detection of pathogens in lettuce rinse water and spent sprout irrigation water. Lettuce inoculated with 0, 1, and 10 CFU of *E. coli* O157:H7 per sample was rinsed with sterile tap water. The rinse water was filtered through standard MF filters. The filters were incubated in enrichment media and the presence of *E. coli* O157:H7 was determined using Neogen's Reveal™ tests. The results showed that integration of MF would improve the detection by 10 fold to as low as 1 CFU/250 g and reduce media use by 100 fold. Similarly, irrigation water collected during sprouting was inoculated with *E. coli* O157:H7 as well as *Salmonella* and was filtered through the MF filters. The presence of the pathogens on the filters was determined using the Reveal™ test for *E. coli* O157:H7 and the Assurance Gold™ EIA for *Salmonella*. Although filtration would result in a detection limit as low as 1 CFU/200 ml and reduce media use by 10 to 100 fold, further improvement was hampered by the limited filterability of sprout water.

P040 DSC Influence of Extended Acid Stressing in Fresh Beef Decontamination Fluids on Sanitizer Inactivation of Acid-adapted *Escherichia coli* O157:H7 Biofilms

J. D. STOPFORTH, P. A. Kendall, G. C. Smith, and J. N. Sofos, Colorado State University, Dept. of Animal Sciences, Ft. Collins, CO 80523, USA

Survival of acid-adapted (grown in 1% glucose) *Escherichia coli* O157:H7 cells in suspension or attached to stainless steel coupons submerged in meat decontamination residual waste fluids (washings), with or without the presence of the natural flora, was evaluated following exposure to sanitizers during storage. *Escherichia coli* O157:H7 was inoculated (10^5 CFU/ml) in unsterilized or sterilized composite hot water and cold water meat washings (HW/CW) mixed with 2% acetic (AA/W) or 2% lactic acid washings (LA/W) at a ratio of 1/99 (vol/vol) or in HW/CW washings. Stainless steel coupons were submerged in the inoculated washings which were stored for up to 14 days at 15°C. Survival of *E. coli* O157:H7 was determined after exposure (15 to 60 s for cells in suspension and 30 to 120 s for attached cells) to two commercial sanitizers [150 ppm peroxyacetic acid (PAA) and 200 ppm quaternary ammonium compound (QAC)] at 2, 7 and 14 days. The attached cells were more resistant to the effects of the sanitizers than the cells in suspension and survival was highest in the presence of the natural flora. Up to day 7 in all washings, the attached cells were more susceptible to the effects of PAA as compared with QAC; however, after 14 days of exposure to the diluted organic acid/water washings the attached cells became more resistant to the effects of PAA as compared with QAC. The results indicate that long term exposure of bacteria to dilute acid meat decontamination washings may increase resistance to sanitizers, which may be avoided by frequent rotation of compounds.

P041 Vanilla and Cinnamon Extracts as Antimycotic Agents in Fruit-based Agar Systems

Victoria Perez-Petrone, Ivonne Audiffred, Fidel T. Vergara-Balderas, ENRIQUE PALOU, and Aurelio López-Malo, Universidad de las Américas-Puebla, Departamento de Ingeniería Química y de Alimentos. Sta. Catarina Mártir., Puebla 72820, Mexico

Debates about the use of antimicrobial agents in foods encourage scientists to search for alternative antimicrobials. In this search, a wide range of natural extracts from plants were evaluated. Our objective was to evaluate the effects of vanilla and cinnamon extracts on the growth of *Aspergillus niger* and *A. flavus* in two fruit (peach and plum) based agars

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at pH 3.5 and 35 Brix (a_w, 0.97). Freeze-dried vanilla and cinnamon (10 g) were extracted with ethanol (150 ml), filtered, concentrated by vacuum evaporation, and dissolved in 100 ml of ethanol. Molds were cultivated on potato-dextrose agar slants and spores harvested (10^6 spore/ml). Fruits were hand peeled, cut into cubes and blanched. Each blanched fruit was aseptically homogenized with 1% w/w of agar, sucrose and citric acid. The fruit based agars were heated (10 min, 110°C), cooled, and 0, 100, 200, 300 up to 1000 ppm of each extract were incorporated. Triplicate plates of every combination and each mold were inoculated with the spore suspension, incubated for 20 days, observed and the colony diameter recorded. The increase in vanilla or cinnamon concentration and the type of fruit significantly ($P < 0.05$) affected radial growth rates. The germination time of each mold depended on the extract concentration being larger as the concentration of the extract increases. The inoculated molds grew faster and in higher concentrations on plum fruit based agars. The results obtained suggest that vanilla and cinnamon extracts could be used as antimicrobial agents to prevent mold spoilage in fruit purées and may be compatible with the fruit characteristics.

P042 Antibacterial Activity of Thymol, Eugenol, Vanillin, Carvacrol, Citral, Potassium Sorbate and Sodium Benzoate against *Staphylococcus aureus* in Culture Medium

Anglica Santiesteban-Lopez, Stella M. Alzamora, Enrique Palou, and AURELIO LOPEZ-MALO, Universidad de las Américas-Puebla, Departamento de Ingeniería Química y Alimentos. Sta. Catarina Mártir., Puebla 72820, Mexico

Several compounds present in extracts and essential oils of plants, such as thymol and carvacrol, have been reported as effective natural antimicrobial agents. However, data comparing their effectiveness with common antimicrobials are scarce. Our objective was to evaluate and compare antimicrobial effectiveness of thymol (Th), eugenol (Eu), vanillin (Vi), carvacrol (Cr), citral (Ci), potassium sorbate (KS) and sodium benzoate (NaB) to inhibit the growth of *Staphylococcus aureus* at a_w 0.99 or 0.97 combined with pH 5.5 or 4.5. Tripticase soy agar (TSA) was adjusted to the desired a_w by adding NaCl, sterilized, pH adjusted, and 50, 100, 150, up to 3000 ppm of each antimicrobial added. TSA plates for every combination were inoculated using a spiral plater with 50 mL of 10^6 cell/ml fresh suspension, incubated for 2 to 3 days at 35°C, and counted. Inhibitory concentrations were determined as those that inhibit bacterial growth. Increasing antimicrobial concentration decreased *S. aureus* counts. At a_w 0.99, no antimicrobial activity (3000 ppm) was observed with KS or NaB at pH 5.5, while 2000 (NaB) and 500 ppm (KS) inhibited growth at pH 4.5. Antimicrobial inhibitory concentrations decreased along with decreased pH and a_w. However, Th, Eu, Vi, Cr and Ci inhibitory concentrations were not as pH dependent as the antimicrobial activity of KS or NaB. Inhibitory concentrations of Cr, Ci, Th, and Eu varied from 100 to 250 ppm and from 1000 to 2500 ppm for Vi. Greater antimicrobial action was observed for natural agents, which were able to inhibit growth of *S. aureus* at lower concentrations than those needed with synthetic antimicrobials.

P043 Marginal Safety of Irradiation Dosage for Reduction and Post-irradiation Survival of *Listeria monocytogenes* in Ready-to-eat (RTE) Meats

SALLY C. C. FOONG, Glenda L. Gonzalez, and James S. Dickson, Iowa State University, Food Safety Research Lab., Dept. of Microbiology, Ames, IA 50011, USA

A five-strain culture of *Listeria monocytogenes*, including serotype 4b, was inoculated onto four different types of ready-to-eat (RTE) meats (frankfurters, ham, roast beef, and bologna). The meats were vacuum-packed and stored at 4°C for 24 h prior to irradiation (targeted levels ranging from 0.5 to 4 kGy). Populations of *L. monocytogenes* were recovered by surface plating on non-selective and selective media. The margins of safety studied include 3D and 5D kill to achieve an optimal level of reduction while retaining organoleptic qualities of the meats. A 3-log reduction of *L. monocytogenes* on the non-selective medium was obtained at 1.5 kGy. On selective medium, the dosages were 1.5 kGy for bologna and roast beef and 2.0 kGy for frankfurters and ham. The D-values ranged from 0.42 to 0.44 kGy. A 5-log reduction of *L. monocytogenes* on the non-selective medium was obtained at 2.5 kGy. On selective medium, the dosages were 2.5 kGy for bologna and roast beef and 3.0 kGy for frankfurters and ham. Survival of *L. monocytogenes* in the same RTE meat types after irradiation was also studied. The meats were irradiated at doses of 2.0 and 4.0 kGy. Recovery of the surviving organisms was observed during storage at temperatures of 40°C and 100°C for 12 weeks. Preliminary results showed no growth in meats irradiated at 4.0 kGy. Survivors were observed for irradiated meats at 2.0 kGy stored at 100°C after the second week. No growth was observed in samples irradiated at 2.0 kGy stored at 40°C until the fifth week.

P044 Effect of Modified Alkaline Cooking on Aflatoxin Content in Contaminated Corn

M. E. ARREOLA, T. M Cabanillas, G. G. Uribe, and S. D. PEÑA BETANCOURT, U.A.M., Calzada del Hueso 1100, Col. Villa Quietud, Mexico, D.F. 04950, Mexico

Alkaline cooking or "Nixtamalización" using 1.0% $Ca(OH)_2$ at 100°C and different exposure times has been tested as a means to destroy aflatoxin. Studies were undertaken to determine the percent of aflatoxin destruction, using three concentrations (0.5, 1.0, 2.5%) of $Ca(OH)_2$ and three exposure times (30, 60, 90 min). Corn contaminated with two concentrations of aflatoxin was used. Aflatoxins and

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protein content were measured by HPLC. Digestibility was determined using an *in vitro* assay. Corn exposed to 1.0% lime contained 164.82 ppb and 51.278 ppb, which represented 37.05 and 11.52% of the initial aflatoxin concentration, with protein loss. Samples treated with 0.5% lime contained 49.3 ppb, and the corn exposed to 2.5% lime contained 13.75 ppb, which represented 66.09 and 9.17% of initial aflatoxin level, respectively. Results indicated that is possible to achieve the best reduction of aflatoxin with a modified alkaline cooking. The protein and digestibility were most affected in samples treated with 2.5% lime.

P045 Bacteriocinogenic *Lactobacillus sake* 1 Inhibits *Listeria monocytogenes* in a Model Meat Gravy System

E. C. P. DE MARTINIS and V. F. Alves, Faculdade de Ciências Farmacêuticas de Ribeirão Preto - USP, Av. do Café, s/n, Ribeirão Preto, São Pãulo 14040-903, Brazil

In the last decade, bacteriocins of lactic acid bacteria have attracted a great deal of interest. In Brazil, the use of 12.5 mg/Kg nisin is allowed for all types of cheeses and it may also be applied (200 ppm) to the surface of vacuum-packaged frankfurters. However, the successful application of those compounds in meat systems has been limited due to interaction with phospholipids, low solubility at meat pH and inactivation by meat endogenous enzymes. *Lactobacillus sake* 1 (isolated from Brazilian fresh pork sausage) presented antiListerial activity in culture media (12,800 UA/ml). Bacteriocin was produced from 4°C to 30°C and was stable at 100°C for up to 20 min. The ability of *L. sake* 1 to inhibit *L. monocytogenes* ATCC 19115 was tested in a model meat gravy system (1.8% proteose peptone, 1.2% meat extract, 0.6% yeast extract, 2.0 % cornstarch). *L. monocytogenes* and *L. sake* 1 were inoculated at initial levels of ca. 10^3 and 10^5 CFU/ml, respectively, and incubated at 8°C for up to 10 days. *L. sake* ATCC 15521 was used as a negative control for bacteriocin production. The populations of *L. monocytogenes* and *L. sake* were enumerated by spread plating on selective media (Oxford and MRS Agar). After 10 days, in the presence of *L. sake* 1, bacteriocin production was observed and the population of *L. monocytogenes* decreased to below detectable levels (<10 CFU/ml), compared to levels of 10^4 CFU/ml when it was co-inoculated with *L. sake* ATCC 15521 and 10^6 CFU/ml for *L. monocytogenes* alone.

P046 Effects of Gamma Irradiation on the Storage Quality of Dry Groats of Coix

FONG-IN CHOU, Hsiao-Ping Chung, and Hsiao-Wei Wen, National Tsing Hua University, Nuclear Science and Tech. Development Center, 101, SEC 2, Kuang-Fu Road, HsinChu, 300, Taiwan, The Republic of China

The storage quality of dry groats of coix (a Chinese medicinal herb and health food in Asia) was improved with gamma radiation doses ranging from 2 to 30 kGy. The number of aerobic microorganisms and viable adult insects largely determined the efficiency of radiation sterilization. Total aerobic plate counts and microscopic investigations were used to enumerate aerobic microorganisms and viable adult insects, respectively. The total aerobic plate counts ranged from 1.8×10^2 to 5.3×10^4 CFU/g, the total number of adult insects reached 3 per gram, and the dry groats of coix were seriously damaged. Experimental results indicate that an increase in irradiation dose resulted in decreases in total aerobic microbial and viable adult insect counts. A highly radiation resistant bacterium, a Gram-positive tetracocci (RC1), was found in three of the twelve samples analyzed and total counts were as high as 1.5×10^1 CFU/g. A 5 kGy radiation treatment was effective in terminating insects in dry groats of coix. A dose of 25 kGy sterilized the groats of coix, whereas an 8 kGy dose eliminated the RC1 from the groats of croix. After radiation sterilization, no revival of microbes or insects was observed during post-irradiation storage at room temperature for three months.

P047 Antagonistic Activity of Natural Herb Product against *Salmonella* and *Escherichia coli* O157:H7

SO HYUN KIM, Nam Hoon Kwon, Ji Yeon Kim, Ji Youn Lim, WonKi Bae, Jun Man Kim, Kyoung Min Noh, Jin Hur, Woo Kyung Jung, Sook Shin, Jong Eun Lee, Jung Chan Ra, and Yong Ho Park, Seoul National University, Dept. of Microbiology, College of Veterinary Medicine and School of Agricultural BioTech., Seo-Doon Dong 103, Kwon-Sun Gu, Suwon, Gyunggi 441-744, Korea

There is increasing interest in natural products such as herb extracts and probiotics as replacements for antibiotics. Antimicrobial activity of a herbal extract against *Salmonella* Enteritidis, *S. enterica* serovar Typhimurium DT104 and *Escherichia coli* O157:H7 was determined *in vitro* and *in vivo*. Though bactericidal activity was not observed, the product had inhibitory activity against *Salmonella* spp. and *E. coli* O157:H7 on Meuller Hinton Agar containing supernatant of the product. To investigate the antagonistic activity, mice were challenged with *S.* Typhimurium DT104 (3.7×10^8 CFU/mouse) after prefeeding with the product for 7 days. One group of mice was fed the product for 3 days after challenge. The fecal shedding of *S.* Typhimurium DT104 and serum IgG and intestinal IgA against the organism were determined. The fecal shedding was dramatically decreased and *S.* Typhimurium DT104 was not detected in feces and intestines 3 days after challenge. Antibody responses of the intestinal IgA were significantly increased in mice fed the product before and after challenge. These findings suggest that the herb extract product has an antagonistic activity against *S.* Typhimurium DT104 *in vitro* and in the gastrointestinal tract of mice. In addition, administration of the product might enhance the mucosal immune response to *S.* Typhimurium DT104.

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P048 Growth/No Growth Interface of Selected Aspergilli as a Function of pH, Incubation Temperature and Vanillin Concentration

Aurelio López-Malo and ENRIQUE PALOU, Universidad de las Américas-Puebla, Departamento de Ingeniería Química y Alimentos. Sta. Catarina Mártir, Puebla 72820, Mexico

Growth/no growth boundary models may predict suitable combinations of hurdles to stop microbial growth for selected probabilities. Probabilistic modeling using logistic regression analysis is a useful tool in predictive microbiology that can be used to describe the growth/no growth interface of spoilage microorganisms. Using a central composite experimental design (5 replicates of central point), the combined effects of incubation temperature (T, from 4.9 to 30.1°C), pH (from 2.7 to 4.3) and vanillin (V) concentration (from 0.033 to 0.117% w/w) were evaluated at a_w 0.99, on a cocktail of *Aspergillus flavus*, *A. ochraceus* and *A. parasiticus* for growth or no growth response. a_w - pH - V adjusted potato dextrose agar was prepared in triplicate for every combination of factors. Solidified plates were inoculated (2 mL of a 10^6 spore/ml suspension), incubated at the evaluated temperatures and observed during one month. If growth was observed, the response was 1; if not, it was registered as 0. Backward stepwise logistic regression was used to develop a simplified model able to predict probability of mold growth. Mold growth was observed in 33 cases from a total of 57 observations. The model shows that T reduction increased the number of combinations of pH and V concentration with probabilities to inhibit growth higher than 0.95. The obtained model predicts the probability of growth under a set of conditions and can be used to calculate critical values of pH, T, or V concentration needed to inhibit mold growth for different probabilities.

P049 Thymol Inhibitory Concentrations of *Aspergillus parasiticus* Growth Determined by Probabilistic Modeling

AURELIO LÓPEZ-MALO and Enrique Palou, Universidad de las Américas-Puebla, Departamento de Ingeniería Química y Alimentos., Sta. Catarina Mártir., Puebla 72820, Mexico

Probabilistic microbial modeling using logistic regression was used to predict thymol inhibitory concentrations of *Aspergillus parasiticus* growth in the presence of growth controlling factors such as a_w (0.99, 0.97 or 0.95) and pH (5.5, 4.5 or 3.5). a_w and pH adjusted potato dextrose agar was prepared and thymol (0, 250, 500, 750 or 1000 ppm) added. Solidified plates (5 replicates) were inoculated (2 mL of a 10^6 spore/ml suspension), incubated and observed up to 10 days. If growth was observed, the response was 1; if not, it was registered as 0. Backward stepwise logistic regression was used to develop a simplified model able to predict mold probability of growth. Model includes the significant ($P < 0.05$) effect of each independent variable as well as a_w - pH and pH - thymol concentration interactions. From a total of 270 observations, in 99 cases growth was observed. The model can be used to calculate critical values of thymol concentration needed to inhibit mold growth for different probabilities. The reduction of pH increased the number of combinations of a_w and thymol concentration with probabilities to inhibit growth higher than 0.95. With a probability of growth of 0.05 and using the logistic model, thymol inhibitory concentrations were higher as pH increases and a_w decreases. Thymol concentration critical values, calculated for selected probabilities of growth, can be used to predict inhibitory concentrations for different a_w and pH values. Logistic regression is a useful tool to predict antimicrobial agents concentrations inhibitory to mold growth.

P050 Antimicrobial Resistance and Plasmid Analysis of *Campylobacter jejuni* Isolated from Clinical Samples

XIAO WANG, Carl Gilbert, Donald Cave, Michael F. Slavik, University of Arkansas, POSC O-114, Fayetteville, AR, USA

Campylobacter jejuni, a leading foodborne pathogen, causes acute bacterial enteritis associated with consumption of a variety of food products. It has been reported that between 19% and 53% of *C. jejuni* contain plasmids, which could contribute to antimicrobial resistance and/or virulence. There is, however, limited knowledge available on genetically characterized *C. jejuni* plasmids and use of plasmid restriction patterns in epidemiological studies of *C. jejuni* strains. The objective of this study was to analyze DNA endonuclease digestion patterns of plasmids purified from *C. jejuni* human isolates. *C. jejuni* plasmids were purified using a modification of the traditional alkaline lysis method, and the sizes of plasmids were determined by pulsed-field gel electrophoresis (PFGE). Plasmid restriction patterns were established based on analysis of *Hind*III digestions, and *Acc*I and *Hinc*II double digestions. Plasmids, present in 17 of the 41 *C. jejuni* human isolates, ranged in size from 4 to 90 kb. Comparisons of plasmid digestion patterns showed substantial genetic variability, but identical plasmid digestion patterns could be found from multiple *C. jejuni* isolates. In continuation of this project, antimicrobial susceptibility testing is being performed using the Kirby-Bauer disk diffusion method. The combination of antimicrobial resistance and plasmid digestion patterns could provide useful information for epidemiological investigations.

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P051 Combined Effect of Lactic Acid and Nisin Solution in Reducing the Levels of Microbiological Contamination in Red Meat Carcasses

YASMINA BARBOZA DE MARTINEZ, Kenna Ferrer, and Enrique J. Marquez, Universidad del Zulia, Facultad de Medicina, Escuxela de Nutrición y Dietética, Maracaibo, Zulia, Venezuela

The change in bacterial numbers on beef carcasses at specific points during slaughter and the effectiveness of nisin, lactic acid, and the combination of the acid and nisin was determined. Swab samples were obtained from the surfaces of randomly selected beef carcasses. Carcasses were swabbed after skinning, splitting, and washing, from the neck, brisket and renal site. Treatments 1.5% lactic acid or 500 IU/ml nisin or a mixture of nisin and lactic acid were applied, after washing the neck area. A control group was not sprayed. Aerobic bacteria, coliforms and *Escherichia coli* recovered from each sample were enumerated. Results indicated that the highest prevalence of aerobic bacteria, total coliforms and *Escherichia coli* was found in the neck site after splitting, and the lowest level of microbial contamination was found after skinning. Washing with water did not significantly reduce bacterial load, but washing with a mixture of nisin and lactic acid produced a 2 log reduction in total bacterial counts, total coliforms and *E. coli*. Therefore, sanitizing beef carcasses with the mixture may be a useful method to reduce contamination on red meat carcasses.

P052 Colonization Property of *Lactobacillus reuteri* and Its Antagonistic Activity in Mice Infected with *Salmonella enterica* serovar Typhimurium DT104

SO HYUN KIM, Nam Hoon Kwon, Ji Yeon Kim, Ji Youn Lim, Jong Hwan Park, Jun Man Kim, WonKi Bae, Kyoung Min Noh, Jin Hur, Woo Kyung Jung, Sook Shin, Byung Woo Yoo, and Yong Ho Park, Seoul National University, Dept. of Microbiology, College of Veterinary Medicine and School of Agricultural BioTech., Seo-Doon Dong 103, Kwon-Sun Gu, Suwon, Gyunggi 441-744, Korea

The aim of this study was to compare the colonization properties of three probiotic strains, *Lactobacillus reuteri*, *L. bulgaricus* and *L. casei*, and their antagonistic activities against *Salmonella enterica* serovar Typhimurium DT104 infection in mice. Mice were fed with one of three probiotic strains (10^9 CFU/mouse) for 7 days and fecal samples were collected daily from day 8 to 11. Mice were challenged with *S.* Typhimurium DT104 (3.7×10^8 CFU/mouse), after prefeeding with one of the above three probiotic strains for 7 days. The fecal shedding of *S.* Typhimurium DT104 and serum IgG and intestinal IgA against the organism were examined. The fecal shedding was dramatically decreased and *S.* Typhimurium DT104 was not detected in feces and intestines 3 days after challenge in mice fed *L. reuteri*. Antibody responses of the intestinal IgA were significantly increased and relatively strong responses were also observed for serum IgG in mice fed with *L. reuteri*. These findings suggest that *L. reuteri* can survive better in the gastrointestinal tract and has superior antagonistic activity against *S.* Typhimurium DT104 compared with the others. Also, administration of *L. reuteri* might enhance the mucosal and systemic immune responses against *S.* Typhimurium DT104. Further studies will be performed to define the mechanism of immunomodulatory effects of *L. reuteri*.

P053 Quantitative Contamination and Transfer from Foods of *Escherichia coli* by Houseflies

ANTONIO J. DE JESUS, Richard C. Whiting, Alan Olsen, and John Bryce, FDA-CFSAN, 5100 Paint Branch Pkwy., College Park, MD 20740-3835, USA

The housefly (*Musca domestica*) is recognized as an important factor in the dissemination of various infectious diseases such as cholera, shigellosis and salmonellosis. However, the potential for bacterial transfer by houseflies has been demonstrated in a qualitative rather than quantitative manner. In this study, the numbers of bacteria a housefly can carry on its body and transfer to a clean surface after exposure to a contaminated food were determined. Two different contamination sources were used, steak and a sugar-milk aqueous solution. Both foods were inoculated with a fluorescent gene *E. coli* (8 $\log_{10}$ CFU/ml). In the first series of experiments to quantify bacterial numbers on the flies, about 20 flies were transferred into a sterile cage and exposed to the food for 30 min. The flies were immobilized and the attached *E. coli* on each fly were enumerated. In the second series of experiments to quantify the bacterial transfer to a clean surface, experiments were performed in 250 ml sterile jar containing a small dish of food. Individual flies were exposed to the contaminated source for different time periods and their numbers of landings on the food were recorded. Then the food was removed and the jar surfaces and flies were separately enumerated. In the first series, individual flies had different levels of activity. Detectable *E. coli* ($> 1.7 \log_{10}$ CFU/fly) were found on 53% (23/43) and 43% (29/67) of the flies in the cages with steak and sugar-milk, respectively. For the positive flies, the mean carriage ($\log_{10}$ CFU/fly) was 3.77 ± 1.28 for steak and 2.93 ± 1.24 for sugar-milk. In the second series, the numbers of *E. coli* transferred to the jar surfaces per landing on the food were 3.9 ± 0.7 for steak and 3.5 ± 0.7 for milk ($\log_{10}$ CFU/fly-landing). This information provides the first quantitative data on contamination of flies and shows they can cross contaminate other surfaces with approximately 10^{-4} g of food per landing.

P054 Survival and Growth of *Listeria monocytogenes* in Stored (4°, 15° or 25°C) Infant Cereal Hydrated with Water, Milk or Apple Juice

A. Abushelaibi, J. Samelis, P. A. Kendall and J. N. SOFOS, Colorado State University, Dept. of Animal Sciences, Ft. Collins, CO 80523-1171, USA

Cereals have been implicated in bacterial foodborne illness in recent years and there is a concern

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for potential bacterial proliferation in hydrated infant cereals that may be stored or temperature abused and become unsafe for consumption. The objective of this study was to assess survival and potential growth of *Listeria monocytogenes* inoculated (mixture of five strains) in rice, oatmeal, and mixed wheat-rice-oatmeal infant cereals, hydrated (0.5/3.0, w/v) with apple juice, pasteurized milk (2% fat) or water. The inoculated products were stored at 4, 15, or 25°C for 0, 8 and 24 h to simulate potential advance home preparation and abuse. Initial inoculum levels were 3.8 to 4.3 and 1.9 to 2.4 log CFU/g for cereals stored at 4°C and 15 or 25°C, respectively. Samples of stored hydrated cereals were analyzed by plating on tryptic soy agar with yeast extract (TSAYE) and on Palcam agar. Bacterial population changes in hydrated cereals stored at 4°C for 24 h were minor, irrespective of hydration liquid. At 15°C, populations increased in cereals hydrated with water or milk to 4.8 to 5.2 log CFU/ml after 24 h. At 25°C, populations in cereals hydrated with water or milk reached 7.7 to 8.3 log CFU/ml in 24 h. In cereals hydrated with apple juice and stored at 15 or 25°C, populations of *L. monocytogenes* fluctuated between 2.0 and 3.0 log CFU/g. Bacterial population changes in TSAYE agar followed similar trends. The results of this study indicate that hydrated infant cereals should be consumed immediately after preparation or held at 4°C and consumed within 8 h.

P055 Growth Potential of *Listeria monocytogenes* in Commercially Prepared Ready-to-eat Deli Salads Stored at Refrigeration Temperatures

BRIAN S. EBLEN, Richard C. Whiting and Arthur J. Miller, FDA-CFSAN, 5100 Paint Branch Pkwy., College Park, MD 20740, USA

The ubiquitous nature of *Listeria* in the environment, its growth potential at low temperatures, and the high mortality rate associated with listeriosis are cause for concern for refrigerated foods that do not require a cooking step before consumption. In this study three *L. monocytogenes* strains were inoculated at a level of 3 $\log_{10}$ CFU/g into either retail store or large-scale processor manufactured deli salads. Salad types included: chicken, tuna, egg, ham, shrimp, crab, imitation crab, potato and cole slaw. Inoculated salads and controls were stored for up to 30 days at either 5°, 7° or 12°C, then sampled and analyzed periodically for *Listeria* levels, titratable acidity, water activity and pH values. Salads produced by large-scale processors uniformly possessed chemical barriers to prevent growth. In these salads, *Listeria* populations either decreased or remained static during the shelf life of the product at all temperatures tested. Salads prepared by retail establishments lacked preservatives and generally had a higher pH. Most of these salads exhibited product decomposition prior to any outgrowth of *L. monocytogenes*. However, shrimp and crab salads exhibited 1 to 2 $\log_{10}$ CFU/g growth in 14 days of storage at 5° and 7°C, and over 3 $\log_{10}$ CFU/g growth in 9 days of storage at 12°C. The results identify the factors influencing *Listeria* growth and control in deli salads. This information can serve as the basis for formulating deli salads with reduced potential for growth and can be applied to estimate *Listeria monocytogenes* growth rates in refrigerated deli salads for quantitative microbial risk assessments.

P056 Evaluation of Coliforms in Bottled Water at Xalapa City, Veracruz, Mexico

PAOLA SABINA CONTRERAS ROMO, Laboratorio de Alta Tecnología de Xalapa, S.C., Calle Médicos no. 5 Col. Unidad del Bosque, Xalapa, Veracruz 91020, Mexico

Coliform organisms evaluation is a tool for identifying potentially dangerous contamination. Its validity is based on the fact that the natural habitat of the *Enterobacteriaceae* family is feces from human beings and other mammals. According to the Mexican bottled water regulation established by the Secretaría de Salubridad y Asistencia, the maximum concentration permitted for total coliforms is 2 NMP/100 ml or 2 UFC/100ml and non detectable fecal coliforms. The bottled water companies are obligated to evaluate the quality of all their presentations to demonstrate that the product respects those limits. To confirm reported values, the Laboratorio of Alta Tecnología de Xalapa, S.C. collected bottled water samples from January, 2000 to December, 2001 to analyze its quality. There were 126 samples, of which 55.5% came from 1.9l capacity bottles, 21.4% from 500 ml, 18.25% from 1.5l and 4.76% from 1l. 80% of the samples had no total coliforms present, although the maximum concentrations obtained were 240,000 UFC/100 ml and 110,000 NMP/100 ml. Likewise, 92% of the samples had no fecal contamination; however, the maximum obtained was 21,000 UFC/100ml and 110,000 NMP/100ml. It is concluded that although purified bottled water companies usually respect the permitted limits of coliforms, other samples indicated that levels are out of limits. Usually these companies work at inappropriated installations and personnel have no sanitary education, which may be two factors contributing to contamination, so it is recommended that assessment of healthful management procedures be improved and periodic analysis of the purifying process be performed.

P057 Assessment of Risks Associated with Consumer Food Handling Practices Using Real-Time Microbiological Analysis

E. C. REDMOND, C. J. Griffith, J. Slader, and T. J. Humphrey, University of Wales Institute-Cardiff, Food Research and Consultancy Unit, UWIC, Colchester Ave., Cardiff, Wales CF23 9XR, UK

Internationally, raw poultry is known to be contaminated with *Campylobacter* (28 to 80%) and *Salmonella* (7% to 48%). Over 700 million chickens are annually sold for consumption in the UK, with many prepared by consumers in domestic kitchens. Epidemiological and observation data from UK and

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USA indicate that food handling actions resulting in cross contamination of pathogens from raw poultry present a serious potential for risk of food poisoning. Actual consumer food preparations of chicken salads were re-enacted in the laboratory, providing data to quantify the risk potential associated with specific food handling behaviors. All chicken pieces used for re-enactments were artificially contaminated with *Salmonella* Typhimurium DT104 (marker organism) and the majority of chicken pieces were naturally contaminated with *Campylobacter*. Sampling of contact surfaces/materials immediately after possible contamination maximized recovery of microorganisms. Results showed that contamination of surfaces was more prevalent with *Campylobacter* than with *S.* Typhimurium, indicating that *Campylobacter* may have a higher spreadability during consumer food handling. Transfer of *Campylobacter/Salmonella* was facilitated when contaminated surfaces were wet and was particularly prevalent on t-towels and cloths (probability 1:5). The probability of contamination of salad vegetables with *Campylobacter/Salmonella* using contaminated, rinsed utensils was 9:11. Generic actions such as inadequate washing/drying of contaminated hands and kitchen utensils, and touching surfaces, e.g., tap handles and bin lids, with contaminated hands followed by retouching with clean hands also were found to be high risk practices. Results will be discussed in terms of probability and percentage risk of contamination. The implications of the results will be discussed within the context of risk communication.

P058 Plant Metabolites Inhibit Growth and Enterotoxin Production of *Vibrio cholerae*

NORMA HEREDIA, Santos Garcia, and Ginebra Alarcon, Universidad Autonoma de Nuevo Leon, Apdo. Postal 124-F, San Nicolas, N.L. 66451, Mexico

Vibrio cholerae has been a recognized problem for food safety and human health. Programs to reduce or inactivate pathogenic microorganisms in foods include the use of herb extracts. Here, ethanolic and aqueous extracts of 25 plants commonly used in Mexican traditional medicine were studied to determine their effects on growth and enterotoxin production by strains of *V. cholerae* O1 and O139. Dilution methods were employed to determine the minimal concentration of extracts that inhibited growth. Growth was determined spectrophotometrically and by viable count in Luria Bertoni broth (LB). Enterotoxin was quantified by an ELISA method. Of the 50 extracts tested, the ethanolic samples from *Artemisia mexicana* (MIC 4 to 6 mg/ml), *Haematoxylon brasiletto* (MIC 0.3 to 0.4 mg/ml) and *Acacia farnesiana* (MIC 4 to 7 mg/ml) were the most effective against growth of this bacterium. For these plants the ethanolic extacts were 10 to 100 more effective compared to the aqueous samples. Enterotoxin production was also inhibited by these extracts. No enterotoxin formation was detected when amounts of extracts lower (75 and 50%) than the minimal inhibitory concentration for growth were added to the media. At 25% of the MIC, *H. brasiletto* caused a reduction in enterotoxin production of 86%, *A. mexicana* of 83%, and *A. farnesiana* of 100%. Due to safety concerns and consumer preference for natural products, the use of these natural compounds could potentially replace synthetic preservatives to avoid contamination of foods by *V. cholerae*.

P059 Adaptation of *Vibrio cholerae* to Acidic and Bile Juice after Sublethal Shock

GENOVEVA ALVAREZ, Norma Heredia, and Santos García, Universidad Autónoma de Nuevo León, Apdo. Postal 124-F, San Nicolás, N.L. 66451, Mexico

Vibrio cholerae causes cholera, a disease that can cause a high morality rate. Many microorganisms develop an adaptive response when grown under moderate stress conditions. Adaptation induces the synthesis of proteins which are believed to protect cells from harsh changes that could be lethal. Acidic pH conditions and bile are stresses to which *V. cholerae* is exposed from food preparation to disease production. Thus, it is important to investigate if this microorganism has the ability to produce stress related proteins, and if the cells become adapted to those stresses. Cells were given a sublethal acid shock (pH 5.5) or human bile juice shock (3 mg/ml) for 20 min. After these treatments, tolerance to acid and bile juice was determined. Cells turned out to be 2 to 3 times more tolerant to pH 4.5 and human bile juice (30 mg/ml); this tolerance was maintained for at least 90 min. On the other hand, when the cells were subjected to acid stress (pH 5.5), they acquired tolerance to human bile juice (30 mg/ml), this tolerance remained for 60 min. No tolerance to acid was detected as a result of bile juice shock. Acid shock did not affect enterotoxin production, but bile juice induced a reduction in the production of this protein. Acid shock induced the synthesis of 4 proteins (106, 94, 88 and 77 kDa). Bile juice induced at least 7 proteins (114, 106, 101, 88, 84, 56 y 46 kDa). This report provides useful information for the control of the diseases caused by this bacterium.

P060 *Haematoxylon brasiletto* Extracts Inhibit Growth, Verotoxin Production and Adhesion of *Escherichia coli* O157:H7

SANTOS GARCIA, Marco Escobar and Norma Heredia, Universidad Autónoma de Nuevo León, Apdo. Postal 124-F, San Nicolás, N.L. 66451, Mexico

Escherichia coli O157:H7 has emerged as an important agent of gastrointestinal disorders by consumption of contaminated food. This microorganism has virulence factors such as the intimin protein, which is involved in adhesion to intestinal epithelial cells, blood cells and neuronal cells; and verotoxins, which act in intestinal epithelial cells. Several researchers have demonstrated that many plant extracts have activity against the growth of *E. coli*; however, there are few reports on the effect on the O157:H7

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serotype. In this work, we studied ethanolic and aqueous extracts of 35 medicinal plants from Mexico against growth, verotoxin production and adhesion of *E. coli* O157:H7. A dilution method in soy trypticase broth was used to determine inhibition of growth. Verotoxins were determined by the VTEC-RPLA method (Oxoid, U.K.). Adhesion of radiolabeled bacteria to HeLa cells was determined in a scintillation counter. Only one out of 35, the ethanolic extract of "palo de Brasil" (*Haematoxylon brasiletto*) had an effect against growth, verotoxin production, and adhesion of *E. coli* to HeLa cells. The minimal concentration of extract that inhibited growth was 4.00 +/- 0.1 mg/ml; lower concentrations inhibited production of verotoxins type I and type II. At 3 mg/ml (75% MIC) inhibition of toxin production of 99% and 98%, respectively, was observed. In addition, adhesion between bacteria and HeLa cells was affected when both were individually exposed to different concentrations of ethanolic extract. Our results open new alternatives in the effort to combat this bacterium, and help to scientifically validate the use of this plant in the traditional medicine in many countries.

P061 Synergistic Effect of Eugenol, Vanillin and Potassium Sorbate Combinations to Inhibit Growth of *Aspergillus flavus*

M. Teresa Jimenez-Munguia, Enrique Palou and AURELIO LÓPEZ-MALO, Universidad de las Américas-Puebla, Departamento de Ingeniería Química y Alimentos. Sta. Catarina Mártir, Puebla 72820, Mexico

There is a growing interest in the use of natural antimicrobials, especially those from plant sources that are traditional ingredients and flavor enhancers. However, to establish the usefulness of natural antimicrobials, they must be evaluated in combination with other antimicrobial agents to determine any synergistic effects. Individual and combined effects of eugenol (Eu), vanillin (Vi) and potassium sorbate (KS) concentrations on the growth of *Aspergillus flavus* in potato-dextrose agar (PDA) adjusted to a_w 0.99 and pH 3.0 were evaluated. PDA agar was prepared with sucrose and citric acid to reach a_w 0.99 and pH 3.0, and the necessary amount of Eu (0, 50, 100, 150, 200 or 250 ppm), Vi (0, 100, 200, 300, 400 or 500 ppm) and/or KS (0, 25, 50, 100 or 150 ppm) was added. Plates of each combination were centrally inoculated with mold spore suspension, incubated at 25°C, and periodically observed. Inhibition was defined as no observable mold growth after 15 days. Minimal inhibitory concentrations (MIC) for Eu, Vi and KS were determined, as well as inhibitory concentrations for each ternary antimicrobial mixture. Fractional inhibitory concentrations (FIC) and FIC Index were calculated. MICs were 250 (Eu), 150 (KS), and 500 ppm (Vi). Calculated FIC Index were lower than 1. FIC index values as well as FIC isobolograms (planes deviated to the left of the additive curve) show synergistic effects on mold inhibition when Eu, V and KS are applied in combination.

P062 DSC The Role of Exopolysaccharide in Protecting *Escherichia coli* O157:H7 from Acidic Conditions in Set and Stirred Yogurt

SHIAO MEI LEE and Jinru Chen, University of Georgia, CFS, 1109 Experiment St., Griffin, GA 30223-1797, USA

Recent studies conducted in our laboratory show that cells of *Escherichia coli* O157:H7 capable of producing colanic acid (CA), the acidic polysaccharide of mucoid slime, survive better under sublethal heat treatment. These cells also have relatively higher tolerance to the extreme pH in microbiological media and in simulated gastric fluid. This study was undertaken to determine the role of CA in protecting *E. coli* O157:H7 during processing and storage of an acidic food: yogurt. Pasteurized milk to make set and stirred yogurt was inoculated, before fermentation, with a wild type *E. coli* O157:H7, its CA-deficient mutant and a mixture of the two strains. Set and stirred yogurt were processed with the inoculated milk according to previously described procedures. Stirred yogurt was also inoculated separately with the 3 inocula after fermentation. The yogurt was stored at 4° or 15°C for 3 weeks. Samples of each yogurt were withdrawn twice a week and plated on tryptic soy agar (TSA) for populations of aerobes, on sorbitol MacConkey agar (SMAC) for levels of *E. coli* O157:H7, and on MRS and M17 agar for counts of starter cultures. It was noticed that injured cells, especially injured mutant cells, of *E. coli* O157:H7 more readily recovered on TSA than on SMAC. Cells of *E. coli* O157:H7 deficient in CA production died off more rapidly than its parental strain. This suggests that CA plays a role in protecting cells of *E. coli* O157:H7 from stress during processing and storage of yogurt.

P063 *Debaryomyces hansenii* Growth/No Growth Interface as Affected by Solute and Acid Type Used to Adjust a_w and pH

ENRIQUE PALOU and Aurelio López-Malo, Universidad de las Américas-Puebla, Sta. Catarina Mártir, Puebla 72820, Mexico

Debaryomyces hansenii is a common contaminant in several types of foods, being responsible for spoilage of meat and dairy products, jam, brines and salad dressings. It is characterized by resistance to salted, sugared and/or acidic environments. Yeast growth in laboratory media formulated at a_w 0.95 or 0.90, pH 4.5 or 3.5 when using sodium chloride (NaCl), sucrose (Su) or glucose (Glu) to adjust a_w, in combination with acetic (Ac), citric (Ci), phosphoric (Pho) or tartaric (Tar) acid to reduce pH, was evaluated. Media formulated with each ingredient combinations was inoculated with 10^3 to 10^4 CFU/ml, and incubated at 27°C. The number of viable cells was determined and compared with the initial population, to determine if growth or yeast death occurred after 120 h. Backward stepwise logistic regression was used to develop a simplified model, which included acid and solute type as categorical variables, to predict the

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probability of yeast growth. In 14 combinations of a total of 48 tested, yeast death was observed. At pH 3.5, Ac promoted cell death at a_w 0.95 or 0.90 independently of solute type. The use of NaCl to adjust a_w causes yeast inhibition or death in more cases than the use of Glu or Su. Ci and Pho allowed growth at a_w 0.95 (NaCl, Glu or Su) or a_w 0.90 (Glu or Su). The model includes several significant ($P < 0.05$) interactions of solute and acid type, permitting calculation of critical pH values for each condition, demonstrating that acid and solute type exhibited an important effect on yeast response.

P064 Death of Pathogenic Bacteria in Yellow Fat Spreads, Margarine, and Toppings as Affected by Temperature

Sarah L. Holliday and LARRY R. BEUCHAT, University of Georgia, CFS and Dept. of Food Science and Tech., 1109 Experiment St., Griffin, GA 30223-1797, USA

A study was done to characterize the death kinetics of *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* (10^6 CFU/g) inoculated into seven yellow fat products (one margarine, one butter and margarine blend, and five dairy and non-dairy spreads and toppings) and held at 4.4, 10, and 21°C for up to 94 days. Neither *Salmonella* nor *E. coli* O157:H7 grew in the test products. Death was more rapid at 21°C than at 4.4 or 10°C. Depending on the product and temperature, rapid reductions of up to 5 $\log_{10}$ CFU/g occurred within 3 to 5 days. Clearance time at 21°C ranged from 5 to 7 to >94 days for *Salmonella,* 3 to 5 to 28 to 42 days for *E. coli* O157:H7, and 10 to 14 to >94 days for *L. monocytogenes.* Death was most rapid in a water-continuous spray product and least rapid in a butter and margarine blend. *E. coli* O157:H7 died more rapidly than *Salmonella* and *L. monocytogenes. Salmonella* died most rapidly in low fat (48%) products. Inhibition of growth is attributed to factors such as acidic pH, salt content, presence of preservatives, emulsion characteristics, and nutrient deprivation. *L. monocytogenes* did not grow in six of the test products but increased in population between 42 and 63 days in a butter and margarine blend stored at 10°C and between 3 and 7 days when storage was at 21°C. This study shows that, under the experimental conditions used, traditional margarine and spreads are not "potentially hazardous" foods in that they did not support the growth of *Salmonella, E. coli* O157:H7, or *L. monocytogenes.*

P065 Advantages and Limitations of a Multiple Hurdle System to Control Food Pathogens and Food Spoilage Organisms

CLAUDIA KOERTING and Carey Walker, University of Connecticut, Nutritional Sciences, 3624 Horsebarn Road Ext., Unit 4017, Storrs, CT 06269-4017, USA

Food preservation generally relies on low pH, high heat or high concentrations of chemicals. A multiple hurdle system for control of food pathogens and food spoilage bacteria which uses reduced concentrations of preservatives, moderate pH and low heat pasteurization was studied in bacterial broths and food. Field isolates of *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* and *Salmonella* biotype pullorum were studied. *Bacillus* species and lactic acid bacteria were from ATCC. Viability was assessed by plate counts. Broth cultures of bacteria (10^5 to 10^6 CFU/ml) observed for >9 days demonstrated that a combination of low concentrations of sodium acetate (<0.25%) and BHA (<0.002%) at pH 5.5 was bactericidal for all pathogens except *Listeria.* The system was bacteristatic for *Listeria* and lactic acid bacteria. Low temperature pasteurization (55°C, 10 to 20 min.) resulted in no detectable *Listeria* and reduced the concentration of viable lactic acid bacteria. A combination of moderate pH with sodium acetate and sucrcose ester or sodium acetate and BHA was sporostatic for *Bacillus* in broth and delayed germination in mashed potatoes and milk. The concentration of BHA to inhibit bacteria was reduced by 50% with sodium acetate and low pasteurization temperature. Tolerance to sodium acetate at low pH was achieved by some bacterial strains, but not in the presence of BHA. The study demonstrates that careful selection of components for a multiple hurdle treatment can be bactericidal and could reduce occurrence of cross-tolerance by pathogens.

P066 Microbial Quality of Groundwater in a Shallow Aquifer Following Hog Manure Application to an Overlying Field

J. Rogasky, G. BLANK, R. Holley, and B. Betcher, Department of Food Science, University of Manitoba, Room 242, Ellis Bldg., Winnipeg, Manitoba R3T 2N2, Canada

The microbiological impact on groundwater resulting from land spreading of liquid hog manure on light soils overlying a shallow sand aquifer was investigated. The site consists of approximately 80 acres of land (divided into four equal north-south fields) and overlies a portion of a large sand aquifer which is considered to be sensitive to contamination. Both groundwater (constant at 5 to 7°C) and unsaturated zone monitoring was performed, the former by installation and periodic sampling of 6 piezometer nests in each of the four fields and the latter by collection and analysis of soil samples. Manure was applied via 4 inch injection. Application rates ranged from 50 to 200 lbs per acre. All monitoring well samples were positive for confirmed *Pseudomonas aeruginosa*, the values of which ranged from 2 to 23 MPN/ml. Highest levels were recorded in March when the soil was snow covered. Confirmed coliforms were detected in 11 of 66 well sites that were evaluated during six separate trials. Values ranged from <2 to 23 MPN/ml. Fecal coliforms were not detected. The presence of confirmed coliforms in the water samples did not appear to correlate with the timing of manure application or major rainfall events. Analyis of soil samples obtained by drilling (0 to 2,

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2 to 4, 4 to 6 and 6 to 8 ft) three weeks following application (ambient air temperature 15 to 25°C) revealed no salmonellae or *Yersinia*. *Escherichia coli* was detected in several soil samples down to a depth of 6 feet. Values ranged from 500 million to 8 billion CFU/g.

P067 Combined Effects of Carbon Dioxide and Temperature in High Pressure Processing of Fluid Food Systems

V. M. (Bala) Balasubramaniam, Sue Keller, Joe Dunn, OMAR MARTIN, and Armand Paradis, Illinois Institute of Technology, 6502 S. Archer, Summit-Argo, IL 60501, USA

High pressure processing (HPP) is a promising technique that can be used towards the goal of satisfying the increasing consumer demand for minimally processed foods. Combining HPP and CO_2 may help to reduce the time and temperature for microbial inactivation. In this study, the inactivation of *Escherichia coli* O157:H7 ATCC 43895 was examined under the combined effects of CO_2: High Pressure (5, 10, 20, 30 and 40 kpsi) at 20°C and 40°C with a 0, 3, and 5 min holding time. The mean CO_2 concentration of the carbonated untreated sample was 7.6%. This value was reduced to 6.85% after processing. The reduction may be attributed to a loss of gas during product handling and storage. The average pH values of the untreated and treated samples were 5.82 and 5.70 respectively. The slight reduction in pH could be attributed to the carbonation process. Increasing pressure increased log reduction in both HPP alone and HPP+ CO_2 treatment conditions. However, the increase in pressure with CO_2 enhanced the inactivation of cells. At 20°C with the addition of CO_2 and constant process time, the pressure required to achieve a given log reduction was reduced.

P068 A Composite Model for Prediction of Bacterial Destruction in Antimicrobial Treatment of Vegetables

HONG YANG, Betty L. Swem, Hong Wang and Yanbin Li, University of Arkansas, Center of Excellence for Poultry Science, Poultry Science Bldg., Fayetteville, AR 72701, USA

Predictive modeling is an important tool to transfer scientific information into the input of microbial risk analysis that is the basis of the food safety system. A composite model, D=f (Bacteria, Food, Treatment), was developed in this study to predict D-values based on three components: bacteria (the characteristics, physiological state), food (the characteristics, bacterial attachment) and antimicrobial treatment (chemical agents, concentration, pH, temperature, treatment time). A second degree polynomial model was applied to describe the continuous variables in each component. The model was evaluated using the data collected for the destruction of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* on fresh-cut vegetables treated with electrochemically activated water (ECAW), chlorinated water (CLW), and cetylpyridinium chloride (CPC). D= 5.80+Bact+Veg+0.43 Temp-0.11Time, accounted for approximately 80% of the variation of the D-value in the ECAW treatment. The coefficient Bact was 0, 0.06 and -0.05 for *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium, respectively, and the Veg was 0, -0.07 and -0.09 for broccoli, lettuce and sprouts, respectively. Coefficients for the effects of concentration and pH of ECAW had values of close to zero. The model could be applied to antimicrobial treatments of fresh-cut vegetables and assist quantitative microbial risk assessment for vegetable processing.

P069 HAV Resistance in Mussels Subjected to Different Kinds of Domestic Cooking

CROCI LUCIANA, Dario De Medici, Simona Di Pasquale, Elisabetta Suffredini, and Laura Toti, Istituto Superiore di Sanità - Laboratorio Alimenti, Viale Regina Elena 299, Rome 00161, Italy

Hepatitis A is a widespread infectious disease world-wide. In Italy, shellfish consumption was shown to be a major risk factor for hepatitis A infection, especially when these products are eaten raw or slightly cooked. Moreover, cooking does not always guarantee the wholesomeness of shellfish. The aim of present study was to evaluate HAV resistance in experimentally contaminated mussels containing 10^4 TCID50 HAV /g subjected to different kind of domestic cooking. The simulation of domestic preparations, such as "mussel hors-d'oeuvre", "mussels au gratin", "mussels in tomato sauce" was made according to traditional Italian cookery and used different time and temperature conditions. The presence of RNA-HAV was evaluated by RT-nested-PCR and the presence of infectious virus was confirmed by an integrated methods cell culture-RT-PCR in the positive samples. The experiments showed that HAV was completely inactivated by cooking only in "mussels in tomato sauce", while it was still present, even if not quantitatively determinable, both in "mussel hors-d'oeuvre" and in "mussels au gratin". The results confirmed that shellfish flesh and the ingredients used for dish preparations may protect the viruses from the heat treatment, preventing a complete decontamination of the product. The present study utilised a higher virus quantity than that usually found within naturally contaminated shellfish, but provide useful indications with respect to the consumption of high-risk food such as shellfish slightly cooked. Then it is advisable to pay particular attention to the timing and temperature setting of the cooking.

P070 DSC Geographic Information System (GIS) and *Listeria* Isolates Recovered from Dairy Cows, Calves, and Farm Environments

K. D. LAMAR, M. Evans, V. Ling, S. P. Oliver, D. A. Golden, and F. A. Draughon, University of Tennessee-Knoxville, Dept. of Food Science and Tech., 2605 River Road, 100 Food Safety and Processing Bldg., Knoxville, TN 37996, USA

Several epidemiological studies have examined links between cases of listeriosis and consumption of

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antimicrobials. Induction of stationary-phase ATR was attempted by both transient low-pH acid shock and acid adaptation. For acid-shock induction, EG medium stationary-phase cultures were resuspended in EG medium adjusted to pH 4.3 for 2 h, while for acid adaptation, strains were grown overnight in tryptic soy broth (TSB) containing 1.0% glucose. Acid-shocked and acid-adapted cultures were challenged for 4 h in EG medium or TSB adjusted to pH 3.0, or 3.0 and 2.3, respectively. Initial populations of 8.2 to 8.7 log CFU/ml were reduced to 3.0 to 4.8 and 2.5 to 3.7 log CFU/ml following 4 h acid challenge for previously acid-shocked susceptible and multi-antimicrobial resistant cultures, respectively; corresponding counts of unshocked cultures were 4.3 to 5.5 and 3.9 to 4.9 log CFU/ml. Populations of acid-adapted susceptible and multi-antimicrobial resistant cultures were acid resistant at pH 3.0, but at pH 2.3 were 6.1 to 6.4 and 6.4 to 6.6 log CFU/ml, respectively, following 4 h acid exposure, while unadapted cultures were 1.8 to 2.0 and 1.8 to 1.9 log CFU/ml. A low-pH inducible ATR was not achieved through transient acid shock, as unshocked populations were higher ($P < 0.05$) following acid exposure. However, an induced ATR was achieved through acid adaptation, as populations prepared in the presence of glucose were 4.2 to 4.8 logs higher than those grown without glucose following acid exposure (pH 2.3). Results of this study suggested no association between antimicrobial susceptibility and the ability to avoid or repair damage associated with acid stress in the *Salmonella* cultures tested under these conditions.

P074 DSC Thermal Inactivation of Susceptible and Multi-antimicrobial Resistant *Salmonella* Grown in the Absence or Presence of Glucose

R. T. BACON, J. N. Sofos, P. A. Kendall, J. R. Ransom, K. E. Belk, and G. C. Smith, Colorado State University, Dept. of Animal Sciences, Ft. Collins, CO 80523, USA

Heat resistance of susceptible and multi-antimicrobial resistant *Salmonella* was determined at 55, 57, 59 and 61°C. Five susceptible and five resistant strains were grown to stationary-phase in glucose-free tryptic soy broth supplemented with 0.6% yeast extract (TSBYE), and in TSBYE with 0.25% and 1.00% glucose. Cells were harvested, washed and resuspended to produce six, five-strain culture composites, diluted to a final concentration of approximately 8.0 log CFU/ml. Cultures were dispensed (50 µl) into capillary tubes, heat-sealed, and immersed in a thermostatically controlled, circulating water-bath. At predetermined time intervals, duplicate capillary tubes (three replicates) were removed and viable cell populations were enumerated on tryptic soy agar supplemented with 0.6% yeast extract and 1.0% sodium pyruvate. Log counts of survivors were plotted against corresponding challenge times, and decimal reduction times (*D*-values) were calculated from more than five values having an $r^2>0.90$. At 59 and 61°C thermal tolerance depended ($P<0.05$) on acid adaptation, as *D*-values increased ($P<0.05$) from 0.50 to 0.66 min and from 0.14 to 0.19 min as glucose level increased from 0.00 to 1.00%, respectively. Collectively, when averaged across *Salmonella* composite, *D*-values ranged from 4.23 to 5.39, 1.47 to 1.81, 0.50 to 0.66, and 0.16 to 0.20 min for cultures inactivated at 55, 57, 59 and 61°C, respectively. Z_D-values were 1.20, 1.48 and 1.49°C for *Salmonella* grown in 1.00, 0.25 and 0.00% glucose, respectively, while corresponding activation energies of inactivation were 497, 493 and 494 kJ/mol. Results suggested a cross-protective effect of acid adaptation on thermal inactivation, and no association ($P>0.05$) between antimicrobial susceptibility and the ability of *Salmonella* to survive or repair damage associated with heat stress.

P075 DSC Genotypic Diversity of *Listeria* Isolates from Dairy Cows, Calves, and the Farm Environment

MATTHEW R. EVANS, F. A. Draughon, S. P. Oliver, and V. Ling, The University of Tennessee-Knoxville, Food Safety and Processing Bldg. 2605 River Drive, Room 105, Knoxville, TN 37996, USA

Listeria isolates (n=48) from a total of 548 cow, 454 calf, and 1,556 University of Tennessee Dairy Farm environmental isolates obtained during a 1999–2001 survey were submitted for automated ribotype analysis utilizing the Riboprinter microbial characterization system, alpha version (E. I. du Pont de Nemours & Co., Inc.). This investigation compared *Listeria* ribotypes (RTs) obtained from dairy cows, calves, and farm environmental isolates to a database of clinical isolates of *Listeria* associated with animal miscarriages and foodborne listeriosis. Riboprint patterns of 17 of the 48 isolates confirmed the identity as *Listeria* spp. based upon the Du Pont (EcoRI) database. The 17 *Listeria* isolates were discriminated as 5 *L. monocytogenes* RTs (1029, 1041, 1051, 1052, and 1XXXX, a confidential isolate held by Du Pont), 5 *L. innocua* RTs (1005, 1006, 1009, 1010, and 1019), and 4 *L. welshimeri* RTs (1072, 1073, 1074, and 1079). According to ribotype analysis, optimal detection sites for the presence of *L. monocytogenes* were from the cows' teats, the oral cavity of calves, from corn silage, and from the river water adjacent to the midpoint of the farm. Knowledge of the agricultural ecosystem, the taxonomy of *Listeria* strains, and careful attention to detailed isolation and confirmation protocols were essential to characterize epidemiological associations among *Listeria* spp. due to the heavy background microflora of farm samples.

P076 DSC Simultaneous Detection of Hepatitis A Virus and Human Rotavirus Using Colorimetric Biplex Nucleic Acid Sequence-based Amplification (NASBA)-Enzyme-Linked Immunosorbent Assay

JULIE JEAN, Burton Blais, André Darveau, and Ismaïl Fliss, Univeriste Laval, Centre de recherche STELA, Dep. Food Science, Universite Laval, Quebec (QC), Quebec G1K 7P4, Canada

Enteric virus transmission due to the consumption of fecally contaminated foods is currently a

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significant public heath concern. Since 1982, 40% of the recognized foodborne outbreaks reported throughout the world have been of viral etiology. Hepatitis A virus, rotavirus and Norwalk virus are the most widely incriminated agents of foodborne viral gastroenteritis. These enteric viruses are commonly isolated from shellfish, fresh fruits and vegetables, which are susceptible to contamination by wash and irrigation water. Epidemiological investigations of outbreaks associated with these viruses have been hindered by the lack of available methods for their detection in foodstuffs. In this study, a biplex nucleic acid sequence-based amplification (NASBA) technique combined to a simple and quantitative colorimetric microtiter plate hybridization and detection system were developed to specifically and simultaneously detect human rotavirus and HAV. Two specific sets of primers were selected from published nucleotide sequences and used for the amplification of specific genes in both viruses. Using these primers, two distinct RNA products with 268 and 474 nucleotides were amplified in rotavirus and HAV, respectively. When combined to the microtiter plate hybridization and detection system, a detection limit of 4×10^2 and 4×10^1 PFU/ml were obtained for HAV and rotavirus respectively. No signal was obtained with non-homologous nucleic acids and non-target microorganisms. The biplex NASBA developed here offers several advantages over monoplex systems for virus detection, namely turnaround time and cost effectiveness. The relative simplicity and speed of this technique make it an attractive tool for the detection and routine monitoring of enteric viruses in environmental and food samples.

P077 DSC Antimicrobial Activity and Mechanisms of Action of Essential Oils and Components

VALERIE W. LING, Katie Davenport, P. Michael Davidson, and F. Ann Draughon, University of Tennessee, Food Safety Center of Excellence, Agricultural Experiment Station, 2605 River Drive, Knoxville, TN 37996-4591, USA

Since consumers are concerned about the safety of synthetic preservatives and prefer natural ingredients, studies reporting the antimicrobial efficacy of essential oils are of interest. The objectives of this study were to evaluate the antimicrobial activity of essential oils and their active antimicrobial components and to investigate their mechanisms of inhibition. The paper disc agar diffusion method (0.024 mg chemical) was used to evaluate the inhibition of growth of *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium DT 104, *Listeria monocytogenes*, and *Staphylococcus aureus*. The essential oils tested were olive, camphor, cinnamon, clove, cranberry, echinacea, garlic, grapefruit seed, juniper berry, lavender, lemon, myrrh, peppermint, tea tree, thyme, and yarrow flower. The essential oil components tested were beta-caryophyllene, beta-citronellol, cymene, eugenol, alpha-humulene, limonene, linolool, myrcene, ocimene, alpha-pinene, beta-pinene, rhodinol, alpha-terpinene, gamma-terpinene, alpha-terpineol, and thymol. Carvacrol, beta-caryophyllene, cinnamic aldehyde, eugenol, alpha-humulene, limonene, linolool, alpha-pinene, rhodinol, and thymol were used to examine the mechanisms of action by studying 1-N-phenylnapthylamine (NPN) uptake and changes in intra- and extracellular ATP concentrations. Gram-positive organisms were more sensitive to essential oils than Gram negatives. Most essential oils were not inhibitory or were only slightly inhibitory to Gram-negative bacteria, except for tea tree, cinnamon, clove (moderate inhibition), and thyme (strong inhibition). Tea tree, juniper berry, peppermint, and thyme completely inhibited the growth of *S. aureus* (zones = 40 to 62 mm). Most essential oil components were slightly inhibitory against Gram-negatives, except for citronellol, limonene, rhodinol, eugenol (moderate), and linolool (strong inhibition, zones = 28 mm). *S. aureus* was strongly inhibited by beta-caryophyllene, rhodinol, alpha-humulene, and eugenol (zones = 30 to 45 mm). Increased uptake of NPN caused by chemicals such as thymol indicated that the bacterial membrane was disrupted by exposure to specific essential oil components.

P078 DSC Distribution of Environmental and Disease Associated *Listeria monocytogenes* Biofilm Phenotypes within rep-PCR Genotype Patterns

JAMES FOLSOM, Gregory Siragusa, and Joseph Frank, University of Georgia, Food Science Bldg., Athens, GA 30602, USA

Rep-PCR, a genotyping tool that amplifies DNA between randomly occurring repetitive elements within the bacterial genome, was used to genotype eight isolates of *L. monocytogenes* from monkeys, twelve monkey environmental isolates, five isolates from food processing areas, and five isolates associated with food borne disease. Primers UprimeDt and UprimeG5, were used and UPGMA-based dendrograms obtained. The percent area covered by cells after overnight biofilm formation was determined using a standardized stainless steel coupon biofilm formation assay. High (n=15) and low (n=15) biofilm producers are defined by the highest and lowest average percent area covered respectively. In dendrograms, all of the high biofilm producers were observed to cluster together, separate from the low producers. Primer Dt separated the serogroup 1/2a isolates into three groups (>90% Pearson coefficient). One group contains all the low producers and the other two groups contain all the high producers. Primer G5 split Serogroup1/2a into 7 groups (>90% Pearson coefficient). Three of these groups contain high biofilm producers, while the other four groups contain low biofilm producers. Because these primers aren't as discriminating for Serotype 4b, those isolates were not resolved into distinct groups of high and low producers with the same level of distinction observed for the serotype 1/2a isolates. Our data indicate that biofilm formation of the 1/2a serogroup is distinguishable both phenotypically and genotypically, leading

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to hypothesis of a genetic basis underlying the complex mechanism of biofilm formation and persistence of *L. monocytogenes* in food environments.

P079 Withdrawn

P080 DSC Bias and Accuracy Values from Ten Years of Predictive Food Microbiology Literature

SIOBAIN DUFFY and Donald W. Schaffner, Yale University, P. O. Box 208106, 165 Prospect St., New Haven, CT 06520-8106, USA

Predictive microbiology is based on the idea that microbial behavior is reproducible, and can be mathematically modeled. Models for both foodborne pathogens and spoilage microorganisms have applications in product formulation, design of challenge studies, development of science-based regulations and HACCP plan design. The bias and accuracy factors designed by Ross (1996) and modified by Baranyi, Pin and Ross (1999) provide indices to measure the predictive power and goodness of fit of microbial models. A handful of predictive food modeling papers have used these factors since they were first proposed. Most published predictive models, however, lack these indices. Bias and accuracy factors (according to Baranyi, Pin and Ross, 1999) were calculated from predictive models published in five leading food microbiology journals (*Applied and Environmental Microbiology*, *Food Microbiology*, *Journal of Food Protection*, *Journal of Food Science* and *International Journal of Food Microbiology*) over the last 10 years. Predicted and observed values for each paper were obtained from either tabular or graphical data. Figures were scanned and converted back to numerical values using SigmaScan software. Some models (e.g., growth/no growth boundary models) could not be included, as bias and accuracy factors are only appropriate for rate and time variables (i.e., not probability of growth). Our results show that some highly biased and inaccurate models do exist in the literature. Over time, however, predictive food microbiology models are improving and the scientific community is producing more accurate and less biased models.

P081 DSC Statistical Distributions Describing Microbial Quality of Surfaces and Foods in a Foodservice Operation

REBECCA MONTVILLE and Don Schaffner, Rutgers University, Food Science Bldg., 65 Dudley Road, Room 207, New Brunswick, NJ 08901, USA

Data on the microbial quality of foodservice kitchen surfaces and ready to eat foods were collected over a period of ten years in dining halls at Rutgers University. Surface counts, total aerobic plate count and coliform counts were determined using standard methods. Analysis was performed on foods tested more than 50 times (primarily lunch meats and protein salads) and on surfaces tested more than 500 times (36 different surfaces types, including pastry brushes, cutting boards, and countertops). Histograms and distributions were determined using Microsoft Excel and Bestfit, respectively. Common trends were seen with both the foods and the surfaces. Total plate counts for the foods ranged from below the level of detection (approximately 175 CFU/gram) to 8 $\log_{10}$ CFU/gram. Mean counts ranged from 2 to 4 $\log_{10}$ CFU/gram, with sealeg salad, shrimp salad, ham, and roast beef having higher means. The counts for the surfaces ranged from 0 CFU/4 cm^2 to too numerous to count. Histograms for all surfaces either contained one peak near 0 CFU/4 cm^2 or were bimodal (with a second peak near our upper limit of detection). Plastic surfaces were more likely to have higher counts than stainless steel surfaces. Plastic hotel pans were more likely to be highly contaminated than stainless steel ones. Pastry brushes and plastic cutting boards had high contaminations rates, whereas countertops had relatively low contamination rates. These data could be used for incorporation in risk assessment or to determine criteria for safe levels of contamination.

P082 DSC Modified RT-PCR to Eliminate False Positive RT-PCR with Inactivated Viruses

SUPHACHAI NUANUALSUWAN, Sakchai Himathongkham, Hans Riemann, MingQi Deng, and Dean Cliver, University of California-Davis, Dept. of Population Health and Reproduction, School of Veterinary Medicine, One Shields Ave., Davis, CA 95616-8743, USA

Human enteric viruses have been recognized as significant causes of foodborne diseases. Many methods are available to detect viruses in food, e.g. susceptible cell culture inoculation, enzyme-linked immunosorbent assay, DNA/RNA probes, reverse transcription-polymerase chain reaction (RT-PCR), etc. RT-PCR can detect only a few copies of virus in food after several amplification cycles of the viral nucleic acid, thus making RT-PCR a very sensitive and leading detection method. Viral foodborne outbreaks are related to consumption of foods contaminated with fecal material from infected humans, so viruses can get into foods either before or after food processing. However, the conventional RT-PCR cannot distinguish whether viruses detected in foods are inactivated or still infectious. In this study, hepatitis A virus, poliovirus 1, and feline calicivirus were inactivated with ultraviolet (254 nm) light, heat (72°C), and chlorine (1.2–1.25 mg/l). The modification is an extra step, just before conventional RT-PCR, called "Digestion." Proteinase K (PK), 20 units, and ribonuclease (RNase), 100 units, were applied to inactivated and infectious viruses at 37°C for 30 min, followed by addition of 40 units of RNase inhibitor. For infectious virus particles, RNA inside the virus was protected from RNase by PK-resistant native coat protein, so the RT-PCR was positive, whereas the coat protein of inactivated virus particles is compromised by inactivation and susceptible to PK digestion. Then, the RNA inside these viruses is unprotected by coat protein and is digested by RNase. RT-PCR of inactivated virus was negative because there was no RNA left in the virus particle.

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P083 Effect of *Lactobacillus rhamnosus* and a Fermented Milk on the Growth of *Aspergillus* and *Penicillium* Species

JITKA STILES, Valerie Carter, and Lloyd B. Bullerman, University of Nebraska-Lincoln, 319 FIC, East Campus, Lincoln, NE 68583-0919, USA

This study was done to investigate the effect of *Lactobacillus rhamnosus* VT1 and skim milk fermented by this strain on growth of *Aspergillus* and *Penicillium* spp. Prior to the experiment, *L. rhamnosus* VT1 was grown in MRS broth and sterile skim milk at 37°C for 18 h. Initially, live bacterial cells (1% v/v inocula), as well as skim milk fermented with *L. rhamnosus* VT1 (0.1% v/v, 1% v/v, and 10% v/v), were added to MRS broth, which was simultaneously inoculated (1% v/v) with *Aspergillus* and *Penicillium* species. The cultures were incubated at 30°C for 10 days. Each day, the growth of each organism and the pH of the broth were determined. Secondly, antifungal effects of live bacterial cells (1% v/w inocula), as well as fermented skim milk (0.1% v/w, 1% v/w, and 10% v/w), were also tested on commercial cottage and mozzarella cheeses. After simultaneous inoculation of cheeses by the antifungal agents and mold species, the cultures were again incubated at 30°C for 10 days. Each day, the inhibition of mold growth was determined visually and by measurement of mold plate counts. *Lactobacillus rhamnosus* VT1 as well as all concentrations of the fermented skim milk were found to significantly affect the growth of both *Aspergillus* and *Penicillium* species. However, *Penicillium* spp. were more sensitive to the antifungal agents tested than *Aspergillus* species. These results suggest that *L. rhamnosus* VT1 has potential as a biocontrol agent of molds in the dairy industry.

P084 Formation of Volatile Compounds by Wild Strains of *Lactococcus lactis* Isolated from Raw Ewes' Milk Cheese

Pilar Morales, Estrella Fernandez-Garcia, Pilar Gaya, Margarita Medina, and MANUEL NUÑEZ, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Carretera de La Coruña Km 7, Madrid 28040, Spain

Strains of lactococci used as starter cultures by the dairy industry have been selected mainly on the basis of acidification rate and phage resistance. Information on their flavoring abilities, essential to define technological interest, is scarce. In the present work, 9 strains of *Lactococcus lactis* subsp. *cremoris* and 23 strains of *L. lactis* subsp. *lactis*, all of different RAPD pattern, isolated from raw ewes' milk cheese, were characterized for their production of volatile compounds. Small cheeses were made from whole pasteurized milk, using each strain as single starter culture, and analyzed 4 days after manufacture. Volatile compounds were concentrated by a Tenax trap in a purge and trap apparatus and determined using gas chromatography, with detection performed by a mass spectrophotometer. Relative abundance was calculated for all compounds, and those of particular interest were quantified by addition of known concentrations of internal standards. Hierarchical cluster analysis, carried out using the relative abundances of 42 volatile compounds, revealed the existence of four main groups of strains. Seven of those compounds achieved by themselves the same separation of strains. Their concentrations in cheese (means of strain groups) were in the ranges 0.027 to 0.157 ppm for 1-propanol, 0.003 to 0.027 ppm for 2-propanol, 0.165 to 7.76 ppm for 2-methyl propanol, 1.29–20.35 ppm for 3-methyl butanol, 0.142 to 1.18 ppm for 3-methyl butanal, 0.784 to 8.23 ppm for 2,3-heptanedione, and 0.001 to 0.011 ppm for isoamyl acetate. When submitted to sensory evaluation of odor characteristics, cheeses with higher levels of 2-propanol and lower levels of the other six compounds were preferred by panelists.

P085 Factors Affecting Inhibition of *Listeria monocytogenes* in Milk by Nisin

MEENA BHATTI and Leora A. Shelef, Wayne State University, Dept. of Nutrition and Food Science, 3009 Science Hall, Detroit, MI 48202, USA

In a previous report on the antilisterial effects of nisin in fresh pasteurized and homogenized milk, *Listeria monocytogenes* was most sensitive to nisin in skim milk (0.1% fat), and was more resistant to the bacteriocin in milk with 2 or 3.5% fat. The purpose of this study was to investigate factors that may contribute to the reduced antilisterial activity of nisin. Raw (R), pasteurized but not homogenized (P), homogenized but not pasteurized (H), and pasteurized and homogenized (HP) milk containing 0.1 and 3.5% fat was obtained from a local dairy. Samples were treated with 125 IU/mL of nisin, alone and in combination with Tween 80 or lecithin, and were challenged with approximately 4 log CFU/ml of *L. monocytogenes* strain Scott A. Cell numbers of the pathogen were determined on PALCAM agar at time intervals during storage at 5°C for up to 15 days. Nisin inhibited growth of the pathogen in skim milk regardless of the treatment, and numbers of the pathogen were <10 per ml after 6 days. In contrast, a decline of the pathogen observed in the homogenized milk (H, HP) during the first week of storage was followed by a gradual increase to the initial or higher *Listeria* numbers. Therefore, homogenization treatment of whole milk appears to cause binding or absorption of nisin thereby making it unavailable for inhibition of the pathogen. Addition of Tween 80, a nonionic emulsifier, to these treatments partially counteracted the loss of the antilisterial activity of nisin, whereas lecithin, an anionic emulsifier, had no effect.

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P086 The Effect of Bacteriocin-producing *Lactococcus lactis* subsp. *lactis* INIA 415 as Adjunct Culture on Proteolysis and Flavor of a Semi-Hard Cheese

Sonia Garde, Javier Tomillo, Pilar Gaya, Margarita Medina, and MANUEL NUÑEZ, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Carretera de La Coruña Km 7, Madrid 28040, Spain

Lactococcus lactis subsp. *lactis* INIA 415, a strain harboring the structural genes of nisin Z and lacticin 481, was used as adjunct culture in the manufacture of a semi-hard cheese from a mixture of pasteurized cows' and ewes' milk (80%–20%), together with a mesophilic starter (*L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*) and a thermophilic starter (*Streptococcus thermophilus*) of high aminopeptidase activity. Addition of the bacteriocin-producer promoted early lysis of mesophilic and thermophilic starter bacteria. Extracellular aminopeptidase activity, determined with Lys-p-NA as substrate, in 7-day-old cheese made using mesophilic and thermophilic starters plus the bacteriocin producer was 3.0-fold the level reached in cheese made without the bacteriocin producer. Overall proteolysis in cheese made with mesophilic and thermophilic starters plus the bacteriocin producer after 25 days of ripening was 1.5-fold the level reached in cheese made without adjunct culture. The level of total free amino acids in 25-day-old cheese made with mesophilic and thermophilic starters plus the bacteriocin producer was 2.9-fold the level found in cheese made without the bacteriocin producer. When cheeses were submitted to sensory evaluation by 16 trained panelists, the cheese made with mesophilic and thermophilic starters plus the bacteriocin producer received significantly higher scores for flavor quality and flavor intensity, and reached in 25 days the flavor intensity score of a 75-day-old cheese made without the bacteriocin producer.

P087 Validation of SL Beta-lactam Test Performance in Goat's Milk

ROBERT S. SALTER, Mary Bulthaus, Frank Fillman, Bob Bonifacio, Phil Kijac, Charm Sciences, Inc., 659 Andover St., Lawrence, MA, 01843, USA

The FDA-CVM (Food and Drug Administration—Center for Veterinary Medicine) and NCIMS (National Conference on Interstate Milk Shipments) oversee third party validation and approval of screening tests for use in US antibiotic milk screening programs. These validations utilize raw commingled bovine milk, but do not address other species milk. Approved NCIMS tests need additional validation testing to be approved for screening other species milk. Previous validation studies of 4 screening tests in goat milk were conducted by Zeng et al. in 1997. The caprine industry has claimed the need for additional screening tests and reported currently approved tests are cost ineffective, prone to false positives during late lactation or time impractical to meet current milk screening regulations. This study was conducted to validate newer screening methods approved after 1997. The SL Beta-lactam test was evaluated for 5 Beta-lactam drug concentration responses using spiked commingled late lactation goat milk. The 90% detection levels with 95% confidence were found to be within 20% of manufacturers claimed detection levels in bovine spiked milk samples. Incurred milk samples using intra-mammary injections of penicillin G, amoxicillin, and cephapirin in goats in late lactation and again in early lactation were compared to spiked samples results using the SL Beta-lactam test and BSDA test. Results were comparable with results from previous studies. As a result of these findings it is likely that the SL Beta-lactam test will be added to the list of goat milk screening tests available to comply with milk screening regulations.

P088 Virulence Attributes of *Escherichia coli* O157:H7 Isolated from Dairies in East Tennessee

PAUL D. EBNER, Shelton E. Murinda, Barbara E. Gillespie, Stephen P. Oliver and Alan G. Mathew, University of Tennessee, Food Safety Center of Excellence, 2505 River Drive, 305 Brehm Animal Science, Knoxville, TN 37996, USA

A study was conducted to examine virulence attributes of *Escherichia coli* O157:H7 isolated from dairies in East Tennessee. Nine of ten test isolates were obtained from cull cow feces whereas the remaining isolate was obtained from bulk tank milk. A multiplex PCR (MP-PCR) was used to determine the presence of five virulence gene sequences (stx_1, stx_2, eaeA, *hly*933 and *fli*C$_h$7) commonly associated with the O157:H7 strain. Each isolate was also tested for susceptibility to 17 antibiotics or antibiotic combinations commonly used in human medicine and/or animal agriculture and screened for class I integrons using an MP-PCR that targeted *intI*, qac_1 and sul_1 gene sequences. Sixty-percent of the O157:H7 isolates tested positive for the presence of stx_1 gene sequences whereas 100% tested positive for stx_2 gene sequences. All isolates tested positive for presence of eaeA, *hly*933 and *fli*C$_h$7 gene sequences. The majority of isolates were susceptible to all test antibiotics and antibiotic combinations, with the exception of one isolate that was resistant to sulfonamides and another isolate that was resistant to both sulfonamides and streptomycin. No isolates were found to carry gene sequences indicative of the presence of class I integrons. These data confirm that *E. coli* O157:H7 isolated from dairy environments commonly possess the stx_2 gene, often in combination with stx_1. Most isolates, however, were highly susceptible to various antibiotics and none were found to carry specific DNA sequences that might aid in the acquisition of antibiotic resistance.

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P089 Incidence of *Brucella* spp., *Listeria* spp. and *Escherichia coli* O157:H7 in Raw Milk from Jalisco State, Mexico

E. CABRERA-DÍAZ, G. Partida-Gutiérrez, R. C. Olivares-Cruz, and M. R. Torres-Vitela, University of Guadalajara, Plan de Ayala #3368, Fraccionamiento Revolución, Guadalajara, Jalisco 45580, Mexico

Consumption of raw milk and unpasteurized milk products is still a common practice in Mexico. This may have a profound impact on public health. However, a careful food safety assessment is necessary for the dairy industry to implement effective measures for pathogen control and minimize the risk for consumers, especially related to products that do not receive a heat treatment. The purpose of this study was to investigate the presence of *Brucella* spp., *Listeria* spp. and *Escherichia coli* O157:H7 in raw milk. One hundred raw milk samples were collected from dairy farms in 19 localities in Jalisco State, Mexico. Testing for *Brucella* spp. was conducted by the WHO recommended method. *Listeria* spp. were tested for by use of USDA's and FDA's methods, whereas testing for *E. coli* O157:H7 was achieved by the method described in the FDA's BAM. *Brucella* spp. were isolated in 9% of the samples, with the species *B. mellitensis*, *B. suis* and *B. abortus* being identified in 6, 3 and 2% of the samples, respectively. Twenty-seven percent of the samples tested positive for *Listeria* spp, and the most frequent species were *L. innocua* (20%), *L. murrayi* (7%), *L. welshimeri* (3%) and *L. monocytogenes* (1%). *E. coli* O157:H7 was isolated in 6% of the samples. The isolation of these foodborne pathogens from raw milk corroborates the potential risk associated with consumption of unpasteurized milk or milk products.

P090 The Use of Ionizable Zinc to Increase the Efficacy of a Chlorhexidine Disinfectant Used in Mastitis Control

Geoffrey Westfall, CLAUDIA KOERTING, and Lynn Hinckley, University of Connecticut, 61 North Eagleville Road, Unit 3089, Storrs, CT 06269-3089, USA

Mastitis results from bacterial infection of the mammary gland. The control of this condition is extremely important to the dairy industry. Chlorhexidine is among a variety of disinfectants routinely used for the prevention of mastitis. An investigation was conducted to determine whether zinc compounds could further enhance the action of chlorhexidine. Major mastitis pathogens obtained from field samples were chosen fc- study: *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli*. Chlorhexidine (0.1%) was combined with 1% zinc acetate, 2% zinc gluconate, 2% zinc EDTA, or water only. Activity of these solutions was compared to iodine and 0.5% chlorhexidine. Possible antagonistic activity of the zinc compounds when combined with the 0.5% chlorhexidine was tested. The disinfectants were combined with bacteria, 10^7 CFU/ml in the presence of milk, and sampled in 15-second time intervals. Plate counts measured cell viability. A micro-well plate reader measured the kinetics of bacteria death. All the zinc compounds improved the activity of 0.1% chlorhexidine compared to the chlorhexidine with no zinc. Zinc gluconate provided the greatest synergistic action, reducing the bacteria count by 1 log in 1 min, with no survivors after one h. There was no decrease in bacterial viability in the presence of the zinc compounds alone within 1 h. The activity of 0.5% chlorhexidine was very rapid and was not affected by the presence of 2% zinc acetate or 4% zinc gluconate. The combinations of zinc compounds with chlorhexidine provide an additional hurdle for bacteria and can reduce the concentration of chlorhexidine required for disinfecting.

P091 A Rapid Screening Method to Test for Alkaline Phosphatase Activity in Cheese

KEN J. YOSHITOMI, FDA, 22201 23rd Drive SE, Bothell, WA 98021, USA

Foodborne outbreaks of disease resulting from improper pasteurization of dairy products have led to concern regarding the safety of dairy foods. Current methods to determine effective pasteurization involve assaying for native alkaline phosphatase (ALP) activity. This study investigated a rapid method to quickly screen for the presence of ALP activity in cheese with the use of a standard laboratory fluorometer. The substrate, 4-methylumbelliferyl phosphate (4MUP), produces a highly fluorescent product, 4-methylumbelliferone (4MU), in the presence of alkaline phosphatase enzyme. This product can be detected upon formation in the cheese homogenate background, negating the need for an extraction or precipitation step. The amount of fluorescence signal generated is directly proportional to the amount of ALP activity in the sample. Rate of fluorescent signal proved to be a better estimate of activity, since cheeses can produce a natural fluorescence background. A cheese sample (0.5g) was homogenized and spiked with 7 levels of ALP enzyme, from 0.0 to 10.0 ng, producing a highly linear curve (R_2 = 0.999). When the same spiked samples were assayed using the colorimetric Scharer Method, the fluorescent assay exhibited good correlation (R_2 = 0.990) with the colorimetric method. Upon sampling a variety of cheeses, increased sensitivity to spikes of ALP enzyme was observed with this fluorometric method than the colorimetric method. Designed as a rapid, inexpensive screening method for the presence of akaline phosphatase activity in cheese, the method can be extended to become quantitative with a standard curve or even adjusted to estimate phenol equivalents.

P092 Effect of Superoxidized Water and Hypochlorite Solutions on the Survival of *Escherichia coli* on Capsicum Fruit

HUGH MARTIN and Jean Taylor, Royal Agricultural College, Stroud Road, Cirencester, Gloucestershire, UK

The production of HOCl in solution has recently been developed through the electrolytic superoxidation of saline solution. This relatively new

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technology, which produces superoxidised water (EW), is now widely adopted for the cold sterilisation of endoscopes and other equipment in clinical practice. This paper explores the application of the biocidal properties of this product in contact with a food surface. A study was undertaken into the effect of EW on the survival of *Escherichia coli* (NCTC 9001) applied to the surface of green pepper (*Capsicum annuum*) fruits. The effectiveness of EW was compared with sodium hypochlorite solutions at pH 7. *E. coli* suspensions were applied using a micro-applicator technique which allowed precise quantities of known bacterial populations in the range of 10^7 to 10^8 CFU to be applied in droplet form to each fruit. Residual bacterial populations washed from the pepper surfaces after treatment showed that EW had significantly higher biocidal activity against *E. coli* than hypochlorite when used at concentrations similar to those used in commercial practice (50 to 150 ppm chlorine). Implications of these results in relation to food hygiene practice are discussed.

P093 DSC Survival of *Escherichia coli* O157:H7 and *Salmonella* Muenchen on Apples as Affected by Application of Commercial Fruit Waxes

STEPHEN J. KENNEY and Larry R. Beuchat, University of Georgia, 365 S. Pope St., Athens, GA 30605, USA

Consumption of unpasteurized apple cider has been associated with outbreaks of *Escherichia coli* O157:H7 and *Cryptosporidium parvum* infections. Some fruit waxes used for fresh-market apples contain chemicals such as alcohols, resins, and surfactants that have potential for antimicrobial activity. The primary objectives of this study were to determine if applying commercial waxes is lethal to *E. coli* O157:H7 and *Salmonella* Muenchen inoculated onto the surface of apples and to monitor survival characteristics of the pathogens during storage. Apples were spot inoculated with the two pathogens and spray coated with waxes. Apples were dried at 21°C or 55°C for 2 min before being subjected to microbiological analysis after storage for up to 12 weeks at 2°C or 21°C. Drying temperature did not significantly influence populations of *E. coli* O157:H7 and *S.* Muenchen. Waxing reduced populations of the pathogens by up to 1.48 $\log_{10}$ CFU/apple. Compared to untreated apples, treatment of apples with water or waxes resulted in significant reductions in populations of the pathogens during storage at 2°C. Reductions on waxed apples stored at 21°C were less compared to reductions on waxed apples stored at 2°C. Wax treatment should not be relied upon to kill or remove *E. coli* O157:H7 and *Salmonella* on raw apples.

P094 Preharvest Assessment of *Salmonella* spp. Contamination of Outer Rind of Cantaloupes in California

TREVOR SUSLOW, Marcella Zuñiga, Lorena Fernandez, and Bradley Butterfield, University of California-Davis, Dept. of Vegetable Crops, One Shields Ave., Davis, CA 95616-8631, USA

Two consecutive multi-state outbreaks of *Salmonella* Poona, linked to imported cantaloupe, during 2000 and 2001 seasons emphasized the need to continue developing regionally specific information about the baseline frequency of *Salmonella* contamination of cantaloupes. A survey of production fields has been conducted from 1999 to 2001 in central California. *Salmonella* spp. was not detectable on 978 individual cantaloupes. One melon from each field was inoculated with log 4.3 CFU stationary phase *Salmonella* Typhimurium LT2rif to establish a positive 'Control'. Air-drying routinely results in a Control population of log 2.5 to 2.7 CFU per melon. Excised rind was vigorously shaken in 200 ml buffered Peptone water 10 times, rubbed forcefully for 30 s, and then shaken 15 times. Wash buffer, 100 ml, was passed thorough a 0.45 mm filter. The membrane was placed in 7.0 ml Luria Broth, at 22°C for 30 min, and then held at 37°C for 4 h. Plating was conducted on Bismuth Sulfite Agar (BSA) and XLD agar. In addition, 5 ml was transferred to 5.0 ml of 2X-RV broth and incubated at 37°C for at least 6 but no more than 12 h. Plating was conducted from RV onto BSA and XLD. Finally, a 1.0 ml aliquot of the of RV broth was centrifuged; the pellet was washed 3X in sterile peptone water (SNPW) and resuspended in 50 ml SNPW, boiled 5 min and subjected to PCR detection using DNA primers for the invA gene of *Salmonella* spp. Positive detection by viable recovery on selective media and by *Salmonella*-specific DNA detection (PCR) was observed for each Control melon.

P095 DSC Inactivation of *Salmonella* during Drying of Roma Tomatoes Treated with Organic Acids

Y. YOON, P. A. Kendall and J. N. Sofos, Colorado State University, Dept. of Animal Sciences, Ft. Collins, CO 80523, USA

The objective of this study was to evaluate the influence of pre-drying treatments including blanching and dipping in organic acid solutions on inactivation, during drying at 60°C for 14 h of Roma tomatoes inoculated with a five-strain mixture of *Salmonella*. Pre-drying treatments included (1) no treatment; or 10 min immersion in (2) water; (3) 3.4% ascorbic acid; or, (4) 0.21% citric acid for both unpeeled-unblanched and unpeeled-blanched (steam-blanched for 3 min) tomato halves. Each tomato half was inoculated (7 log CFU/g) and left for 15 min to allow attachment before samples were treated with the appropriate solutions for 10 min, after which they were dehydrated. During dehydration and storage, samples were spread-plated on tryptic soy agar with 0.1% pyruvate (TSAP) and XLT-4 agar for bacterial enumeration. Bacterial populations decreased after dipping in water, ascorbic acid and citric acid for both unpeeled-unblanched and unpeeled-blanched tomato halves by 0.4 to 1.5 log CFU/g (TSAP) and 0.7 to 1.4 log CFU/g (XLT-4). Bacterial populations in control samples were reduced by 3.1 (TSAP) and 3.8 (XLT-4) log CFU/g in unpeeled-unblanched halves, and 4.0 (TSAP) and 4.4 (XLT-4) log CFU/g in unpeeled-blanched halves after 14 h of drying. Reductions of

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bacterial populations in water-treated unpeeled-unblanched and unpeeled-blanched halves were 3.3 to 3.6 (TSAP) and 5.0 to 5.6 (XLT-4) log CFU/g, while reductions achieved by drying of unpeeled-unblanched and unpeeled-blanched samples previously immersed in acid solution were 5.2 to 6.1 (TSAP) and 5.5 to 5.8 (XLT-4) log CFU/g. Results suggest that dipping in organic acid solutions may enhance inactivation of *Salmonella* during dehydration of tomato halves.

P096 Assessment of the Viral Quality of Reclaimed Wastewater for Food Crop Irrigation

DIMA KAYED, Pablo Gortares, Martin M. Karpiscak, Robert P. Freitas, Ralph Meer, and Charles P. Gerba, University of Arizona, Dept. of Soil, Water, and Environmental Science, Tucson, AZ USA

Norwalk-like viruses belong to the family Caliciviivadae and are an important cause of water- and food-borne gastroenteritis. The goal of this project was to assess the occurrence of human caliciviruses and enteroviruses in chlorine disinfected activated-sludge treated reclaimed wastewater that is being considered for food crop irrigation. One-hundred liter samples of the wastewater were concentrated using positively charged 1MDS or MK filters. Reconcentration of the filter eluates was accomplished by organic flocculation using beef extract. The viral RNA in the final 20 to 25 ml volume was then extracted and tested using primers for the Norwalk-like virus and enteroviruses (these primers were capable of detecting polio, echo, and Coxsackie viruses) using semi-nested PCR. Out of a total of 16 samples, two were positive for enteroviruses and one for Norwalk-like virus. Although viability of the viruses could not be assessed by the methods used to detect the viruses, the results suggest that both of these viruses are detectable in the reclaimed water. Studies are now underway to assess the presence of infectious enteroviruses in the reclaimed wastewater, using animal cell culture.

P097 DSC Reduction of *Escherichia coli* O157:H7 on Alfalfa Seeds Following Exposure to Trans-2-Nonenal

M. AUCHTER and M. C. Newman, University of Kentucky, Dept. of Animal Science, 204 W. P. Garrigus Bldg., Lexington, KY 40546-0215, USA

The volatile trans-2-nonenal was evaluated for its ability to eliminate *E. coli* O157:H7 on alfalfa seeds. The seeds were inoculated with approximately 2.5×10^2 CFU/g *E. coli* O157:H7. The analyses were performed in triplicate with 2 grams alfalfa seeds on one side of a split petri dish and the volatile on the other side. The petri dishes were then placed in plastic bags, sealed and held at room temperature for 2, 4, 6, 24, and 48 h. The treatments were 0, 10, 25, and 50 micrograms trans-2-nonenal for each time interval. The most effective treatments were 25 and 50 micrograms of trans-2-nonenal, reducing the *E. coli* O157:H7 by 53.67% and 68.15%, respectively. These volatile concentrations had no effect on the percent germination, with the average germination rate of both the inoculated and control seeds approaching 69%.

P098 Comparision of Subsurface and Furrow Irrigation in the Viral Contamination of Iceberg Lettuce

SCOTT STINE, Inhong Song, Faezah Manshadi, Jose Pimentel, Christopher Y. Choi, and Charles P. Gerba, University of Arizona, Dept. of Soil, Water, and Environmental Science, Tucson, AZ 85721, USA

The goal of this project was to evaluate the effectiveness of subsurface drip irrigation (SDI) in preventing plant and soil contamination by enteric viruses during irrigation of iceberg lettuce in arid- and semi-arid lands. Coliphages similar in shape and size to human enteric viral pathogens were used in this study. A total of eight plots of iceberg lettuce were grown, four irrigated by furrow irrigation (FI) and four by SDI. Once the lettuce heads had formed, the plots were irrigated with dechlorinated reclaimed domestic wastewater to which coliphage MS-2 and/or PRD-1 had been added. The viruses were added to the irrigation water to achieve concentrations of between 10,000 and 1,000,000 PFU/ML. Soil and lettuce samples were collected at various times after irrigation and tested for the coliphage. The coliphage was eluted from the surface of the outer lettuce leaves and soil using 3% beef extract. Background samples were also collected of the irrigation water for the presence of coliphage. The coliphage survived longer in soil samples near the surface and 10 cm below the surface in SDI plots than in the FI plots. A greater amount of lettuce contamination also occurred with the SDI. These results suggest that less contamination by viruses occurs during FI irrigation.

P099 *Salmonella* Enteritidis Infections Associated with Mung Bean Sprouts, California, 2000

J. C. MOHLE-BOETANI, J. Farrar, P. Bradley, M. Miller, K. Cummings, and S. B. Werner, California Dept. of Health Services, 2151 Berkeley Way, Berkeley, CA 94704, USA

We investigated an outbreak of *Salmonella* Enteritidis (SE) gastroenteritis phage type 33 (PT33), a type rarely recovered in the US. We identified 67 laboratory-confirmed case-patients in 2 adjoining California counties; 3 patients were hospitalized. Additional cases resided in Nevada, Oregon, and Massachusetts. To identify a source, we conducted 2 case-control studies. The first study identified an association with eating a cold appetizer at a Vietnamese restaurant chain (odds ratio (OR) =45, P=0.001). The second study demonstrated that eating a specific appetizer ingredient, raw mung bean sprouts, was associated with disease (90% of cases vs. 5% of controls, OR= 171, $P < 0.001$). This is the first time that mung bean sprouts have been implicated in an outbreak in the US. A traceback investigation revealed that the sprouts were from a single sprout grower.

An environmental investigation at the sprout grower recovered SE PT33 from irrigation water and a drain. The sprout grower inadequately decontaminated seeds and continued to use seed lots found to yield *Salmonella* on routine cultures of irrigation water in the weeks before and during the outbreak. Five

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subsequent outbreaks of SE associated with raw mung bean sprouts occurred in the US, Canada, and the Netherlands. These outbreaks highlight the need for all sprout growers to follow FDA guidelines, including disinfection of sprout seeds and testing of irrigation water. Public health officials should continue to advise the public, especially those at high risk of complications from *Salmonella* infections, of the risk of eating all raw sprouts, including bean sprouts.

P100 Inactivation of GFP-Transformed *Escherichia coli* O157:H7 by Sanitizers on Lettuce and Strawberries as Determined by Confocal Scanning Laser Microscopy

DSC

STEPHANIE L. RODGERS, Joanne H. Whallon, and Elliot T. Ryser, Michigan State University, Dept. of Food Science and Human Nutrition, East Lansing, MI 48824-1225, USA

Attachment and viability of GFP-transformed *E. coli* O157:H7 (strain E318) on leaf lettuce and strawberries were evaluated in response to a 5-min exposure to water and four different sanitizers —Fit™ (as recommended), sodium hypochlorite (200 ppm chlorine), chlorine dioxide (3 ppm) and ozone (2 ppm)—using standard plating techniques and confocal scanning laser microscopy (CSLM). Whole lettuce leaves and strawberries were inoculated by submersion in an *E. coli* O157:H7 suspension prepared from an 18 to 24 h broth culture to obtain 10^8 CFU/g. Visualization of *E. coli* O157:H7 on the surface of lettuce and strawberries by CSLM at an excitation wavelength of 488 nm revealed generalized non-specific attachment to surface structures with some penetration through intact surfaces up to approximately 20 mm. *E. coli* attachment to both products was more strongly influenced by water deposition and pooling than by any affinity to stomates or other surface structures. Fit™ was the least effective (~1.25 log reduction on either product) sanitizer tested and was not significantly different from water, while ozone and chlorine dioxide were most effective, yielding reductions of 3.77 and 3.55 logs on lettuce and 3.28 and 3.17 logs on strawberries, with these differences not significant. Sodium hypochlorite was significantly different from the other treatments, giving reductions of 2.95 and 2.34 logs for lettuce and strawberries, respectively. Based on CSLM analysis, viable *E. coli* cells that survived sanitizer treatments did so by organizing into groups or clusters in areas of pooling rather than by penetrating through intact produce surfaces.

P101 An Outbreak of *Salmonella* Serotype Kottbus Infections Associated with Raw Alfalfa Sprouts

K. L. Winthrop, M. S. PALUMBO, J. A. Farrar, J. Mohle-Boetani, S. Abbott, G. Inami, and S. B. Werner, California Dept. of Health Services, Food and Drug Branch, 601 N. 7th St. MS 357, P.O. Box 942732, Sacramento, CA 94234, USA

Raw sprouts have frequently been implicated as a vehicle in food-borne disease outbreaks. Because contaminated seeds usually cause these outbreaks, many sprout producers disinfect seeds before germination and attempt to detect sprout contamination during the production. In March 2001, we detected an increased number of *Salmonella* serotype Kottbus isolates. Overall, we identified 31 cases from three western states. To identify the cause, we conducted a case-control study with the first 10 identified cases and 20 controls matched to cases by age, sex, and residential area. Our case-control study found illness to be statistically associated with alfalfa sprout consumption. The traceback investigation implicated a single sprouter, where environmental studies yielded *S.* Kottbus from ungerminated seeds and floor drains within the production facility. Pulsed field gel electrophoresis (PFGE) patterns of all patient, seed, and floor drain *S.* Kottbus isolates were indistinguishable. Most implicated sprouts were from seeds that underwent heat pasteurization and soaking with a 2,000 ppm sodium hypochlorite solution. Other implicated seeds had been soaked in a calcium hypochlorite solution that, when tested, measured only 11,000 ppm. Producer screening tests of irrigation water detected *Salmonella* in January and, if properly confirmed, may have averted this outbreak. Although negative, confirmatory tests of these samples inappropriately utilized refrigerated irrigation water instead of the positive samples' enrichment broth. Producers should properly perform these screening tests, and until effective technologies are developed, they should adhere to FDA recommendations for sprout seed disinfection with 20,000 ppm calcium hypochlorite.

P102 Survival and Growth of *Salmonella* spp. on Fresh-cut Cantaloupe Cubes and Rind Following Electron Beam Irradiation

AUBREY MENDONCA, Maria Romero, Ainura Orozalieva, and Floyd Woods, Iowa State University, Dept. of Food Science and Human Nutrition, 2313 Food Sciences Bldg., Ames, IA 50011, USA

The effect of irradiation on survival and growth of salmonellae on cubes of cantaloupe flesh and external rind surface was investigated. Fresh-cut cubes and rind were inoculated with a mixture of *Salmonella* (*S.* Typhimurium, *S.* Chester, *S.* Poona, and *S.* Stanley) to give ~ 2.0×10^6 CFU/g and 5.0×10^6 CFU/rind, respectively. Inoculated samples in sterile plastic containers were irradiated with 0.0 (control), 0.5, and 1.0 kGy, then held at 5°C (14 days) or 25°C (24 h). Viable salmonellae were enumerated by surface plating samples onto Hecktoen enteric agar (HEA) and incubating inoculated HEA plates at 35°C for 48 h. Initial populations on cubes and rind decreased with increasing radiation dose. Numbers of survivors on cubes and rind were 3.9×10^2 CFU/g and 9.1×10^2 CFU/rind, respectively, following irradiation with 1.0 kGy. Growth of survivors on cubes (25°C) was rapid. At 12 h viable counts on cubes irradiated at 0.0, 0.5, and 1.0 kGy, increased to 1.5×10^9, 1.7×10^7, and 2×10^6 CFU/g, respectively; viable counts on rind were 2.1×10^8, 3.2×10^6, and 1.7×10^6 CFU/rind, respectively. Final numbers were about 1.2 $\log_{10}$ greater on cubes than on rind. Numbers of survivors

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on cubes or rind did not increase during 14 days of storage at 5°C. These results indicate that electron beam irradiation (1.0 kGy), followed by low temperature (5°C) storage, seems to have good potential for control of salmonellae on fresh-cut cantaloupe.

P103 Inactivation of *Salmonella* during Drying and Storage of Gala Apples Treated with Acid or Sodium Metabisulfite Solutions

Patricia Di Persio, P. A. Kendall, M. Calicioglu, and J. N. SOFOS, Colorado State University, Dept. of Animal Sciences, Ft. Collins, CO 80523-1171, USA

The recent association of foodborne illness associated with dried foods has indicated a need for research in home food drying. Apple slices are often pre-treated with acid or metabisulfite solutions to retain color during dehydration. The objective was to determine whether pre-treating inoculated Gala apple slices with acid or metabisulfite solutions altered survival of *Salmonella* during dehydration and storage. The inoculated (five strain mixture, 7.6 log CFU/g) slices were pre-treated, dried for 6 h at 60°C, and stored aerobically at 25°C for 30 days. Pre-drying treatments included: (1) no treatment; or 10 min immersion in: (2) sterile water, (3) 3.4% ascorbic acid (AA), (4) 0.21% citric acid (CA), or (5) 4.18% sodium metabisulfite (SM) solutions. Samples were plated on tryptic soy agar with 0.1% pyruvate (TSA), BG sulfa (BGS), and XLT-4 agar for enumeration of bacteria. Populations were not (P >0.05) reduced by immersion in water but were reduced by 0.4 (TSA), 1.3 (BGS), and 5.4 (XLT-4) log CFU/g by immersion in SM. Immersion in acidic solutions reduced populations by 0.7 to 1.1 log CFU/g. After 6 h of dehydration, bacterial populations on untreated or water treated slices were reduced by 2.7-2.8 (TSA), 2.7-2.9 (BGS), and 4.0-4.2 (XLT-4) log CFU/g. In acidic and SM treatments, populations were reduced by 3.8 to 5.2 (TSA), 3.9 to 5.5 (BGS), and 5.5 to 5.7 (XLT-4) log CFU/g after 6 h dehydration. Bacterial populations were detectable after 30 days except on slices treated with AA. Results suggest that acid or sodium metabisulfite pre-treatments may enhance inactivation of *Salmonella* during drying of apple slices.

P104 DSC Attraction of a Free-living Nematode, *Caenorhabditis elegans*, to *Escherichia coli* O157:H7 and *Salmonella*, and Its Potential as a Vector for Preharvest Contamination of Fruits and Vegetables

KRISHAUN N. CALDWELL, Gary L. Anderson, Phillip L. Williams, and L^rry R. Beuchat, University of Georgia, CFS, Dept. of Food Science and Tech., 1109 Experiment St., Griffin, GA 30223-1797, USA

A bacterivorous free-living nematode found in soil, *Caenorhabditis elegans*, was studied to determine its potential role as a vector for preharvest contamination of fruits and vegetables with foodborne pathogens. Initial studies revealed that *C. elegans* is attracted to *Listeria innocua* and avirulent strains of *Escherichia coli* and *Salmonella* Typhimurium. The attraction of worms to seven strains of *E. coli* O157: H7 and eight serotypes of *Salmonella* was then determined. Adult worms (20 to 30) were placed on K-agar plates 0.75 cm from a 24-h bacterial colony; 10 µl of tryptic soy broth (TSB) was placed 0.75 cm from the worms as a control. The number of worms that migrated to colonies and TSB after incubation of plates at 37°C for 5, 10, 15, and 20 min was determined, followed by incubation at 20°C for up to 7 days to determine the ability of *C. elegans* to survive and reproduce in bacterial colonies. The nematode was attracted to colonies of all test strains and serotypes. The percentages of worms attracted to *E. coli* O157:H7 and *Salmonella* colonies within 20 min were 31 to 67% and 65 to 87%, respectively. *C. elegans* survived and reproduced within colonies of all strains and serotypes for up to 7 days. Results indicate that *C. elegans* is capable of feeding on and surviving in colonies of pathogenic bacteria, and may play a significant role in the preharvest dispersal of incidental human pathogens in soil to raw fruits and vegetables.

P105 Cross-contamination of Lettuce by *Escherichia coli* O157:H7 via Contaminated Ground Beef

MARIAN R. WACHTEL, James L. Mc Evoy, Yaguang Luo, Anisha Williams-Campbell, and Morse B. Solomon, USDA-ARS-BARC-W, Produce Quality and Safety Lab, Bldg. 002, Rm. 117, 10300 Baltimore Ave., Beltsville, MD 20705, USA

Bacterial pathogens may cross-contaminate produce via a number of scenarios. The cross-contamination of lettuce was simulated using ground beef containing enterohemorrhagic *Escherichia coli* O157:H7 (EHEC). A nalidixic acid-resistant derivative of EHEC strain F6460, previously associated with an outbreak linked to lettuce in a restaurant setting, was used to contaminate ground beef for these studies. F6460 NalR multiplied 2 to 3 log CFU/g in retail 80% and 93% lean ground beef stored at room temperature for 16 h. Background microflora detected in retail ground beef was typical for fresh meat. Significant numbers of F6460 NalR were recovered from latex-gloved fingers after the formation of contaminated hamburger patties. The pathogen was consistently recovered from a plastic cutting board after contaminated ground beef patties contacted this surface. Upon 16 h incubation at room temperature, the pathogen level on cutting boards decreased ~ 1 log CFU. F6460 NalR w^s easily transferred to iceberg lettuce leaves that had contacted the contaminated cutting board surface. Because the infectious dose for EHEC O157:H7 may be as low as 50 CFUs, the numbers of pathogens transferred to lettuce via contaminated cutting boards or hands may represent a true food safety risk for the preparation of raw vegetable salads.

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P106 The Microbiological Examination of Prepared Ready-to-eat Salad Vegetables from Retail and Catering Premises in the United Kingdom

CHRISTINE L. LITTLE, Satnam K. Sagoo, and Robert T. Mitchell, Public Health Laboratory Service, Environmental Surveillance Unit (CDSC), 61 Colindale Ave., London NW9 5EQ, UK

Prepared salad vegetables have become increasingly popular due to consumer demand for fresh, healthy, convenient and additive-free foods that are safe and nutritious. During 2001, two studies of prepared ready-to-eat salad vegetables were undertaken to determine the microbiological quality of these vegetables on sale in the UK. Examination of pre-packed prepared salad vegetables from retail premises and open prepared salad vegetables from customer self service bars and food service/preparation areas in catering and retail premises revealed that most (98.4%; 6655/6766) were of satisfactory/acceptable microbiological quality, and 104 (1.5%) were unsatisfactory. However, seven (0.1%) samples were of unacceptable microbiological quality due to the presence of *Salmonella* spp. (*S.* Newport (1); *S.* Umbilo (3); *S.* Durban (1) and *L. monocytogenes* (2) above 100 CFU/g indicating a risk to health. In each case the retailer and the UK Food Standards Agency were immediately informed and remedial action taken. Twenty cases of *S.* Newport were subsequently identified throughout England and Wales. The outbreak strain of *S.* Newport isolated from the salad and human cases had a unique plasmid profile. Unsatisfactory results were due to *Escherichia coli* and *Listeria* spp. (not *L. monocytogenes*) levels above 10^2 CFU/g. *Campylobacter* spp. and *E. coli* O157 were not detected in any of the samples examined. The presence of *Salmonella* spp. and high levels of *L. monocytogenes* is unacceptable and highlights the necessity of applying good hygiene practices during production and processing and at retail and catering to prevent cross-contamination and/or bacterial growth occurring in these salad products.

P107 DSC Passage of *Escherichia coli* O157:H7 from Contaminated Water to Lettuce is Dependent on Irrigation Methodology

ETHAN B. SOLOMON and Karl R. Matthews, Rutgers University, Cook College, Dept. of Food Science, 65 Dudley Road, New Brunswick, NJ 08901, USA

In 1999, 5 of the 18 foodborne outbreaks of *Escherichia coli* O157:H7 in the US were linked to the consumption of fresh fruits and vegetables. The number of outbreaks traced to fresh produce continues to increase. Contamination of produce may occur during on-farm contact with contaminated irrigation water. In our study, we demonstrate the transmission of *E. coli* O157:H7 from contaminated irrigation water to lettuce plants by both spray and surface irrigation methods using marker bacteria. *E. coli* O157:H7 expressing green fluorescent protein were utilized, enabling easy detection with UV illumination. Three lots of 48 lettuce plants were started at 10-day intervals so that on the day of the experiment, plants aged 20, 30, and 40 days were available. Each lot was divided into two groups of 24 plants and irrigated either by applying water containing 10^7 CFU/ml of *E. coli* O157:H7 to the soil only (ensuring that no inoculum contacted the edible portion of the plant), or by spraying the edible portion of the plant. The forty-day-old plants were processed the following day to detect the presence of *E. coli* O157:H7. Twenty and thirty day old plants were grown to 40 days of age (while being irrigated with potable water) before being processed. For all treatments combined, a greater number of plants were positive following a single exposure to *E. coli* O157:H7 through spray irrigation (51 of 61) than via surface irrigation (8 of 64). *E. coli* O157:H7 persisted for 20 days following spray irrigation. Immersion of contaminated lettuce into 200 ppm chlorine was not effective in eliminating *E. coli* O157:H7.

P108 Effect of Acid Adaption on Inactivation of *Escherichia coli* O157:H7 during Drying of Apple Slices

Suman Priya Lakkakula, PATRICIA A. KENDALL, John Samelis, and John N. Sofos, Colorado State University, Dept. of Food Science and Human Nutrition, Ft. Collins, CO 80523-1571, USA

Acid-adapted *Escherichia coli* O157:H7 may pose a threat to the food industry due to increased survival under acid stress. While the combined treatments of acidity and drying inactivate nonacid-adapted *E. coli* O157:H7, no work has evaluated these effects on acid-adapted cultures during apple drying. The objective was to assess bacterial survival on Gala apple slices inoculated with 5-strain acid-adapted or nonadapted cultures of *E. coli* O157:H7 and not pre-treated, or pre-treated with sterile water or 1.7% citric acid for 10 min or 18 h (4°C), followed by drying at 57.2°C for 6 h. Samples were plated on tryptic soy agar (TSA), sorbitol MacConkey agar with cefixime and tellurite supplement (SMAC-CT), and modified eosin methylene blue agar (MEMB). Bacterial changes following pre-treatment (10 min and 18 h) were +0.2 to -0.6 log CFU/g and -0.8 to -1.5 log CFU/g for inoculated slices pre-treated in sterile water and citric acid, respectively. After 6 h dehydration, bacterial populations on control and sterile water treated slices were reduced by 1.7 to 2.8 (TSA), 2.3 to 3.3 (SMAC-CT) and 1.5 to 1.7 (MEMB) log CFU/g when inoculated with nonadapted cultures, and by 2.4 to 3.2 (TSA), 3.1 to 4.3 (SMAC-CT) and 2.6 to 4.3 (MEMB) log CFU/g when inoculated with acid-adapted cultures. In comparison, bacterial reductions on citric acid treated slices following 6 h dehydration were 5.1 to 5.4 log CFU/g for both acid-adapted and nonadapted cultures with one exception (3.6 log CFU/g reduction for nonadapted cells recovered on TSA). Results suggest that pre-treating apple slices with citric acid enhances inactivation, while acid adaptation does not affect inactivation of *E. coli* O157:H7 during drying.

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P109 Inhibition of Sprout Pathogenic Fungi Growth Using Allyl Isothiocyanate Vapor

KANAKO FURUYA, Shigeo Miyao, and Kenji Isshiki, Daikin Environmental Laboratory, Limited, 3 Miyukigaoka, Tsukuba, Ibaraki 305-0841, Japan

Sprout seeds contaminated with pathogenic fungi cause putrefaction of sprouts. Sterilization treatment for seeds should be done safely because sprouts are usually consumed within 10 days after the treatment. We tried sterilization of sprout seeds using allyl isothiocyanate (AIT), the main flavoring component of wasabi (Japanese horseradish) and mustard. Maximal AIT dose that does not inhibit sprout seed germination was determined first. Sprout seeds were then exposed to AIT under 20 ppm, 24 h, RH100%, which is within the maximal possible dose obtained, after being inoculated with pathogenic fungi isolated from mung bean sprouts. After exposure, they were incubated for 14 days at 25°C to determine the ratio of disease. Growth of mung been sprouts was affected by dose of AIT, AIT concentration multiplied by exposure time. AIT dose less than 600 ppm/h did not inhibit sprouts growth. AIT exposure under 20 ppm RH100% for 24 h prevented the damage caused by *Colletotrichum gloeosporioides*, *Alternaria alternata* and *Fusarium oxysporum* without influence on sprouts growth. It is desirable to use chloride treatment and AIT treatment together. AIT treatment could inhibit chloride resistant disease, but AIT treatment alone did not prevent some diseases for which chloride treatment was effective.

P110 Reduction of *Escherichia coli* K12 on Alfalfa Seeds by Supercritical Carbon Dioxide Treatment

Angela M. Mazzoni, ALI DEMIRCI, Gregory R. Ziegler, and Ratna R. Sharma, Pennsylvania State University, 231 Agricultural Engineering Bldg., University Park, PA 16802, USA

The supercritical carbon dioxide (SC-CO_2) process involves pressurizing CO_2 in a chamber which generates liquid phase of carbon dioxide. Pressurized liquid CO_2 has a strong extraction capability of organic and inorganic compounds. Recent studies have also demonstrated that the antimicrobial effect of SC-CO_2 is due to extraction of some cellular components of microorganisms. The efficacy of a supercritical carbon dioxide treatment on alfalfa seeds contaminated with *Escherichia coli* K12 was tested at 2000, 3000, and 4000 psi at 50°C. Samples were treated for 15, 30, and 60 min at each pressure. After pummeling of the seed samples in 0.1% peptone water, the initial and final *E. coli* on the seeds were determined by plating on 3M Petri Films. After 48 h of incubation at 37°C, the colonies were enumerated. Treated seeds were evaluated in terms of germination characteristics. Experiments were repeated for statistical purposes. The reductions of *E. coli* for 2000, 3000, and 4000 psi treatments at 15 min were 26.6, 68.1, and 81.3%, respectively. As the time was increased from 15 to 60 min at 4000 psi, the percent *E. coli* reduction increased from 81.3% to 92.8%. The percent germination for all treatments was over 90%. Supercritical carbon dioxide treatments demonstrated a reduction of *E. coli* without affecting the germination characteristics of alfalfa seeds ($P < 0.05$). This study was a step in the direction of improving safety of alfalfa seeds used to produce fresh sprouts, which have been the cause of several outbreaks.

P111 DSC Efficacy of Chlorine and Calcinated Calcium Treatment of Alfalfa Seeds and Sprouts to Eliminate *Salmonella*

MEGHA GANDHI and Karl R. Matthews, Rutgers, The State University of New Jersey, Cook College, Dept. of Food Science, 203 Food Science Bldg., 65 Dudley Road, New Brunswick, NJ 08901, USA

A number of recent outbreaks of foodborne illness have been linked to the consumption of contaminated alfalfa sprouts. To control pathogens associated with sprouts, various sanitizing schemes have been tried but ultimately have proven to be of limited efficacy. In the present study, the efficacy of spray application of chlorine (100 ppm) to eliminate *Salmonella* during germination and growth of alfalfa was assessed. Alfalfa seed artificially contaminated with *Salmonella* was treated at germination, on day 2 or day 4, or for the duration of the growth period. Spray application of 100 ppm chlorine at germination, day 2, or day 4 of growth was minimally effective, resulting in approximately a 0.5 log decrease in population of *Salmonella*. Treatment on each of the four days of growth reduced populations of *Salmonella* by only 1.5 log. *Salmonella* populations reached >7.0 log on sprouts grown from seeds artificially contaminated with *Salmonella* and then treated with 20,000 ppm $Ca(OCl)_2$. Combined treatment of seeds with 20,000 ppm $Ca(OCl)_2$ and followed by treatment with 100 ppm chlorine during germination and sprout growth did not eliminate *Salmonella*. Studies are in progress evaluating the efficacy of applying 20,000 ppm $Ca(OCl)_2$ to seeds followed by germination and growth of sprouts in water containing calcinated calcium. Based on preliminary studies the combination of these treatments should control *Salmonella* associated with alfalfa sprouts.

P112 Inactivation of *Escherichia coli* O157:H7 on Alfalfa Seeds and Sprouts by Ozonation

Ratna R. Sharma, ALI DEMIRCI, Larry R. Beuchat, and William F. Fett, Pennsylvania State University, 231 Agricultural Engineering Bldg., University Park, PA 16802, USA

Chemical and heat treatments to eliminate pathogens on inoculated seeds and sprouts have shown little success. This study investigated the antimicrobial potential of ozone on alfalfa seeds and sprouts. Alfalfa seeds inoculated with *Escherichia coli* O157:H7 were immersed in water containing 4, 8, 10, and 21 ppm ozone for 2, 4, 8, 16, 32, 64 min at

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4°C. Population reductions ranged between 0.40 and 1.75 log_{10} CFU/g. Alternatively, direct ozone sparging of seeds in water, for similar time intervals, reduced populations by 2.21 log_{10} CFU/g for a 64 min treatment. Subsequent exposure of ozone sparged seeds to heat at 40, 50 and 60°C for 3 h reduced populations to levels undetectable by direct plating (4 to 4.8 log_{10} CFU/g) at 60°C. Direct sparged seeds were subjected to low hydrostatic pressure of 12 psi for 5 min to increase accessibility of ozone into the cracks of seeds. The highest inactivation was 2.03 log_{10} CFU/g and was not significantly different from control. Addition of surfactants during pressurization significantly reduced ozone's effectiveness. Hydrostatic pressure treatments at 8 and 12 psi without direct ozone sparging were not significantly different. The reductions ranged between 0.72 and 1.62 log_{10} CFU/g for the 12 psi treatment. Ozone did not have a detrimental effect on seed germination. Immersion of inoculated alfalfa sprouts in water containing 21ppm ozone for 64 min reduced the populations by 0.85 log_{10} CFU/g. Direct ozone sparging resulted in 2.19 log_{10} CFU/g reduction, while direct ozone sparging followed by exposure to low hydrostatic pressure at 12 psi resulted in 2 log_{10} CFU/g. This study did not demonstrate ozone's lethality in eliminating *E. coli* O157:H7 on alfalfa seeds and sprouts. Further investigation is required on decontamination of sprouting seeds to reduce health risk.

P113 UV-C Destruction of *Salmonella* spp. and *Escherichia coli* O157:H7 on the Surface of Agar and Fresh Produce

B. R. YAUN, S. S. Sumner, J. D. Eifert, and J. E. Marcy, Virginia Tech., Dept. of Food Science and Tech. (0418), Blacksburg, VA 24061, USA

Application of UV-C has been shown to be effective against common foodborne pathogens such as *Campylobacter*, *Salmonella*, and *Escherichia coli*. To apply this technology to fresh fruits and vegetables, studies were undertaken to determine the efficacy of direct UV-C radiation on the surface of fresh produce. The antimicrobial wavelength of ultraviolet light at 254.7nm (UV-C) was applied to cocktail strains of naladixic acid resistant *Salmonella* and *Escherichia coli* O157:H7. To determine a baseline for the efficacy of the UV-C treatment on microbial reductions, Tryptic Soy Agar + 50 ppm naladixic acid (TSAN) plates were inoculated with known concen-trations of cells and subjected to different UV-C treatments. Doses applied ranged from 1.5 to 30 mW/cm^2. Serial dilutions were performed and inoculation levels tested included 10^1 to 10^8 CFU/ml for each of the selected pathogens. Results for both organisms yielded sigmoidal inactivation curves. A 5 log reduction of *Escherichia coli* O157:H7 was observed at doses exceeding 8.36 mW/cm^2, while a 5 log reduction for *Salmonella* spp. was observed at doses exceeding 14.2 mW/cm^2. Spot inoculation of apples, tomatoes and lettuce with *E. coli* O157:H7 or *Salmonella* spp. treated with a dose range of 1.5 to 30 mW/cm^2 resulted in at least a 3 log reduction of the pathogen on the surface of the produce. Results demonstrate that UV-C could be effective when used in conjunction with other treatments to produce a 5 log reduction of pathogens on the surface of fresh produce within a processing facility.

P114 Influence of Calcium Lactate on the Fate of Pathogenic and Spoilage Microorganisms in Orange Juice

JINRU CHEN, Joy G. Adams, and Jui-Yueh Yeh, CFS, University of Georgia, 1109 Experiment St., Griffin, GA 30223, USA

Calcium lactate is used by the beverage industry as a source of calcium to fortify fruit juice. The objective of this study was to investigate the influence of calcium lactate on the fate of pathogenic and spoilage microorganisms in orange juice. Non-fortified orange juice was supplemented with calcium lactate at a concentration equivalent to 5, 10, 15, 20, 25, or 30% Reference Dietary Intake (RDI). Juice pH was adjusted to 3.6 or 4.1. Each fortified juice was inoculated separately with a 3-strain mixture of *Salmonella*, a 3-strain mixture of spoilage yeasts, and 3 single strains of spoilage bacteria, including *Alicyclobacillus acidoterrestris*, *Lactobacillus plantarum*, and *L. sake*. Contaminated juice was stored at 4 or 10°C for 7 weeks. Juice was assayed once a week for populations of *Salmonella*, spoilage yeasts and spoilage bacteria. *A. acidoterrestris* was inhibited in all juice at 4°C and in low pH juice at 10°C. But the bacterium was able to grow at 10°C in high pH juice containing calcium lactate equivalent to 5% RDI. *L. sake* declined and eventually died off in low, as well as in high, pH juice at 4 and 10°C. Calcium lactate inhibited *L. plantarum*, which did not increase in any juice at either storage temperature. While inhibited in all juices at 4°C, spoilage yeasts grew in low and in high pH juices at 10°C. *Salmonella* died off in all juices at 4°C and in low pH juice at 10°C. However, cells persisted at 10°C in high pH juice.

P115 Evidence of *Salmonella* Internalization into Fresh Mangoes during Simulated Post Harvesting Procedures

Ana L. Penteado, BRIAN S. EBLEN, and Arthur J. Miller, CFSAN-FDA, 5100 Paint Branch Pkwy., College Park, MD 20740, USA

Concern about tephritid fly larvae has resulted in an importation requirement for heat treatment of all mangoes exported from the West Indies, South America, and Central America. Although only a heat treatment is required for importation, processors often use cooling procedures to extend the shelf life of the fruit. Recently, two US salmonellosis outbreaks were epidemiologically associated with consumption of fresh mangoes. Here, studies simulated heat disinfestations required by the US and product cooling procedures, to assess their potential to promote *Salmonella* infiltration into mangoes. The conditions modeled were those used by one farm/packer, which was implicated with one outbreak using traceback procedures. Untreated green and ripened

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Tommy Atkins variety mangoes (n=60) were immersed in water at 46°C for 90 min, followed by immersion at 22°C for 10 min into water containing 10^7 CFU/ml GFP labeled *S.* Enteriditis. Fruit was then stored at 10, 20, or 30°C for up to 1 wk. Green and ripened mangoes were positive for infiltration at a rate of 80% and 87%, respectively. Infiltration frequency into the stem portion (83%) was significantly higher ($P < 0.01$) than infiltration into the middle (19%) or bottom (9%) sections. The degree of fruit ripeness, post treatment holding temperature, or duration of storage had no significant effect on infiltration or survival of *Salmonella* inside mangoes. This study demonstrates potential pathogen internalization if heat-treated mangoes are cooled using contaminated water. Results demonstrate the need for further research to determine fruit processing parameters that eliminate fruit fly larvae and protect human public health.

P116 Screening of Potential Bacterial Pathogen Surrogates for Antibiotic Resistance

DSC

ARUNA PERI, Claudia Rodriguez, Nicole Maks, Jodie Ulaszek, Susanne Keller, Sadhana Ravishankar and Peter Slade, The National Center for Food Safety and Technology, IIT Moffett Campus, 6502 South Archer Road, Summit-Argo, IL 60501, USA

The objectives of this study were to isolate antibiotic resistant *Enterobacteriaceae* from organic produce and to determine if the resistance for each isolates is plasmid- or chromosomally-mediated. Organic produce (various salad mixes) was washed by agitating in buffered peptone water (w/v) for two min. The wash was then screened for antibiotic resistant *Enterobacteriaceae* by plating on Violet Red Bile Agar containing appropriate concentrations of one of the following antibiotics: ampicillin, erythromycin, gentamycin, streptomycin and tetracycline. Following confirmation of the antibiotic resistance and screening for possible multiple antibiotic resistance by use of the disk diffusion assay, isolates were further characterized using API 20E test strips. Identified isolates were screened to determine whether antibiotic resistance was plasmid- or chromosomally-mediated. Plasmids were extracted and then detected by agarose gel electrophoresis. Curing of putative plasmids mediating antibiotic resistance was attempted by growing each isolate in BHI+acriflavin followed by plating on BHI and BHI containing the appropriate antibiotic(s). The bacteral isolates identified from organic produce were *Pantoea* spp., *Enterobacter cloacae, Enterobacter sakazakii, Citrobacter freundii, Serratia odorifera, Serratia marcescens, Vibrio fluvialis, Klebsiella pneumoniae* and *Klebsiella oxytoca*. Those isolates determined to have chromosomally-mediated antibiotic resistance will be used in further stress tolerance studies and compared to foodborne pathogens. Isolates that compare favorable may provide appropriate surrogates for future food safety related studies where the actual use of pathogens may provide a great risk to laboratory or pilot plant personnel.

P117 Incidence and Growth of *Salmonella* and *Listeria* in Melon

ANA LUCIA PENTEADO and Mauro Farber Freitas Leitao, Dept. of Food Tech., State University of Campinas, Rua Monteiro Lobato, 80, Barao Geraldo, Caixa Postal 6121, Campinas, São Pãulo 13083-970, Brazil

The occurrence of *Listeria* and *Salmonella* on melon (variety Valenciano Yellow) surface was verified in forty fruits collected at the marketplace and open markets in different periods of the year. The product was analyzed by Bacteriological Analytical Manual (BAM 1998) and TECRA Visual Immunoassay. *Salmonella* spp. and *Listeria monocytogenes* were not isolated, and *L. welshimeri* was the only species detected. In a second experiment the ability of *S.* Enteritidis and *L. monocytogenes* to grow in melon pulps collected aseptically were studied at three different temperatures (10°C, 20°C and 30°C). Fruits without any defects (breaks in the peel, bruised areas) were used in the experiment. Pulps were aseptically removed and homogenized in a previously sterilized shaker. Melon portions were inoculated with saline suspensions of the test organisms yielding final populations of approximately 100 CFU/ml. Generation times of 7, 1.77 and 0.85 h were obtained for *L. monocytogenes* at temperatures of 10°C, 20°C and 30°C, respectively. The results for *S.* Enteritidis were similar to the one obtained for *Listeria* with generation times of 6.75, 1.46 and 0.57 at temperatures of 10°C, 20°C and 30°C, respectively. The study indicates that *Salmonella* and *Listeria* can grow well in homogenized melon pulp at different temperatures.

P118 Evaluation of Factors that Influence the Recovery of *Listeria monocytogenes* from Lettuce Treated with Sanitizers

Andrea B. Burnett, Montserrat H. Iturriaga, Eduardo F. Escartin, Charles A. Pettigrew, and LARRY R. BEUCHAT, University of Georgia, CFS, Dept. of Food Science and Tech., 1109 Experiment St., Griffin, GA 30223-1797, USA

A study was undertaken to evaluate some of the factors that may affect the recovery of *Listeria monocytogenes* from iceberg lettuce treated with sanitizers. The efficacy of chlorine (200 mg/ml) and a produce wash (Professional Line Fit®, tested at 5 g/liter) (PW) in killing *L. monocytogenes* on iceberg lettuce (14 cm^2) was not influenced by the composition of the medium used to culture cells for use in inocula, the number of strains in the inoculum, or the recovery medium used to enumerate the pathogen. Drying the inoculum on lettuce for 45 min at 37°C caused more cells to die or not be retrieved compared to drying for 30 min at 25°C. However, the percentages of cells recovered from lettuce treated with chlorine or PW were not significantly different, regardless of the drying method. Treatment with chlorine or PW resulted in decreases in populations of 1.76 and 1.51 $\log_{10}$ CFU/lettuce, respectively, regardless of variations in test parameters. Reductions were significantly greater ($a = 0.05$) than those

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observed for water but not significantly different from each other. Evaluation of sanitizers for their efficacy in killing *L. monocytogenes* on lettuce can be determined by spot inoculating 50 ml of a five-strain mixture of 24-h cultures suspended in 5% horse serum albumen, followed by drying the inoculum for 45 min at 37°C, treatment by immersing in 50 ml of sanitizer for 5 min, stomaching samples in 50 ml of a neutralizer broth for 2 min, and enumerating survivors on modified Oxford medium.

P119 A Receptor Assay for the Detection of Sulfamonomethoxine and Sulfadimethoxine Residue in Live Pigs

S. H. LEE, S. J. Park, H. Lee, H. J. Lee, C. C. Chae, Y. Jin, J. H. Choi and M. H. Lee, College of Agriculture and Life Sciences, Seoul National University, College of Veterinary Medicine and School of Agricultural BioTech., 103 Seodundong, Suwon, Kyonggi-do 441-744, South Korea

Sulfamonomethoxine (SMM) and sulfadimethoxine (SDM) have been widely used in pigs for prevention or treatment of infections. To prevent unwanted drug residues entering the human food chain, both government authorities and industries have established extensive control measures. In this study, we established a rapid predictive test for detection of pigs with unacceptable tissue residues of SMM and SDM. The recommended therapeutic doses of SMM (withdrawal time, 5 days) and SDM (withdrawal time, 14 days) were administered to two groups of 20 pigs each. Blood was sampled before drug administration and during the withdrawal period. The counts per min (CPM) ratio of internal standard to sample (B/Bs) was employed as an index to determine whether drug residues in pig tissues were negative or positive. That is, B/Bs ratio less than 1 was considered residue positive and that larger than 1 negative. The concentration of internal standard (500 ppb) of each drug was determined from the plasma drug residue on the last day of withdrawal period. All 20 plasma samples from non-treated pigs were negative to SMM and SDM. SMM and SDM were detected in plasma of treated pigs until the last day of the withdrawal period. The present study showed that a receptor assay can be adapted for use in prediction of tissue residues of SMM and SDM in live pigs by measuring plasma concentration with the modificated charm test, based on the definition of the withdrawal time.

P120 National Animal Health Monitoring System Swine 2000: A Surveillance Study of *Escherichia coli* O157 in Swine

INGRID FEDER, Jeffrey T. Gray, Rachel Pearce, Eric Bush, Pina Fratamico, F. Morgan Wallace, Anna Porto, Paula Fedorka-Cray, Richard Perrine, Robert Dudley, and John B. Luchansky, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

As part of a national surveillance system (National Animal Health Monitoring System Swine 2000) to monitor the presence of pathogens in swine, 2,526 swine fecal samples were collected from 57 sites in the top 17 swine producing states to investigate the natural occurrence of *Escherichia coli* O157 in healthy swine. Ten grams of feces were placed into brilliant green bile broth for a 6h enrichment and incubated at 37°C. One ml aliquots were removed and added to a suspension of *E. coli* O157 antibody coated immunomagnetic beads. Beads processed by immunomagnetic separation were plated onto sorbitol MacConkey (SMAC) agar and cefixime/tellurite SMAC agar. Non-sorbitol fermenting colonies were tested for the presence of ß-glucuronidase and the ability to ferment lactose using *Escherichia coli* broth containing 4-methylumbelliferyl-ß-D-glucuronide (MUG) and MacConkey broth, respectively. Lactose positive and MUG negative colonies were tested for O157 latex agglutination. The O157 latex agglutination positive isolates were tested for the presence of genes encoding for the O157 somatic antigen, *Shiga* toxins 1 and 2, intimin protein, hemolysin, and the H7 flagellum by PCR analysis. Of the 2526 samples tested, 102 *E. coli* O157 isolates were recovered and were positive by PCR for the O157 gene. Of these isolates, none were positive for the H7 flagellum or any of the virulence factors by PCR. The *E. coli* O157 genotype was found in the feces of healthy swine. However, none of the isolates obtained in this study contained the virulence pattern associated with pathogenic *E. coli* O157:H7.

P121 DSC Evaluation of Enrichment Methods for Recovery of *Yersinia enterocolitica* 0:3 and 0:8 from Swine Feeds

JOO-SUNG KIM, Alan Mathew, and F. Ann Draughon, The University of Tennessee Food Safety Center of Excellence, Dept. of Food Science and Tech., 2605 River Road, Knoxville, TN 37996, USA

Swine are major reservoirs of *Yersinia enterocolitica* and pigs are easily colonized through oral infections. The objective of this study was to evaluate five different enrichment methods to recover *Y. enterocolitica* from swine feeds since optimal methods for isolation of *Y. enterocolitica* are not available for farm samples having a heavy background microflora. Recovery of *Y. enterocolitica* from swine feeds using enrichment was evaluated with an inoculum level of 1000 CFU/g feeds. Enrichment media evaluated included: phosphate buffered saline (PBS) with and without sorbitol and bile salts, ITC broth and tryptic soy broth with polymyxin (5 IU/ml) and novobiocin (10 mg/ml) (TSBPN). Incubation periods for enrichment were evaluated from 24 h up to 6 weeks and incubation temperature from 4 to 30°C. CIN, MacConkey and *Salmonella-shigella* deoxycholate calcium (SSDC) agar plates were used for differential plating. KOH treatments at concentrations of 0.25% to 0.13% were evaluated. PBS with sorbitol (1%) and bile salts (0.15%), at 4°C or 21°C, was not effective for recovery of *Y. enterocolitica* 0:3 and 0:8 from swine feeds. KOH treatment did not improve recovery of *Y. enterocolitica* in the methods studied and often significantly ($P < 0.05$) reduced recovery. TSBPN at 18°C with 24 h incuba-

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tion was better than other methods evaluated for recovery of both 0:3 and 0:8 with recovery percentage of 100% and 60%, respectively. ITC broth gave 100% recovery of serotype 0:3 but was not effective for recovery of 0:8. Overall, optimal recovery of *Y. enterocolitica* from swine feeds, based on these data, was achieved using TSBPN enrichment at 18°C with incubation for 24 h followed by differential plating on CIN at 30°C for 24 h.

P122 Genomic Fingerprinting of *Salmonella* Recovered from Swine Carcass and Fecal Samples at a Slaughterhouse

LAURA WONDERLING, Rachel Pearce, F. Morgan Wallace, Jeffrey E. Call, Mark Tamplin, Robert Dudley, Ingrid Feder, Samuel Palumbo, Alan Oser, Lisa Yoder, and John B. Luchansky, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Previously, 100 swine carcasses were swabbed and 60 corresponding fecal samples were collected on 10 days over a 30-day period. From carcass (73%) and fecal (33%) samples that were positive for *Salmonella*, a total of 582 isolates were characterized by PFGE. There were 13 unique *XbaI* restriction profiles, with the majority (71%) of the isolates having profile types "F" (36%) or "B" (35%). While both the "F" (*S.* Typhimurium DT104-like PFGE profile) and "B" profiles were found on at least 8 of 10 sampling days, profile "I" was found on 3 days, profiles "A" and "D" on 2 days, and the remaining 8 profiles on only 1 of the 10 sampling days. A single isolate obtained from a carcass 18 h post-chill during the survey period also displayed profile type "F". Examining multiple isolates from a given carcass revealed a single profile in 40 of 57 (70%) samples tested. Similarly, multiple isolates from a given fecal sample displayed a single profile in 16 of 19 (82%) samples tested. In general, analyses of the 15 paired carcass-fecal samples revealed that clonal types found in feces were typically found on the carcass and vice-versa, suggesting that fecal isolates from a given animal contaminated the carcass. These data indicate that although each group of animals can introduce new clonal types into the slaughterhouse, the majority of *Salmonella* associated with a given animal exhibit low genomic diversity.

P123 Evaluation of Methods for Recovery of *Salmonella* from Swine Feces

PHILIPUS PANGLOLI, F. Ann Draughon, Alan Mathew, and Omaima Ahmed, The University of Tennessee, Food Safety Center of Excellence, 100 Food Safety and Processing Bldg., Knoxville, TN 37996, USA

Improved detection and isolation of *Salmonella* in farm environments is essential to establish baseline data on its occurrence in the animal environment. The objective of this study was to determine optimal methods for detection of *Salmonella* from swine feces. Pre-enrichment in lactose broth (LB) and direct enrichment in Rappaport-Vassiliadis (RV), selenite cystine (SC), and tetrathionate at 35°C (TT35) and at 42°C (TT42) in conjunction with differential plating on brilliant green agar (BG), bismuth sulfite agar (BS), Hektoen enteric agar (HE) and xylose-lysine-tergitol 4 agar (XLT4) were evaluated for their ability to recover *Salmonella* from feces of farrowing sows and grower/finisher pigs. The samples were artificially contaminated with *Salmonella* Typhimurium (ATCC 14028) at low levels (~10 CFU/25 g of sample). The optimal methods (> 78% recovery of 10 CFU/25 g) to recover *Salmonella* from feces of farrowing sows were pre-enrichment in LB followed by enrichment in RV with differential plating on BS, HE or XLT4 and enrichment in TT42 with plating on XLT4. None of the direct enrichment media evaluated recovered 10 CFU/25 g in feces of farrowing sows. However, several media combinations consistently recovered *Salmonella* at 10 CFU/25 g from feces of grower/finisher pigs. Direct enrichment of grower/finisher pig feces in SC with plating on BS, HE, or XLT4, direct enrichment in TT35 with plating on BS or XLT4, and direct enrichment in TT42 with plating on XLT4 gave 100% recovery of samples spiked with 10 CFU of *S.* Typhimurium/25 g feces. Recovery of *Salmonella* from feces of grower/finisher pigs was not improved by pre-enrichment in LB. These data show that selection of appropriate protocols to isolate *Salmonella* from swine farm samples such as feces is strongly dependent upon the type of animals being sampled.

P124 Examination of Class I Integrons in *Escherichia coli* Isolated from Pigs on US Swine Farms that Use or Exclude Antibiotics

PAUL D. EBNER and Alan G. Mathew, University of Tennessee, Dept. of Animal Science, 2505 River Drive, 305 Brehm Animal Science Bldg., Knoxville, TN 37996, USA

A study was conducted examining class I integrons in *E. coli* isolated from pigs on US swine farms. A multiplex PCR targeting conserved elements of class I integrons (*intI*, $qacE_1$ and sul_1) was used to screen 153 *E. coli* isolates obtained from farms that use (ab+; n=4) or exclude (ab-; n=3) antibiotics. Eleven isolates contained all three gene sequences. Three isolates contained only $qacE_1$ and sul_1 sequences and two isolates contained only the *intI* sequence. Of the integron positive (+) isolates, 12 were obtained from pigs on ab+ farms while the remaining four isolates came from pigs on ab- farms. All isolates were resistant to tetracyclines while 13 were resistant to sulfonamides. Several isolates were resistant to as many as five different antibiotics or antibiotic combinations including streptomycin, gentamicin, apramycin, ampicillin, cephalothin, trimethoprim/sulfamethoxazole and kanamycin. Macrorestriction profiling of the integron+ isolates revealed several unrelated biotypes except for three isolates obtained from one ab- farm and two isolates from one ab+ which proved to be two distinct clonal strains, respectively. DNA hybridization suggested that in 11 cases the integrons were part of plasmid DNA. Sequencing of inserted cassettes showed that in five cases the inserted sequences shared similarity to *aadA2*, a gene conferring resistance to streptomycin/spectinomycin. These data suggest that several

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different bio-types of *E. coli* isolated from swine carry class I integrons. Further research is needed to ascertain whether the use of antibiotics on swine farms selects for such isolates as these data suggest.

P125 Prevalence of *Trichinella* spp. in Farmed Wild Boars in Alberta

JOHN T. Y. WU, Ken H. Dies, Eva Y. W. Chow, Evelyn E. Bowlby, and Lester S. Y. Wong, Alberta Agriculture Food and Rural Development, Immunology and Virology, Food Safety Division, 6909-116 St., Edmonton, Alberta T6H 4P2, Canada

Trichinella spp. have been found in different wild animal populations in the northwestern parts of Alberta, Canada. The management practices and locations of the farmed wild boar populations in the province expose them to *Trichinella* transmission and infection from wildlife species. Since consumer interest in wild boar meat in Alberta has been increasing steadily, wild boar carcasses in provincial abattoirs are carefully inspected for presence of trichinae. This is to prevent the possibility of human consumption of infected wild boar meat. No prevalence study of *Trichinella* spp. in Alberta has been reported. The objective of this study was to determine the prevalence of this parasite in farmed wild boars in Alberta. Tongue (N=174), diaphragm (N=181) and serum (N=167) were harvested from the wild boars slaughtered in provincially inspected abattoirs during a period of one year. Both the digestion test and an enzyme-linked immunosorbent assay (ELISA) were used in this survey. No larvae were isolated by direct examination. On the other hand, two seropositive pigs have been identified. This is the first time in Alberta that the exposure of the wild boar population to the parasite was demonstrated serologically. However, in the absence of isolation of the larvae from these wild boar tissues, it can be concluded that *Trichinella* infection is still not a problem in Alberta. The ELISA used in this surveillance project was automated using the Biomek 2000 robotic workstation. A quality assurance system based on the requirement of the ISO/IEC 17025 standards has been adopted throughout this project.

P126 Screening for Cephalosporins Plasma Residues in Live Pigs by Receptor Assay

S. J. Park, S. H. LEE, H. Lee, H. J. Lee, C. C. Chae, Y. Jin, J. H. Choi and M. H. Lee, College of Agriculture and Life Sciences, Seoul National University, College of Veterinary Medicine and School of Agricultural BioTech., 103 Seodundong, Suwon, Kyonggi-do 441-744, South Korea

Cephalosporins, a group of semisynthetic antibacterial agents, are chemically related to penicillin. Cephalosporin antibiotics have been used in pigs for prevention or treatment of infections. To prevent unwanted drug residues entering the human food chain, both goverment authorities and industries have established extensive control measures. In this study, we established a screening test to predict tissue residues of cephalosporin antibiotics (cefazolin, ceftiofur, cephalexin) in live pigs by measuring plasma concentration. The recommended therapeutic doses of cefazolin (withdrawal time, 1 day), ceftiofur (withdrawal time, 2 days) and cephalexin (withdrawal time, 4 days) were administrated to three groups of 20 pigs each. Blood was sampled and analyzed before drug administration and during the withdrawal period. The plasma concentration of the last day of the withdrawal period was about 40 ppb, which was calculated on each standard curve, so the concentration of internal standard solution was set at 50 ppb. The count per min (CPM) ratio of internal standard to sample (B/Bs) was introduced as an index to determine whether drug residues in pig tissues are negative or positive. All 60 plasma samples of non-treated pigs were negative for all cephalosporins. Cephalosporins were detected until the last day of the withdrawal period whereas no cephalosporins were detected after the withdrawal time. The present study showed that the receptor assay can be adapted for use in prediction of plasma residues of cephalosporin antibiotics in live pigs by measuring plasma con-centration with the modificated charm test, based on the definition of the withdrawal time.

P127 Enzyme-linked Immunosorbent Assay for the Detection of Sulfamonomethoxine and Sulfadimethoxine Residue in Live Pigs

S. H. LEE, S. J. Park, H. Lee, H. J. Lee, C. C. Chae, Y. Jin, J. H. Choi and M. H. Lee, College of Agriculture and Life Sciences, Seoul National University, College of Veterinary Medicine and School of Agricultural BioTech., 103 Seodundong, Suwon, Kyonggi-do 441-744, South Korea

Sulfamonomethoxine (SMM) and sulfadimethoxine (SDM) have been widely used in pigs for prevention or treatment of infections. To prevent unwanted drug residues entering the human food chain, both government authorities and industries have established extensive control measures. In this study, we established a rapid prediction test for the detection of pigs with unacceptable tissue residues of SMM and SDM. The recommended therapeutic doses of SMM (withdrawal time, 5 days) and SDM (withdrawal time, 14 days) were administered to two groups of 20 pigs each. Blood was sampled before drug administration and during the withdrawal period. The absorbance ratio of internal standard to sample (B/Bs) was employed as an index to determine whether drug residues in pig tissues were negative or positive. That is, a B/Bs ratio less than 1 was considered residue positive and that larger than 1 negative. The concentration of internal standard of each drug was determined from the plasma drug residue on the last day of the withdrawal period. All 20 plasma samples from non-treated pigs were negative to SMM and SDM. SMM was detected in plasma of treated pigs until the 5th day of withdrawal period and SDM was detected until the 14th day of its withdrawal period. The present study showed that the semi-quantitative ELISA could be easily adapted for use in predicting tissue residues of SMM and SDM in live pigs.

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P128 A Comparison and Development of Isolation Protocols for Recovery of *Escherichia coli* O157:H7 from Swine Feces

PHILIPUS PANGLOLI, F. Ann Draughon, David Golden, Alan Mathew, and Omaima Ahmed, The University of Tennessee, Food Safety Center of Excellence, 100 Food Safety and Processing Bldg., Knoxville TN, 37996, USA

This study was designed to compare, modify, and optimize methods for detection and recovery of *E. coli* O157:H7 from swine feces, based upon published data, FDA BAM and USDA protocols. Enrichment media evaluated for recovery of *E. coli* O157:H7 from swine feces included: modified trypticase soy broth containing 20 mg/liter novobiocin (mTSB+N), modified EC medium with 20 mg/liter novobiocin (mEC+N) and enterohaemorrhagic *E. coli* enrichment broth (EEB). Selective/differential plating media evaluated for each enrichment broth included: sorbitol MacConkey agar (SMAC), SMAC supplemented with cefixime and potassium tellurite (CTSMAC), hemorrhagic coli agar (HC) and modified Levin's eosin methylene blue agar (mEMB). The efficacy of immunomagnetic separation (IMS) was compared to non-IMS recovery. Swine feces from farrowing and grower/finisher pigs were artificially contaminated with *E. coli* O157:H7 at low (~10 CFU/25 g), medium (~100 CFU/25 g) and high (~1000 CFU/ 25 g) levels. Enrichment for 14 h improved recovery of *E. coli* O157:H7 from swine feces of farrowing sows but recovery of *E. coli* O157:H7 from feces of grower/finisher pigs was best after 6 h. The use of IMS increased recovery of *E. coli* O157:H7 from feces of grower/finisher pigs but did not improve recovery from feces of farrowing sows. Maximum recovery of *E. coli* O157:H7 at 10 and 100 CFU/25 g was 17% for farrowing sows and 55% for grower/finisher pig feces. Based on our data (inoculation level 1000 CFU/25 g), methods recommended for recovery of *E. coli* O157:H7 from feces of grower/finisher pigs are 6 h enrichment in mEC+N with plating on SMAC or CTSMA (78% recovery), or HC (83% recovery), in EEB with plating on HC (89% recovery) or mEMB (78% recovery), and in EEB with IMS prior to plating on SMAC (89% recovery) or HC (100% recovery). The most effective method to recover *E. coli* O157:H7 from farrowing sows was 14 h enrichment in mEC+N followed by plating on SMAC, HC or mEMB and 14 h enrichment in EEB with plating on SMAC (83% recovery).

P129 Influence of pH on Retail Shelflife of Pork

DSC

B. L. KNOX, R. L. J. M. van Laack, P. M. Davidson, E. Spencer, and R. E. Klont, University of Tennessee, Dept. of Food Science and Tech., Knoxville, TN 37996-4539, USA

Pork pH is an important characteristic affecting sensory quality and shelflife. Higher pH is associated with reduced incidence of PSE. However, higher pH may result in shorter microbial shelflife. In this study, the effect of pH on microbial counts and color of retail cuts from pork loins was evaluated. Thirty-six loins were collected at a commercial slaughter facility one day post-slaughter, vacuum-packaged, and stored at 4°C. All pigs were from the same genetic line. At 6, 14, 24 and 34 days post-packaging, pork loins were sliced and grouped by pH (group:pH range): 1:5.41 to 5.65; 2:5.66 to 5.80; 3:5.81 to 5.95; 4:5.96 to 6.10; 5: >6.11. Loin slices were packaged and stored under retail conditions at 5°C for 5 days. Aerobic (APC) and *Enterobacteriaceae* (EC) counts and color analysis were performed at 0 and 5 days. Data were analyzed using ANOVA. At day 5, the APC were lower for pH group 1 and 2 than for group 4 and 5 over the storage time of the vacuum-packaged loins ($P < 0.05$). Group 1 was 1.0 to 1.5 logs lower than group 5. Retail samples reached log 7.0 CFU/cm^2 at day 5 only after 24 days vacuum-packaged storage. Groups 1 and 2 had lower ($P < 0.05$) EC than groups 3 to 5 at day 0 for all sampling times but, at day 5, counts were similar over storage. Based on hue, chroma and L* values, the color stability of retail cuts decreased during storage. L* values were higher at lower pH. Results demonstrate that higher pH pork has a slightly shorter retail shelflife.

P130 The Impact of Starvation on the Resistance of *Salmonella* Typhimurium to Irradiation in 0.85% Saline and in Ground Pork

AUBREY MENDONCA, Maria Romero, and Makuba Lihono, Iowa State University, Dept. of Food Science and Human Nutrition, 2313 Food Sciences Bldg., Ames, IA 50011, USA

The impact of starvation on the resistance of *Salmonella* Typhimurium to electron beam irradiation was investigated. Exponential- or stationary-phase cells (controls) were grown at 35°C in tryptic soy broth (TSB). Washed exponential phase cells were starved in 0.85% (w/v) saline (25°C) for 14 days. Tubes of fresh saline and packages of sterile ground pork were inoculated with control or starved cells. Inoculated samples were irradiated at doses ranging from 0.0 to 1.0 kGy (saline) and from 0.0 to 2.0 kGy (ground pork). *S.* Typhimurium survivors were enumerated by plating diluted samples on tryptic soy agar (TSA) or on xylose lysine desoxycholate (XLD) agar, and counting bacterial colonies following 48 h of incubation at 35°C. Cells exhibited the highest irradiation resistance at two days of starvation. Irradiation (1.0 kGy) of *S.* Typhimurium in saline resulted in 6.7 and 3.0 $\log_{10}$ reduction in controls and starved cells, respectively. Irradiation of pork at 2.0 kGy reduced controls by 4.5 logs, whereas starved cells were reduced by 2.6 logs. Numbers of surviving stationary-phase cells were not significantly different ($P > 0.05$) from those of exponential-phase cells. Starved *S.* Typhimurium exhibited significantly ($P < 0.05$) higher irradiation D_{10} values than controls. Exponential, stationary, and starved cells had D_{10} values of 0.12, 0.14, and 0.25 kGy, respectively, in saline. In pork, D_{10} values for exponential, stationary, and starved cells were 0.40, 0.43, and 0.71 kGy, respectively. These results demonstrate that starvation increases the radiation resistance of *S.* Typhimurium and should be considered when determining the irradiation D_{10} value for this pathogen.

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P131 Detection and Enumeration of *Listeria monocytogenes* in Battered and Breaded Seafood

FLETCHER ARRITT, Joseph Eifert, and Michael Jahncke, Virginia Tech., Dept. of Food Science and Tech., Mail Code 0418, Blacksburg, VA 24061, USA

The traditional frozen, battered and breaded seafood portion is a low risk food safety concern. This product is usually fully cooked prior to consumption by the consumer. In addition, pathogen growth is unlikely to occur during frozen storage. Nevertheless, an increasing number of supermarkets and convenience stores are interested in selling raw or fully-cooked unfrozen battered and breaded portions. Many types of refrigerated foods such as ready-to-eat sandwiches, meat salads, frankfurters, and cold smoked fish products have been recalled from the marketplace because of contamination with *Listeria monocytogenes.* This survey examined frozen and refrigerated, further processed battered and breaded fish portions for the presence of this bacterial pathogen. Battered and breaded fish portion samples (n=112), primarily pollock, cod, or whiting, were collected over one year from both retail and commercial markets. Qualitative test results revealed 16 of the 112 (14.3%) were positive for *Listeria* species. *L. monocytogenes* was identified among 9 (8.0%) of the battered and breaded seafood products. Separate 50g portions of the *L. monocytogenes*-positive samples were analyzed quantitatively by dilution in buffered peptone water and direct plating onto PALCAM agar. The concentration of *L. monocytogenes* was less than 50 CFU/g for all positive samples. Since *Listeria* can survive in these products, other processing interventions, including antimicrobial additives and modified atmosphere packaging, should be considered for these products if they are sold unfrozen in retail markets.

P132 DSC The Fate of *Escherichia coli* O157:H7 on Channel Catfish Fillets with and without Skin Packaged under Modified Atmosphere

RICO SUHALIM and Yao-wen Huang, The University of Georgia, Food Science Bldg., Dept. of Food Science and Tech., Athens, GA 30602, USA

The survival of *Escherichia coli* O157:H7 on channel catfish (*Ictalurus punctatus*) fillets packaged under modified atmosphere was investigated. Catfish fillets, with and without skin, were inoculated with approximately 10^6 CFU/ml *E. coli* O157:H7 containing green fluorescent protein, ampicillin and nalidixic acid resistant genes. Fillets were packaged under a modified atmosphere environment (30% CO_2 and 70% N_2), and stored at 0 and 10°C for 15 days. *E. coli* O157:H7 were enumerated on Brain Heart Infusion (BHI) Agar supplemented with 100 mg/ml ampicillin and nalidixic acid. BHI and aerobic (APC) plates were incubated at 37°C for 48 h, while psychrotrophic plates were incubated at 4°C for 10 days. *E. coli* O157:H7 counts decreased by 3 and 4 log CFU/g after the 5th day of storage on fillets stored at 10° and 0°C, respectively. Only 1 log CFU/g of *E. coli* O157:H7 was detected by the 15th day of storage at 0°C. However, no significant difference between the survival of *E. coli* O157:H7 on fillets both with skin and without skin was observed. Surface pH of fillets stored at 0 and 10°C throughout the 15-day storage decreased from 6.6 to 6.4 and from 6.7 to 6.1, respectively. Both aerobic and psychrotrophic plate counts for fillets with skin stored at 0°C increased from 4 log to 6 log CFU/g after the 15 days storage. These findings suggest that *E. coli* O157:H7 can survive on channel catfish fillets packaged in a modified atmosphere up to 15 days.

P133 DSC Microbial Validation of *Sous Vide*-like Cooking Process for Lamb in Curry Sauce

L. S. VANDERWAL, H. Thippareddi, C. L. Kastner, R. J. Danler, P. Udomvarapont, D. H. Kropf, R. K. Phebus, and E. A. Boyle, Kansas State University, 216 Weber Hall, Manhattan, KS 66506, USA

A *sous vide*-like cooking process was used to produce a lamb in curry sauce product to provide consumers with a flavorful, convenient, and economical gourmet lamb entrée. A microbial validation study evaluated the safety of the process. Steam cooking the packaged product at 85°C for 2 h reduced aerobic plate count (APC) and anaerobic plate count (ANA) by ca. 4 log CFU/g. Lactic acid-producing bacteria (LAB) were reduced by 4.56 log CFU/g and *Clostridium sporogenes* populations by 0.52 log CFU/g. When the product was cooked at the optimum temperature for quality (90°C for 2 h), ANA and APC were reduced by ca. 5 log CFU/g. LAB and *C. sporogenes* populations were reduced by 5.08 and 1.38 log CFU/g, respectively. Chilling *C. perfringens* inoculated product according to the USDA recommendations (1.5 h from 54.4 to 26.7°C and 5 h from 26.7 to 4.4°C) reduced *C. perfringens* population 1 log CFU/g ($P < 0.05$) compared to heat shocked controls. Chilling the product according to industry conditions of 2 h through the first temperature phase and 5 h through the second phase resulted in no change in *C. perfringens* ($P > 0.05$), meeting USDA regulations. Levels of ANA, APC, CSP, and LAB did not change ($P < 0.05$) in non-inoculated product cooked at 90°C for 2 h and stored at either 4 or 10°C (abusive temperature) for at least 150 days. Accordingly, the described process produced a safe product for consumers.

P134 Human BSE Exposure Risk and Direct Detection of Abnormal Prion Protein in Meat Products

E. LÜCKER, M. Hardt, and M. H. Groschup, Institute of Food Hygiene, Veterinary Faculty, University of Leipzig, An den Tierkliniken 35, Leipzig D-04103, Germany

Several methods for the detection of tissues of the central nervous system (CNS) in meat products have been developed for use in official food control as pertaining to human BSE exposure risk. However,

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methods for the detection of abnormal prion protein (PrP-res) were not evaluated for their potential applicability to the matrix of heat treated meat products. We developed a micro technological procedure for the preparation of meat products suitable for high security laboratories, as masses were 6 to 8 orders of magnitude lower than in conventional meat technology. This material showed all characteristics of normal meat products and a homogeneous distribution of brain as indicated by NSE and GFAP western immunoblotting and GFAP immunometric analyses. Thus it was possible to produce standard micro-sausages containing defined amounts of bovine BSE-positive brain. Using a certified immunometric assay for detection of PrPSc in untreated brain, it was possible to detect BSE-positive CNS down to a content of 0.25% in heat treated meat products. We found a high correlation (r=0.99) between PrP-res immuno reactivity and BSE-positive brain content in a range up to 10% CNS. Furthermore, we will report the results of PrP-res testing of a large sample collective of retail meat products from various European food outlets.

P135 The Role of the *sigB* Gene in Stress Survival of *Listeria monocytogenes* Strains of Meat and Clinical Origin

SANDRA M. MOORHEAD and Gary A. Dykes, AgResearch, Private Bag 3123, Hamilton, New Zealand

The ability of *Listeria monocytogenes* to survive and grow under adverse conditions encountered in many foods is of public health concern. Knowledge of the physiological and genetic mechanisms of the stress survival in this pathogen are necessary to control it. The alternative sigma factor (*sig*B gene) has been shown to be involved in the stress response of *L. monocytogenes* 10403S, a commonly used laboratory strain of clinical origin. The current study was undertaken to determine if this feature was also apparent in a strain of meat origin and a further strain of clinical origin. This was achieved by inducing null mutations in the *sig*B genes of both these strains and comparing mutated and wild-type strains under a variety of stress conditions. Parameters studied included the ability to survive low pH, carbon and starvation stress, the presence of ethanol and bacteriocins, as well as the ability to utilise osmolytes. Results indicated that the relative importance of the *sig*B gene in the stress response is not the same in all strains of *L. monocytogenes*. Specifically, the meat-isolated wild-type and corresponding null mutant used in our study exhibited similar responses to those of the previously studied 10403S strain, while the clinical strain behaved differently. In particular, the clinical isolate displayed a lesser dependence on the *sig*B product under conditions of stress. The results of the present study indicate that genetic variation between strains should be taken into consideration when examining the survival of stress conditions by *L. monocytogenes* on food.

P136 Genotypic Variability and Antibiotic Resistance Profiles of *Escherichia coli* O157:H7 Isolates from Downer and Healthy Dairy Cattle

CAITRIONA M. BYRNE, Irfan Erol, Jeffrey E. Call, Dennis Buege, Charles W. Kaspar, Clayton Hiemke, Paula Fedorka-Cray, Jovita Hermosillo, Takiyah Ball, Andrew K. Benson, Morgan Wallace, Marcus Handy, and John B. Luchansky, USDA-ARS-ERRC, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA

While cattle have been identified as a reservoir of *Escherichia coli* O157:H7, there is limited data regarding the prevalence and clonality of this pathogen in downer dairy cattle and the contribution of this source of ground beef to human health. A study conducted in a mid-western state between April and October of 2001 established a three-fold higher prevalence of *E. coli* O157:H7 in fecal samples obtained aseptically from intact colons of downer (12/201, 6.0%) than in healthy (4/200, 2.0%) dairy cattle. Analyses of 67 isolates, representing these 16 positive samples (1 to 5 isolates per sample), by PFGE revealed 17 distinct XbaI profiles. Isolates from different animals displayed distinct profiles. However, in one sample, three different, but related, XbaI profiles were displayed by the isolates recovered. In the remaining samples, the isolates from the same fecal/colon sample displayed indistinguishable profiles. Moreover, at least 15 of 67 isolates from 3 (1 downer and 2 healthy animals) of the 16 positive samples contained isolates that were resistant to at least 3 of the 18 antibiotics tested. Also, isolates with the same PFGE profile displayed the same antibiotic resistance profile. It was not possible to distinguish between isolates recovered from downer or healthy cattle based on their XbaI or antibiotic resistance profiles. However, the results establish that downer cattle had a greater prevalence of *E. coli* O157:H7 than healthy cattle within the time frame and geographic scope of this study.

P137 Dried Distiller's Grains with Solubles in Finishing Cattle Diets: A Preharvest Strategy against Acid Resistant *Escherichia coli* and Coliforms

MICHELLE GORDON, R. K. Phebus, J. S. Drouillard, H. Thippareddi, D. L. Lambert, K. Kerr, N. Pike, J. J. Sindt, and J. J. Higgins, Kansas State University, 201 Call Hall, Manhattan, KS 66506, USA

Study 1. Finishing beef heifers (345 head) were used in a 153 day finishing trial to evaluate the effects of feeding dried distiller's grains with solubles (DDGS), 0%, 15%, 30%, 45%, 60%, 75% dry basis (DMB), on acid resistant *Escherichia coli* and coliforms. Concurrently, a similar study (Study 2) was conducted on canulated Jersey steers (18 head) using 0%, 30%, and 75% (DMB) of DDGS. From Study 1, fecal samples were taken on days 65 and 100, at 2 and 20 h after feeding, and analyzed for acid resistant *E. coli* and total coliforms, pH and fecal VFAs. From

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Study 2, groups of six steers were assigned to each of the three treatments using a 3 × 3 Latin square. Fecal and rumen samples were obtained after 4-week adaptation periods 2, 4, 6, 8, 12, 18, and 24 h post-feeding. For Study 1, a linear increase in fecal pH at both 2 and 20 h post-feeding was observed ($P < 0.05$). Total coliforms and acid resistant *E. coli* at 2 and 20 h post feeding were not affected by dietary treatment ($P > 0.05$). Total fecal VFAs were not affected by dietary treatment or the sampling time ($P > 0.05$). For Study 2, fecal and rumen pH were not affected by dietary treatment, yet an hour affect was noted. There was no dietary treatment effect on *E. coli* or total coliforms in the rumen fluid or the feces ($P > 0.05$). Future research is needed to determine the components of diet affecting acid resistance in *E. coli* and coliforms.

P138 Characterization of *Escherichia coli* O157 Isolated from Slaughterhouses and Retail Stores in Korea

JI YEON KIM, Won ki Bae, So Hyun Kim, Nam Hoon Kwon, Ji Youn Lim, Jun Man Kim, Kyoung Min Noh, Woo Kyoung Jung, and Yong Ho Park, Seoul National University, Dept. of Microbiology, College of Veterinary Medicine, and School of Agricultural Biotech., Seo-Doon Dong 103, Kwon-Sun Gu, Suwon, Gyunggi 441-744, Korea

A total of 11 *Escherichia coli* O157 isolates were identified from 623 fecal samples and 172 meat samples at 3 slaughterhouses and random retail houses between April 2000 and August 2001. The bacteria were detected using the immunomagnetic separation procedure, followed by selective plating on Sorbitol MacConkey agar and Fluorocult agar. They were identified as *E. coli* serotype O157 using 4 primer pairs that amplified fragments of stx_1, stx_2, eaeA and *hly* genes in PCR assays. The study has shown they all carried stx_1 gene, alone or in combination with the stx_2 gene. Testing for antimicrobial susceptibility to 23 antibiotics showed that all isolates were resistant or intermediately resistant to two or more antibiotics tested. As for plasmid profiling, they all had the most common plasmid of 60MDa. Further, we used DNA fingerprinting by randomly amplified polymorphic NA(RAPD) to compare the identified *E. coli* O157 strains, and we observed these strains had relatively high similarity. The results indicate that the identification and typing methods used in the study of *E. coli* O157 might be useful for the epidemiological analysis of *E. coli* O157 outbreaks.

P139 DSC Preparation of Ground Beef Samples for Detecting *Escherichia coli* O157:H7 by PCR

SHENGHUI CUI and Jiang Hong Meng, University of Maryland, Dept. of Nutrition and Food Science, 3304 Marie Mount Hall, College Park, MD 20742, USA

Escherichia coli O157:H7 has become a significant foodborne pathogen since it was identified in 1982. Ground beef is the most important single product that has been associated with outbreaks of *E. coli* O157:H7 infection. Rapid and specific detection of *E. coli* O157:H7 in food will contribute to the control of illness caused by this pathogen. We developed a sample preparation procedure for detecting *E. coli* O157:H7 in ground beef by PCR. This procedure was able to concentrate *E. coli* O157:H7 cells present in ground beef, and to remove PCR inhibitors. Ground beef samples were homogenized in a filtered two-chamber stomach bag, followed by centrifugation at different speeds. The samples were treated with lysozymes and proteinase K, and freezing-thawing to release bacterial DNA. A nalidixic acid resistant *E. coli* O157:H7 strain inoculated in ground beef was used to determine effectiveness of the treatments. Centrifugation at different speeds resulted in a recovery rate of 70% *E. coli* O157:H7 from ground beef based on colony plate counts. After concentration by centrifugation and DNA release by freezing and drying procedures, a PCR assay specific for gene encoding Shiga toxin 1 was able to detect *E. coli* O157:H7 at 100 CFU/g in ground beef without enrichment. The sample processing method has potential for use in molecular detection of *E. coli* O157:H7 in food.

P140 DSC Vancomycin Resistant Enterococci Possessing vanA Gene Isolated from Beef Imported to Malaysia

NIMITA FIFADARA, Gulam Rusul, Son Radu, Zaiton Hassan, and Larry R. Beuchat, University Putra Malaysia, Dept. of Food Science, Faculty of Food Science and BioTech., Serdang, Selangor, D.C. 43400, Malaysia

Foods of animal origin have been reported to harbor vancomycin resistant enterococci (VRE) that may be transferred to humans via the food chain. Beef (87 frozen beef and 63 processed beef samples) imported to Malaysia was analyzed. Seventeen (11.3%) were positive for VRE. Sixty VRE isolates were identified as *Enterococcus faecium* (35), *E. faecalis* (22), and *E. pseudoavium* (3). All 60 isolates were resistant to 10 of the 14 antibiotics tested. All were resistant to streptomycin, teicoplanin, and vancomycin; 94 to 97% of the isolates were resistant to 8 other antibiotics. Resistance to penicillin, ampicillin, and chloremphenicol was least, 26.8, 38.8 and 58.2%, respectively. Hemolytic activity on horse blood agar showed that 28 of 60 isolates (46.7%) were β hemolytic, indicating potential to be pathogenic. Plasmid profiling revealed that 36 (60%) of 60 isolates had plasmids ranging from 1.5 to 54 Kb. By use of a specific PCR assay, *van*A gene was detected in all isolates. RAPD-PCR analysis provided high discrimination among all 60 strains. The 60 isolates showed clusters with 10 to 100% similarity levels. It is concluded that beef can serve as a vector for VRE in Malaysia. Data also support previous findings indicating that VRE originate from foods of animal origin.

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P141 Identification of Spoilage Microorganisms in Ground Beef Treated with Diacetyl and Hydrodynamic Pressure Processing, Alone or in Combination

CHERYL MUDD, Anisha Williams-Campbell, and Morse Solomon, USDA-ARS, Food Tech. and Safety Laboratory, 10300 Baltimore Ave., BARC-East, Bldg. 201, Room 8, Beltsville, MD USA

Both diacetyl and hydrodynamic pressure processing (HDP) have been shown to reduce spoilage microorganisms in ground beef. The purpose of this study was to identify microorganisms recovered from ground beef treated with either HDP or diacetyl, alone or in combination. After mixing, retail ground beef (11g; 30 per treatment) was divided and assigned to one of the following treatments: untreated controls, diacetyl (100 mg/g), HDP, and diacetyl combined with HDP. Samples were wrapped in a plastic film, placed in a multilayer barrier bag and vacuumized. HDP samples were placed into the bottom of a water-filled shockwave container with a binary explosive (100 g) that was detonated. On days 0 and 7, bacterial enumeration was done by spiral plating onto plate count agar (PCA) with incubation at 30°C for 48 h. Following incubation, colonies from each treatment (controls, n=19; diacetyl, n=14; HDP, n=26; diacetyl combined with HDP, n=10) were isolated on PCA and identifed using the VITEK 32. On day 0, the predominant organism recovered from most treatments was *Streptococcus* (60.0%). On day 7, the most frequently recovered organisms in controls were *Streptococcus* (36.8%), *Gemella* (26.3%), *Serratia* (10.5%), *Acinetobacter* (10.5%), and *Staphylococcus* (10.5%); for diacetyl, *Gemella* (35.7%), *Streptococcus* (21.4%), and *Serratia* (14.3%); for HDP, *Serratia* (26.9%), *Klebsiella* (19.2%), and *Streptococcus* (15.4%); for diacetyl plus HDP, *Rahenella* (50.0%), *Serratia* (30.0%), *Acinetobacter* (10.0%) and *Staphylococcus* (10.0%). The results suggest that HDP and diacetyl may inhibit indigenous microorganisms found in meats, which could extend the shelf-life of meat products.

P142 Improving the Safety of Harvest Practices for Strawberries for Processing

MARY PALUMBO, Nancy Nagle, Cindy Jewell, Michael Gutierrez, and Jeff Farrar, California Dept. of Health Services, Food and Drug Branch, 601 N. 7th St. MS 357, P.O. Box 942732, Sacramento, CA 94234, USA

Frozen strawberries have been epidemiologically linked to several outbreaks of hepatitis A. Established harvest practice involves removal of the calyx (cap) in the field. If the berry is sufficiently soft, the calyx remains on the plant when the berry is pulled off. Historically when self-capping did not occur, the calyx was removed by the picker's thumbnail, possibly introducing pathogens into the flesh. The California Strawberry Commission (CSC) worked with the California Department of Health Services (CDHS) and other regulatory agencies to establish procedures for safer harvest practices in California. The CSC Quality Assurance Program (QAP) was introduced to California growers in regional meetings in October 1999. Safe harvest practices include frequent, thorough hand-washing and optional use of a tool or gloves for calyx removal. Financial incentives for tool-cut berries are offered to growers by some processors. Other reported incentives are less dockage from caps remaining on berries and better strawberry yield when the calyx is removed from the plant. Impediments to implementation are the cost of tools or gloves, and potentially slower pace of harvest and accidental cuts on hands when using tools for calyx removal. CDHS monitored implementation of the CSC QAP by field audits and interviews with processors during the 2000 and 2001 harvest seasons. We estimate that a tool was used in harvesting 60% of strawberries for processing in 2001 compared to < 5% in 1999. Implementation of safe harvesting practices is expected to reduce the probability of a foodborne illness outbreak from frozen strawberry products.

P143 DSC Efficacy of an FDA Approved Peroxyacid-based Sanitizer to Inactivate *Escherichia coli* O157:H7 in Artificially Contaminated Alfalfa Seeds

PASCALE M. PIERRE, Elliot T. Ryser, and Jerry N. Cash, Michigan State University, Dept. of Food Science and Human Nutrition, East Lansing, MI 48824-1225, USA

Alfalfa seeds (Caudill Seed Co., Louisville, KY) were inoculated with a 3-strain cocktail of *Escherichia coli* O157:H7 by immersion so as to contain ~4.0 log CFU/g. After 10 min, the seeds were dried under a laminar flow hood for 24 h and held at ~23°C for up to 4 days for experiments. The inoculated seeds were treated with an FDA-approved sanitizer containing 250 ppm peroxyacid, 1000 ppm fatty acid, 1000 ppm lactic acid and 500 ppm glycerol monolaurate at concentrations of 1x, 5x and 10x for 5 and 10 min. Appropriate decimal dilutions in neutralizing buffer or 0.1% peptone were spiral plated on Cefixime Tellurite Sorbitol McConkey Agar and Plate Count Agar to enumerate *E. coli* O157:H7 and mesophilic aerobic bacteria (MAB), respectively. Using a concentration of 1x, *E. coli* O157:H7 populations decreased 0.99 and 1.14 logs following 5 and 10 min of exposure, respectively. At concentrations of 5 and 10x, numbers of *E. coli* O157:H7 decreased > 1.85 and > 3.24 logs, respectively, regardless of the length of sanitizer exposure. MAB decreased from an initial population of 5.68 log CFU/g on inoculated seeds to 4.41 and 4.46, 3.35 and 3.51, and < 1.30 and < 1.30 log CFU/g following 5 and 10 min exposures to sanitizer concentrations of 1x, 5x and 10x, respectively. Seed germination rates were 95.7% for the control (untreated seeds) and 95%, 92% and 83% for the 1x, 5x and 10x concentrations following 10 min of exposure.

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P144 Combination Treatments of Ozone, Chemical Preservatives, and Refrigerated Storage for Inactivation of *Escherichia coli* O157:H7 and *Salmonella* in Apple Cider

Charity A. Lakins, DAVID A. GOLDEN, and Susan S. Sumner, University of Tennessee, Dept. of Food Science and Tech., 2605 River Drive, Knoxville, TN 37996-4591, USA

Inactivation of nalidixic acid-resistant *Escherichia coli* O157:H7 and *Salmonella* (7 log CFU/ml) in apple cider treated with ozone (O_3; 0.9 g/h) in combination with dimethyl dicarbonate (DMDC) or hydrogen peroxide (HP) was evaluated. For Part 1 of the study, cider was ozonated for 30, 45, or 60 min followed by addition of no preservatives (control), 250 ppm DMDC (250DMDC), or 75 ppm DMDC+200 ppm HP (DMDC/HP) and stored at 4°C for up to 90 min. For Part 2, these preservatives were added first, and cider was stored at 4°C for up to 4 h. During storage, samples were removed and ozonated for 30, 45, or 60 min. Surviving populations of the pathogens were determined using TSA containing 50 ppm nalidixic acid. Significance was defined at $P < 0.05$. Part 1: In control cider, *E. coli* O157:H7 was reduced by 1.02, 3.09, and 4.51 log CFU/ml, and *Salmonella* was reduced by 1.36, 2.48, and 3.60 log CFU/ml after 30, 45, and 60 min of O_3 treatment, respectively. *E. coli* O157:H7 was undetectable after 75, 15, and 30 min storage with 250DMDC and 75, 15, and 15 min storage with DMDC/HP. Results were similar for *Salmonella*. Part 2: *E. coli* O157:H7 and *Salmonella* populations were undetectable after a 30 min O_3 treatment of cider containing 250DMDC or DMDC/HP and stored for 15 min or less. Treatment of cider with O_3 in combination with chemical preservatives and cold storage provides > 5-log reduction of *E. coli* O157:H7 and *Salmonella* spp. Addition of preservatives followed by brief storage before O_3 treatment enhances sensitivity to O_3.

P145 Irradiation Temperature Influences Product Quality Factors of Frozen Vegetables and Radiation Sensitivity of Inoculated *Listeria monocytogenes*

Brendan A. Niemira, Xuetong Fan, and CHRISTOPHER H. SOMMERS, USDA-ARS-ERRC, Food Safety Intervention Technologies Research Unit, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Four frozen vegetables (broccoli, corn, lima beans and peas) were gamma irradiated at various frozen temperatures ranging from -5 to -20°C to determine a) the radiation sensitivity of an inoculated outbreak strain of *Listeria monocytogenes* (ATCC 49594), b) the effect of changing irradiation conditions, i.e temperature, and c) the effect of the recommended radiation dose on the texture and color of irradiated frozen vegetables. The D_{10} (the amount of radiation necessary to reduce the bacterial population by 90%) for *L. monocytogenes* differed significantly among the vegetables at each irradiation temperature. D_{10} increased significantly with decreasing temperature for all vegetables, with each vegetable showing a different pattern of response. At an irradiation temperature of -5°C, D_{10} ranged from 0.505 kGy on broccoli to 0.613 kGy on corn. At -20°C, D_{10} ranged from 0.767 kGy on lima beans to 0.916 kGy on peas. At -20°C, radiation doses sufficient to achieve a 5 $\log_{10}$ kill (3.9-4.6 kGy) caused significant softening of peas and broccoli stems, but not of corn or lima beans. Lower doses of comparable antimicrobial efficacy delivered at -5°C (2.5 to 3.1 kGy) did not cause significant changes in texture in any vegetable. The color varied significantly among the dose-temperature combinations only for broccoli florets; this variation did not demonstrate a clear pattern of quality changes in response to irradiation. These results clearly demonstrate the potential impact of processing conditions on the antimicrobial efficacy and sensory impact of ionizing irradiation on vegetable products.

P146 Effect of Citric Acid on the Radiation Resistance of *Listeria monocytogenes* and Frankfurter Quality Factors

CHRISTOPHER SOMMERS, Xuetong Fan, A. Philip Handel, and Kimberly Sokorai, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor PA 19038, USA

Listeria monocytogenes is a common contaminant of ready-to-eat meat products, including frankfurters. Ionizing radiation can eliminate *L. monocytogenes* from frankfurters. Citric acid (CA) is an antioxidant synergist and antimicrobial agent that can be applied to the surfaces of cured meat products prior to packaging. The effect of CA on the radiation resistance of *L. monocytogenes* that was surface-inoculated onto frankfurters was determined. The D_{10} values, the radiation doses required to inactivate 90% of viable *L. monocytogenes*, were 0.61, 0.60, 0.54, and 0.53 kGy on frankfurters dipped in 0, 1, 5 or 10% CA solution, respectively. CA, although an antioxidant synergist, did not increase antioxidant activity (AA) on frankfurter surfaces as determined by the Ferric Reducing Antioxidant Power (FRAP) assay. Lipid oxidation, as determined by the Thiobarbituric Acid Reactive Substances (TBARS) assay, was not affected by CA or ionizing radiation. Color of frankfurters, determined by Hunter L, a, b, indicated that ionizing radiation induced a small, but visually imperceptible, loss of redness (a-value). Frankfurter firmness, as measured by maximum shear force, was not affected by ionizing radiation or CA. CA enhanced the lethality of ionizing radiation without negatively impacting the frankfurter quality factors tested.

P147 DSC Efficacy of Detergents to Enumerate Pathogenic Microorganisms from the Surface of Fresh Strawberries

RENEE M. RAIDEN, Susan S. Sumner, Merle D. Pierson, and Joseph D. Eifert, Virginia Tech, 22 Food Science and Tech. Bldg., Blacksburg, VA 24061-0418, USA

Fresh produce has been implicated in several foodborne outbreaks. A primary site of contamination for produce occurs on the surface during production

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and handling. An approach to reduce such contamination is to treat fresh produce with rinsing agents. This study used different detergent rinse agents at room temperature and 40°C to determine their efficacy for removal of *Salmonella* and *Shigella* spp. from the surface of fresh strawberries. Strawberries were dip inoculated at room temperature with a 7×10^6 CFU/ml cocktail of nalidixic acid resistant organisms and allowed to dry. Strawberries were rinsed with either a 0.1% solution of Tween 80, 0.1% sodium lauryl sulfate (SLS), or water. Rinse solutions were plated onto Tryptic Soy agar with 50 ppm nalidixic acid. Detergents were no more effective in the removal of *Shigella* than sterile water. In each trial, almost 1×10^4 CFU/ml were recovered from each sample. Similar results were obtained with water and Tween 80 for *Salmonella*. However, *Salmonella* inoculated strawberries rinsed with SLS displayed minimal recovery of about 5×10^1 CFU/ml at room temperature, and no recovery at 40°C. When the whole strawberries treated with SLS were analyzed, no *Salmonella* was recovered. Lack of recovery of *Salmonella* rinsed with SLS suggests that SLS may be inactivating *Salmonella*, especially when a 40°C solution is used. Results indicate that the use of SLS or Tween 80 alone would not enhance the removal of *Shigella*; to be effective, rinse agents would need to be used in combination with another treatment.

P148 Non-thermal Pathogen Reduction for *Escherichia coli* O157:H7 on Apple Surfaces Using Chlorine Dioxide Gas

J. DU, Y. Han, and R. H. Linton, Purdue University, 1160 Food Science Bldg., W. Lafayette, IN 47907, USA

Outbreaks of *Escherichia coli* O157:H7 have been associated with apple-based products, leading to an interest to develop non-thermal pathogen reduction processes. Our objective was to determine the efficacy of chlorine dioxide (ClO_2) gas to accomplish a 3 to 5 log reduction for *E. coli* O157:H7 on pulp skins, calyx cavities and stem cavities of apples. A mixture of three strains (C7927, EDL933, 204P) were spotted onto each site at 1 to 8 log CFU/site, air dried, then treated with ClO_2 gas (1.1 to 18.0 mg/l for 10, 20, or 30 min) at 21°C and 90% relative humidity. Bacterial log reduction was determined by surface plating, a membrane transfer plating, a most probable number, and an end point method. A significantly higher ($P < 0.05$) log reduction was observed for the pulp skins, followed by the calyx and stem cavities. A > 5 log reduction was obtained for pulp surfaces at 7.2, 3.3, and 1 mg/L ClO_2 gas for 10, 20, and 30 min, respectively. A > 3 log reduction was observed for the a) calyx at 12, 3.3, and 3.3 mg/l ClO_2 gas and b) stem cavity at 12, 4.8, and 3.3 mg/l ClO_2 gas for contact times of 10, 20, and 30 min, respectively. The optimal processing conditions ($P < 0.05$) for pathogen reduction for all three sites were 12 mg/l for 10 min, 4.8 mg/l for 20 min, and 3.3 mg/l for 30 min. ClO_2 gas is a promising non-thermal pathogen reduction technique. Further research to optimize conditions for pathogen reduction while maintaining product quality is warranted.

P149 Evaluation of Good Agricultural Practices and Good Manufacturing Practices in Export Growers and Packaging Houses of Fresh Fruits and Vegetables in Costa Rica

CARMELA VELÁZQUEZ and Fernando Aguilar, National Center for Food Science and Technology—CITA, Ciudad Universitaria Rodrigo Facio, University of Costa Rica, San José, Costa Rica

Fruits and vegetables are the main export of Costa Rica to Europe, the US, and the rest of the countries in Central America. The growers and packers for this products are about 50% small and medium producers (less than 20 employees). With the intense Regulatory Food Safety issues stated in the US and the EC, a need developed to evaluate the Good Agricultural Practices (GAP) for the growers and the Good Manufacturing Practices (GMP) of their packaging houses. This survey was encouraged by the FDA in order to apply this tool in a developing country. A group of 209 packaging houses and 300 growers were evaluated by use of a modified version of the "Fruits and Vegetables Agricultural Practices Survey" developed by the National Agricultural Statistics Service (NASS) of USDA. The growers and packagers were selected randomly, considering the risk associated with the product and the exported volume reported in the year 2000. The evaluated products were cabbages, blackberries, strawberries, tomato, lettuce, cantaloupe melon, parsley, cilantro, bananas, pineapples, cassava, chayote and mangos. The data (230 variables for growers and 250 variables for packers per survey) was analyzed with an statistical program, SPSS. The growers are following only about 30% of the GAP recommendations stated in the FDA guidelines, and the packaging houses are applying about 65% of the GMP. Results are analyzed per variable. A great deal of work remains to be done with the growers and packagers, and current training and technical assistance programs are including the findings of this evaluation.

P150 Internalization of *Escherichia coli* in Apples

DSC

B. K. SEEMAN, S. S. Sumner, M. Pierson, R. Worobo, and D. Kang, Virginia Tech., 22 Food Science and Tech. Bldg., Blacksburg, VA 24060, USA

The purpose of this study was to study bacterial internalization in apples during growing conditions, using *Escherichia coli* ATCC 25922 as a surrogate for *E. coli* O157:H7. Five varieties of apples were studied: Redfree, Rome, York, Golden Delicious, and Red Delicious. Redfree was used in a long-term study in which individual apples were spray-inoculated at the beginning of the growing season with *E. coli* ATCC 25922 at 10^4 CFU/apple. The apples were picked on days 1, 30 and 60, and sectioned into skin, flesh, inner and outer cores. The remaining four apples species were used in an intensive two-week study. Apples were inoculated two weeks prior to harvest and picked every other day until harvest. In the long-term study, the surrogate *E. coli* was not found in the apples after day 1. Other coliforms, such as *E. vulneris*, *Klebsiella pneumoniae* and *Kl. ozaenae*, were present in each pick. The two-week study showed higher rates

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of internalization in Red and Golden Delicious than in Rome and York, with the *E. coli* present in all four sections of the apples. Red Delicious apples showed a trend of increasing counts of bacteria over the two-week period with initial counts ranging from less than one to 1.9×10^2 bacteria/ml and final counts as high as 1.3×10^3 bacteria/ml. Again, *Klebsiella* species and *E. vulneris* were found in the apples. Electron and laser confocal microscopy techniques will be used to compare differences of internalization rates of *E. coli* O157:H7 in confined laboratory conditions.

P151 Reduction of *Salmonella* spp. in Aqueous Treatments Used to Pack Fresh-Market Oranges

JENNIFER E. SNART, Mickey Parish, and Linda J. Harris, University of California-Davis, Dept. of Food Science and Tech., One Shields Ave., Davis, CA 95616, USA

Fresh citrus products have not been associated with outbreaks of foodborne illness. However, outbreaks associated with other fresh fruits and unpasteurized apple and orange juices suggest that contamination of the fruit is possible. Fresh oranges are subject to a variety of aqueous spray or tank treatments designed to clean the fruit and reduce the potential for mold development during storage. Although control of fungal contaminants by these treatments is well documented, survival of foodborne pathogens is not. Our objective was to determine the potential for aqueous treatments commonly used to pack fresh-market oranges to harbor *Salmonella*. A cocktail of four ampicillin-resistant, orange-juice outbreak-associated serovars of *Salmonella* was inoculated at 10^6 CFU/ml into several treatment solutions at concentrations and temperatures typically employed by the industry. *Salmonella* was enumerated for up to 48 h using ampicillin-containing bismuth sulfite agar. A 5 log CFU/ml reduction in *Salmonella* was observed within 20 s in 50 ppm chlorine with or without 3% sodium bicarbonate (25°C, pH 8.2) and within 10 min in 0.5% sodium ortho-phenylphenate tetrahydrate (25°C, pH 11.7), 250 ppm imazilil (35 to 46°C, pH 7.3) or 2% soda ash (40°C, pH 11.3). In contrast, *Salmonella* declined by 4 log CFU/ml over 48 h in 4% borax/2% boric acid solution (40°C, pH 8.3). Increasing concentration, temperature, or pH increased the rate of decline of *Salmonella* for all treatments while the effect of organic load was treatment dependent. Aqueous treatments used in fresh-market packing houses pose a low risk for contamination of fruit with *Salmonella*.

P152 Inactivation of *Escherichia coli* O157:H7 and *Salmonella* in Apple Cider and Orange Juice by Combination Treatments of Ozone, Chemical Preservatives, and Refrigerated Storage

ROBERT C. WILLIAMS, David A. Golden, and Susan S. Sumner, Virginia Tech., Dept. of Food Science and Tech., Blacksburg, VA 24061-0418, USA

Inactivation of *Escherichia coli* O157:H7 and *Salmonella* in apple cider and orange juice treated with ozone (O_3), in combination with chemical preservatives and refrigerated storage, was evaluated. A five-strain mixture of nalidixic acid-resistant *E. coli* O157:H7 or *Salmonella* was inoculated (7 log CFU/ml) into cider and orange juice. O_3 (0.9 g/h) was pumped into juices (4°C) containing no preservative (control), dimethyl dicarbonate (DMDC; 250 and 500 ppm), or hydrogen peroxide (HP; 300 and 600 ppm) for 60 min followed by 24 h storage at 4°C. Surviving populations of the pathogens were determined using TSA containing 50 ppm nalidixic acid. Significance was defined at $P < 0.05$. All combination treatments, in conjunction with refrigerated storage for 24 h, provided > 5 log reduction of both pathogens in cider and orange juice, except the O_3/DMDC-250 treatment in orange juice, which reduced *E. coli* O157:H7 populations < 5 log CFU/ml. Both pathogens were reduced by 0.4 to 1.8 log CFU/ml in control juices after 24-h storage. Overall, and with some minor variation in the order of combined treatments, inactivation of *E. coli* O157:H7 and *Salmonella* in cider and orange juice followed the order: O_3/DMDC-500 > O_3/HP-600 > O_3/HP-300 = O_3/DMDC-250 > O_3 only > air. This study indicates that O_3 treatment in combination with DMDC or HP, followed by refrigerated storage, could serve as an alternative to thermal pasteurization to meet the FDA's 5-log reduction standard for apple cider and orange juice.

P153 The Effect of Gamma Irradiation on *Escherichia coli* O157:H7 and *Salmonella* Inoculated on Strawberries

A. KILONZO, J. Kim, T. S. Huang, M. Carter, S. J. Weese, and C. I. Wei., Auburn University, 328 Spidle Hall, Auburn, AL 36849, USA

Treatments with chemical disinfectants show limited effectiveness in killing foodborne pathogens on fruits and fresh produce. Therefore, it is necessary to explore other feasible means that can effectively eliminate these microorganisms in fruits. Irradiation technology, due to its ability to penetrate through the food, can be used to effectively control foodborne pathogens in fresh produce. The purpose of this study was to determine the effect of low dose gamma radiation on nalidixic- acid and novobiosin-resistant *Escherichia coli* O157:H7 and nalidixic acid-resistant *Salmonella* spp. on strawberries. Fresh strawberries were immersed in the bacterial suspensions for 10 min to give an initial population of about 5 log CFU/g. After drying in a biological safety hood, the strawberries were subjected to irradiation at 0, 0.14, 0.24, 0.375, 0.50, and 0.75 kGy. After 0, 7, and 14 days of storage at 4°C, the treated samples were individually blended with 9 volumes of Butterfield's phosphate buffer for 2 min, and the suspensions subjected to bacterial enumeration on appropriate selective media, by use of both spread and pour plating methods. Irradiation at 0.14, 0.24, and 0.375 kGy caused a 1 to 3 log reduction of both pathogens. *E. coli* O157:H7 and *Salmonella* were reduced to non-detectable levels at 0.5 kGy and 0.75 kGy, res-

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pectively. Thus, gamma irradiation can be used as a tool for reducing or eliminating these pathogens on strawberries. The results also show that *Salmonella* spp. is more resistant than *E. coli* O157:H7 to low dose gamma irradiation.

P154 DSC Differential Killing Activity of Cetylpyridinium Chloride (CPC), with or without Neutralizing Buffer Quench, against Firmly Adhered *Salmonella* Gaminara on Lettuce Stored at 4°C

MOEZNIMANWATY OSMAN, M. E. Janes, R. Story, R. Nannapaneni, and M. G. Johnson, University of Arkansas, Dept. of Food Science, 2650 N. Young Ave., Fayetteville, AR 72704, USA

CPC, a chemical in oral-hygiene products, was used to reduce firmly adhered *Salmonella* Gaminara inoculated onto the surfaces of chopped iceberg lettuce stored at 4°C for 0, 2 and 4 days. Lettuce samples were inoculated with 10^8 CFU/ml of *S.* Gaminara, rinsed twice (1 min) with sterile dH_2O, leaving 10^5 CFU/ml firmly attached bacteria, then rinsed with 0.05% to 0.4% CPC (1 min). Next, drained samples were stomached immediately or quenched with a CPC neutralizing buffer (NB) (1 min), rinsed with water (1 min) then stomached in 0.02 M Phosphate Buffered Saline. Bacterial counts were determined on XLD. The minimum level of detection was 2 CFU/g (non-quenched lettuce) and 10 CFU/g (NB-quenched lettuce). With a quench step, there was no reduction of *S.* Gaminara counts even with 0.4% CPC for 0, 2 or 4 day samples. At day 0, 0.1% CPC without quench reduced 4-5 logs of *S.* Gaminara to non-detectable levels. After day 2 at 4°C, the 0.1% and 0.2% CPC without quench gave 3-4 logs reductions, leaving 1-1.5 logs of survivors, which required 0.4% CPC to give non-detectable counts. At day 4, at least 0.2% CPC without quench was needed to give non-detectable counts. These data suggest that 1 min treatments with CPC are only effective against reducing firmly adhered *S.* Gaminara on the surfaces of lettuce if no quenching and rinsing steps are used before enumeration.

P155 The Risk of Salmonellosis Associated with the Consumption of Raw Alfalfa Sprouts: An Exposure Assessment

LYNETTE KLEMAN and Lee-Ann Jaykus, North Carolina State University, Dept. of Food Science, Box 7624, Raleigh, NC 27695-7624, USA

Raw seed sprouts (particularly alfalfa) represent a substantial food safety concern largely because germination conditions are optimal for bacterial growth. The purpose of this project was to estimate the *Salmonella* contamination levels to which a consumer of raw alfalfa sprouts may be exposed. The prevalence of seed contamination with *Salmonella* spp. was estimated to be 1 to 5% with levels of < 1 to 6 CFU/100 g seed; the average concentration of total bacteria in alfalfa seed was estimated at 7.9×10^4 CFU/g. The USDA Pathogen Modeling Program (PMP) was used to model the growth of *Salmonella* during germination. Because the PMP does not consider the influence of competing microflora on *Salmonella* growth rate, it was necessary to estimate the levels of *Salmonella* based on proportion of total bacteria. Using Monte Carlo simulation methods, the mean proportion of *Salmonella* contamination was estimated at 0.028% of total bacterial population on seed. By assuming that the level of *Salmonella* increases proportionally with the growth of total bacteria, the model revealed that *Salmonella* levels reach upper limits ranging from 10^2 to 10^8 /serving within 12 h of sprout seed germination. This is significant since the germination time for commercial production of alfalfa sprouts ranges from 2 to 7 days. This exposure model reaffirms the high degree of foodborne disease risk associated with the consumption of raw seed sprouts and supports the conclusion that mitigation strategies should continue to focus on all aspects of the farm-to-fork continuum, with specific emphasis on the decontamination of seed.

P156 Ultrasonic Treatment of a Rinse Solution to Enhance Enumeration of *Salmonella* spp. from Produce Surfaces

GABRIEL SANGLAY, Joseph Eifert, Merle Pierson, and Susan Sumner, Virginia Tech, Dept. of Food Science and Tech., Mail Code 0418, Blacksburg, VA 24061, USA

Fresh fruits and vegetables have been increasingly associated with outbreaks of food borne illness. Produce can harbor a wide variety of naturally occurring microorganisms, some of which may be pathogenic to humans. Microorganisms on the surface of raw produce may be difficult to remove for decontamination or microbial sampling due to porous surfaces and microbial attachment. Our objective is to determine if ultrasonic treatment (40 kHz) of a rinse solution can enhance the removal and recovery of *Salmonella* spp. from different produce surfaces. Strawberries and apples were spot inoculated with approximately 10^5 cells of a five-strain cocktail of nalidixic acid-resistant *Salmonella* spp. The produce (25°C) was immersed in 0.1% buffered peptone water in either a Whirl-Pak bag for manual shaking (60 s) or a sterile beaker for sonication (60 or 120 s). Additionally, diluent temperatures of either 25°C or 40°C were used with these sampling protocols. Diluents were spiral plated onto tryptic soy agar with nalidixic acid (50 ppm). Plates were incubated at 35°C for 48 h. Ultrasonic treatment of rinse solutions did not enhance recovery of microorganisms. *Salmonella* enumerated after sonication was 0.1 to 0.2 log CFU/ml lower than the level recovered after manual shaking. Additionally, *Salmonella* recovery from diluent at an elevated temperature (40°C) was approximately 0.5 log CFU/ml lower than from sample diluents at 25°C. Since organism recovery was only slightly reduced by sonication, additional research could examine the effect of other ultrasonic energy frequencies, ultrasonic exposure times, and alternative diluents.

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P157 Microbial Profile of Conventionally and Organically Grown Spring Mix

Christie A. Phillips and MARK A. HARRISON, University of Georgia, CFS, Dept. of Food Science and Tech., Room 211, Athens, GA 30602, USA

In recent years, consumption of vegetable salads has increased significantly due to the health interest and diet trends of consumers. Demand for organic food is growing steadily, with sales increasing at 20% per year since 1990. There is little data available comparing the microbial quality of organically grown produce to produce grown by conventional methods. The purpose of the research was to determine the microbiological composition of spring mix grown by either conventional or organic farming practices. Spring mix, or mesclun, is a mixture of multiple salad ingredients. The samples were tested for mesophilic, psychrotrophic, yeasts and molds, lactic acid bacteria, *E. coli* and coliforms counts and for the presence of *Listeria monocytogenes* and *Salmonella* spp. When the sizes of the populations of microbial groups were compared, washed organic and conventional spring mixes were not significantly different ($P < 0.05$). Populations of all the microbial types tested were significantly ($P < 0.05$) lower on chlorine washed spring mix than on unwashed spring mix. *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7 were not isolated from the product. The results can be used by the industry and consumers To judge whether claims concerning microbiological quality and safety of produce grown by organic or conventional means are valid.

P158 DSC The Analysis of Pathogens in Chicken Manure Fertilizer

TRISTIN CRENSHAW, Christopher Choi, and Charles Gerba, University of Arizona, 1117 E. Lowell St., Room 407, Tucson, AZ 85721, USA

Chicken manure from egg ranches in Ramona, California, is being used as a fertilizer on lettuce crops in Yuma, Arizona. The proposed threat of lettuce crops fertilized with chicken manure being contaminated with fecal material has scared wholesalers into refusing to purchase lettuce grown in this manner. A study was set up to examine the validity of this threat by analyzing pathogenic bacteria in chicken manure over time. A 100 m² experimental plot was fertilized with 89.64 kg of chicken manure to mimic a standard lettuce field. Soil/chicken manure and control samples were taken over a fifteen-week period. Initial chicken manure samples were also taken from egg ranches at the beginning of the project. Each sample was analyzed using standard methods for *Escherichia coli* sp., total coliforms, *Clostridium perfringens*, *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7, percent moisture, and temperature. *L. monocytogenes* and *E. coli* O157:H7 were not detected in any of the samples. *Salmonella* was detected three times, twice in the initial chicken manure samples and once in the soil/chicken manure samples. *E. coli* O157:H7 counts reached zero after three weeks. Total coliform counts from the control plot and the experimental plot were equal after eleven weeks. *C. perfringens* persisted in initial, soil/chicken manure, and control samples randomly, suggesting that *C. perfringens* is not related to the presence of chicken manure.

P159 Comparison of Dipping, Spotting, and Spraying Methods to Inoculate *Listeria monocytogenes* on Green Pepper Surfaces

Y. HAN and R. H. Linton, Purdue University, 1160 Food Science Bldg., W. Lafayette, IN 47907, USA

It is difficult to compare or interpret the efficacy data of antimicrobials that use different inoculation procedures. The objective of this research was to compare dipping, spraying, and spotting for inoculating *Listeria monocytogenes* on the calyx cavity, stem cavity, and skin of green peppers. Peppers were dipped, spotted, or sprayed with a mixture of *L. monocytogenes* (Scott A, F5069, and LCDC 81-886) at a level of 10^7 CFU/ml. Recovered and attached (after water washing) bacterial populations on different surfaces were enumerated by a surface-plating method with modified Oxford agar. Samples were examined at the following time points: after inoculation, 2 h drying, 1 day storage and 3 day storage at 4°C. The highest bacterial recovery and attachment for the three inoculation methods were on the pepper stem cavity and the least on the skin. The stem cavity showed no significant differences ($P < 0.05$) in populations after inoculation, 2 h drying, 1 day and 3 day storage for dipping and spotting. However, spraying showed no significant difference ($P < 0.05$) in the populations after 2 h drying, 1 day and 3 day storage. On skin surface, the recovered and attached populations after 1 day or 3 day storage were significantly ($P < 0.05$) lower than the populations after inoculation or 2 h drying for the three inoculation methods. Spotting and spraying on the calyx surface showed significant differences between the populations after 1 day or 3 day storage and after inoculation or 2 h drying; dipping exhibited no significant difference. Of the three methods examined, spotting with 1 day storage at 4°C provided optimum levels of attachment and recovery.

P160 Physical Means of Inactivating *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on Alfalfa Seeds

Chih-Ying Lu, Stuart O. Nelson, Larry R. Beuchat, and MARK A. HARRISON, University of Georgia, CFS, Dept. of Food Science and Tech., Room 211, Athens, GA 30602, USA

Outbreaks of *Escherichia coli* O157:H7 and *Salmonella* infections have been associated with consumption of contaminated alfalfa sprouts; while *Listeria* has not been implicated yet, the risk remains high. Physical and chemical means to reduce pathogen contamination levels on sprout seeds without adversely affecting the seed germination rate were investigated. Dielectric heating was evaluated as a physical means to kill pathogens on the seeds.

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Contaminated seeds with varying moisture contents were subjected to various dielectric heating time/temperature treatments. As an alternative, chemical treatments with or without heat and ultrasound treatments were also evaluated. Dielectric heating significantly ($P < 0.05$) reduced pathogen populations without adversely affecting the germination percentage. Treatment of seeds with 6.5% moisture content for 20 s at 89°C reduced *E. coli* O157:H7 and *Salmonella* populations by 1.40 and 1.06 logs, respectively. Treatment of seeds with 6.4% moisture for 12 s at 82°C reduced *L. monocytogenes* populations by 0.89 logs. Ultrasound treatment at 38.5 to 40.5 kHz in combination with heat and chemical sanitizers did not appear to substantially add to the overall lethality. Treatments with 1% $Ca(OH)_2$ and 8% H_2O_2 were effective in significantly reducing ($P < 0.05$) *Salmonella* populations by over 4 logs at either 23° or 55°C without reducing seed germination rates. Tsunami® 200 (330 ppm) and Sanova® (1200 ppm) at both temperatures reduced *Salmonella* populations significantly, although to a lesser degree. While not eliminating pathogen populations totally from initial levels, these treatments may provide seed growers alternatives to consider for their pathogen reduction programs.

P161 Fate of *Salmonella* in Homemade Unpasteurized Fruit and Vegetable Juices

Claudia M. Cornwell and MARK A. HARRISON, University of Georgia, CFS, Dept. of Food Science and Tech., Athens, GA 30602, USA

The effects of acid adaptation on the survival of *Salmonella* spp. in 4 different acidic unpasteurized fruit and vegetable juices stored at 3 temperatures and the survival of *S.* Poona in unpasteurized cantaloupe juice were determined. Juices with pH ranges of 3.0 to 4.3 were inoculated with 10^8 CFU/ml of acid adapted (grown overnight in tryptic soy broth supplemented with 0.4% dextrose) and non-adapted (grown overnight in TSB) nalidixic acid resistant *Salmonella* stored at 4°, 10°, and 20°C, and sampled every 24 h up to one week. Samples were enumerated on bismuth sulfite (BSA) and plate count agar supplemented with nalidixic acid. The same procedure was followed with cantaloupe juice (pH 6.5) except only non-adapted cells were used and samples were enumerated only on BSA. There were no differences between acid adapted and non-acid adapted *S.* Montevideo and *S.* Gaminara survival patterns in highly acidic unpasteurized juices. The apple-celery juice mix experienced a large drop in *S.* Gaminara numbers mainly because of its pH lethality (pH 3.0). However, a few cells were detected by enrichment even after 48 h. *Salmonella* Montevideo and *S.* Gaminara populations were maintained throughout the entire sampling period in tomato juice and in the carrot juice mix, and *S.* Poona was able to survive and grow in cantaloupe juice at 20°C. The results obtained in this study confirm that unpasteurized juices, regardless of their acidity, pose a risk to consumers who either buy them or make them at home.

P162 Withdrawn

P163 Reduction of Microbes Attached to Fresh-cut Lettuce Using Electrochemically Activated Water Spray

BETTY L. SWEM, Hong Yang, Yulai Cheng, and Yanbin Li, University of Arkansas-Fayetteville, O420 POSC, University of Arkansas, Fayetteville, AR 72701, USA

Fresh-cut lettuce, obtained from local markets on the day of the test, was sprayed in a spray chamber with 300 ppm electrochemically activated water (ECAW), 300 ppm chlorinated water or tap water, at a pH of 7 for 90 s with a pressure of 10 or 50 psi, a temperature of 22°C and a conductivity of 7.22 kmS. Fresh-cut lettuce (400 g) was placed on a conveyor and passed through the spray chamber for spray treatment, and was then sent through a rinse chamber for 30 s to rinse off any ECAW residue. Samples were stomached in 225 ml of buffered peptone water and plated using an automatic spiral plater. Subsequent growth colonies were counted with a ProtoCol colony counting system. The spray chamber, designed for laboratory use, consisted of two overlapping conveyors set at a 45° angle inside a plastic case. There were a total of 6 nozzles installed in the spray chamber, 4 of them for the first conveyor and 2 for the second conveyor. Results indicated that the total aerobic plate count of the ECAW spray treatments was reduced by 2.14 and 2.26 log for 10 and 50 psi, respectively, compared to the no-spray control. The ECAW treatment at a pressure of 50 psi resulted in 0.88 and 1.75 log reduction, more than the chlorinated water and tap water treatments, respectively, under the same test conditions.

P164 Survival of *Shigella flexneri* on Strawberries Stored at -20, 4, and 24°C

STEPHAN FLESSA and Linda J. Harris, University of California-Davis, Dept. of Food Science and Tech., One Shields Ave., Davis, CA 95616, USA

Foodborne shigellosis is often associated with consumption of raw foods, including fresh vegetables that have been handled by infected workers or washed in contaminated water. Although growth of *Shigella* species has been shown for a number of vegetables, little is known about the behavior of this organism on fresh fruits. To determine the influence of inoculum level and storage temperature on the survival of *Shigella flexneri* on strawberries, whole berries were spot inoculated at levels of approximately 10^5 and 10^7 CFU/berry with rifampicin-resistant *S. flexneri* and stored at 4°C and 24°C. Sliced berries were inoculated at levels of 10^5 and 10^7 CFU/25-g sample and stored at -20°C. *Shigella* were enumerated on rifampicin-containing MacConkey agar incubated at 37°C for 24 to 48 h. When inoculated at 10^7 CFU/berry and stored at 4°C, populations decreased by approximately 1.5 log and 3 log CFU/berry within 24 h and 5 days of storage,

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respectively. In contrast, when inoculated at 10^5 CFU/berry, populations declined by > 4 log CFU/berry within the first 24 h of storage at 4°C. Similar trends were observed at room temperature and during frozen storage. Populations declined approximately 2 log CFU/berry within the first few days at the higher inoculum level. At the lower inoculum level, decreases of > 4 log CFU/berry were observed within 72 h of storage at 23°C and 48 h of storage at -20°C. Inoculum level significantly impacts the ability of *Shigella* to survive on strawberries. These data have implications for experimental design, interpretation of data, and risk assessments.

P165 Growth of *Salmonella* Enteritidis PT 30 on Almond Hulls and Shells

LINDA J. HARRIS, Solymar Ontiveros, and Aaron Uesugi, University of California-Davis, Dept. of Food Science and Tech., One Shields Ave., Davis, CA 95616, USA

An outbreak of *Salmonella* Enteritidis Phage Type 30 (SEPT30) associated with consumption of raw almonds was reported in the spring of 2001. Significant rainfall occurred during the usually dry harvest at the time the outbreak-associated almonds were collected. The resulting wet condition of the almonds (a nutmeat surrounded by a shell and hull) necessitated their drying in stockpiles or in a commercial dryer before the hulls and shells were removed. To investigate the potential contribution of this excess moisture to the outbreak, two varieties of naturally-dried almond hulls and shells were obtained from a commercial huller/sheller. When hulls and shells were soaked in water for 30 min, drained, and excess water removed, the weight increased 64 to 78% for hulls and 86 to 106% for shells. To determine the ability of wet almond hulls and shells to support the growth of SEPT30, dry hulls and shells, both mixed and alone, were coarsely ground and 5 g was suspended in 20 ml of sterile water. These suspensions were inoculated with approximately 10^4 CFU/ml SEPT30 and incubated at 37°C. Significant increases (approximately 3 log CFU/ml) in the population of SEPT30 were observed for all combinations of hulls and shells within 24 h with maximum populations of approximately 10^8 CFU/ml observed at day 3. Wet almond hulls and shells provide favorable conditions for the growth of SEPT30 and this may have contributed to the outbreak.

P166 Contamination of Vegetable Crops Irrigated with Dairy Wastewater

FAEZAH MANSHADI, Pablo Gortares, Martin M. Karpiscak, Robert J. Freitas and Charles P. Gerba, University of Arizona, Dept. of Soil, Water, and Environmental Science, Tucson, AZ 85721, USA

The goal of this project was to assess the contamination of various vegetable crops irrigated with dairy wastewater by enteric bacteria and coliphage. Plots of carrots, lettuce and bell pepper were furrow irrigated with dairy wastewater diluted with groundwater throughout the growing season. Control plots of the vegetables were irrigated with groundwater only. The irrigation water and the outer surface of the vegetables were tested on a regular basis for coliform bacteria, *Escherichia coli*, *Clostridium perfringens*, *Listeria monocytogenes* and coliphage. While *L. montocytogenes* was detected in the wastewater, it was never detected on any of the crops. Coliphages were rarely detected in the wastewater and on the crops. *E. coli* concentration in the diluted wastewater ranged from 10 to 100,000 per ml, while *C. perfringens* ranged from 1 to 10 per ml. *E. coli* and *C. perfringens* were always detectable on the wastewater irrigated carrots and lettuce. *E. coli* was occasionally detected on the bell peppers, while *C. perfringens* was almost always detected. *E. coli*, *C. perfringens* and coliphage were rarely detected on the vegetables from the adjacent control plots. The results suggest that *C. perfringens* may be a potentially useful indicator of crop contamination by fecally contaminated irrigation water.

P167 Survival of Acid-adapted or Nonadapted *Escherichia coli* O157:H7 in Apple Wounds following Chemical Treatments and Storage of Samples

J. Ikeda, J. D. STOPFORTH, P. A. Kendall, and J. N. Sofos, Colorado State University, Dept. of Animal Sciences, Ft. Collins, CO 80523, USA

This study evaluated survival/growth of *Escherichia coli* O157:H7 inoculated (4 log CFU/wound) in wounds (generated with 6mm diameter nails) of apples. Wounds were inoculated with a green fluorescent protein-expressing derivative of *E. coli* O157:H7 ATCC43895 and allowed to attach (1 h). Apples were dipped (2 min) in water (W), 5% acetic acid (AA), 5% hydrogen peroxide (HP), 0.02% sodium hypochlorite (SH), or not treated, and stored at 25°C. Survivors were determined in cores of the apple wounds (12mm; inner core) and surrounding tissue (18mm; outer core) after the samples were homogenized in neutralizing broth and plated on tryptic soy agar (TSA) and TSA supplemented with 100 μg/ml rifampicin (35°C, 48 h) after 0, 2 and 5 days. Bacterial populations at day-0 were 4.0 and 2.0 logs in the inner and outer core, respectively. In the inner core of the untreated apples populations increased to 7.0 logs after day-2, while counts did not exceed 3.0 logs in the outer core during storage of all treatments. Acid-adaptation did not affect survival of the pathogen. Dipping in W, AA and SH did not reduce initial counts, while at day-2 of storage counts in W, AA and SH reached 7.1, 5.5 and 6.9 logs, respectively. In contrast, HP reduced initial counts in the inner core by approximately 1.5 logs, but they increased to 7.0 logs by day-2. Populations of all treatments reached 6.6 to 7.1 logs by day-5. Thus, sanitizer treatment did not effectively reduce or inhibit growth of *E. coli* O157:H7 contamination in apple wounds and surrounding tissue.

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P168 Efficacy of Calcinated Calcium in Killing *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* on the Surface of Tomatoes

M. L. BARI, Y. Inatsu, S. Kawasaki, E. Nazuka, and K. Isshiki, National Food Research Institute, Food Hygiene Research Team, Kannondai 2-1-12, Tsukuba 305-8642, Japan

A study was conducted to evaluate the efficacy of calcinated calcium, 200 ppm chlorine water (1% active chlorine) and sterile distilled water in killing *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on the surface of tomatoes (spot inoculated). Inoculated tomatoes were sprayed with calcinated calcium, chlorinated water or sterile distilled water (control) and hand rubbed for 30 s. Each tomato was then placed in a plastic bag and rinsed with 200 ml of sterile water by vigorously agitating for 30 s. Each tomato was then transferred to a second bag, and 20 ml of sterile 0.1% peptone was added; tomatoes were rubbed by hand for 40 s. Populations of *Escherichia coli* O157:H7, *Salmonella* or *L. monocytogenes* in the rinse water and the residual (0.1% peptone) wash solution were determined. Treatment with 200-ppm chlorine and calcinated calcium resulted in > 4.00 and > 7.85 $\log_{10}$ reductions, respectively, in *Escherichia coli* O157:H7. Treatment with 200-ppm chlorine and calcinated calcium resulted in > 3.00 and > 7.36 $\log_{10}$ reductions, respectively, in *Salmonella*. Treatment with 200 ppm chlorine and calcinated calcium reduced the number of *L. monocytogenes* by > 4.00 and > 7.59 $\log_{10}$ CFU/tomato, respectively. The findings suggested that calcinated calcium could be useful in controlling pathogenic microorganisms in fresh produce.

P169 Survival of *Listeria monocytogenes* in a Simulated Recirculating Brine Chiller System

J. K. GAILEY, J. S. Dickson, and W. Dorsa, Iowa State University, 207 Science I, Ames, IA 50011, USA

Contamination by *Listeria monocytogenes* of processed meats following cooking presents a significant food safety risk. The purpose of this study was to determine the survival of *L. monocytogenes* in a simulated recirculating brine chiller system accounting for pH values of 5, 6, and 7 with free chlorine levels of 0, 3, 5, 10 ppm in a 20% salt brine at -12°C. At pH values of 5, 6, and 7 with chlorine levels of 2 and 3 ppm, using 10^8 CFUs in a test tube system, an immediate drop of 0.28 logs was observed, with no significance between treatments ($P > 0.05$), followed by a steady survival phase with a slope near zero. In brine of pH 5 with 5 and 10 ppm chlorine, an initial drop of .8 logs was observed, followed by a steady survival phase with a destruction slope close to zero. At an inoculation level of 10^2 in a test tube system (pH 5 and 7 with zero and 10 ppm chlorine), the average initial drop for all treatments was 0.1 logs, followed by a steady survival phase. In a recirculating system, it was found that very few cells are destroyed during the brine chilling process but that only low numbers of *L. monocytogenes* were recovered from the brine and uninoculated hot dogs. Although little destruction of *L. monocytogenes* was noted, the dilution effect observed during the study indicates that environmental contamination of a brine chiller system poses little danger of post-cooking contamination for processed meats.

P170 Investigation of Potential Sites of Microbial Contamination of Sliced Ready-to-eat Meat Products

L. PEDROSO, A. Louçã, and J. Sofos, Instituto Superior de Ciências da Saúde - Sul, Campus Universitário, Quinta da Granja, Monte de Caparica, Caparica 2829-511, Portugal

The strategies for control of post-processing contamination of ready-to-eat meats with pathogens such as *Listeria monocytogenes* may depend on the potential sites of contamination in the food chain. The aim of this study was to evaluate potential sources of microbial contamination of sliced ready-to-eat meat products, mostly cooked hams, in meat plants and at supermarkets. Levels of contamination were also compared between two regions of Portugal and between two seasons (summer and winter). We analyzed 337 samples (297 cooked hams and 40 other ready-to-eat meat products such as salami and chorizo) for total plate counts, total coliforms, *Escherichia coli*, *Listeria* spp., *L. monocytogenes*, *Staphylococcus aureus* and *S. aureus* enterotoxin. Samples were studied in two separate groups: those that were sliced and packaged by meat companies and those sliced at supermarket deli counters. Results obtained for total plate counts, total coliforms, *E. coli* and *S. aureus* suggest levels of contamination did not particularly differ between samples sliced in meat companies or by those sliced in supermarkets. *S. aureus* enterotoxin was not detected in any of the samples analyzed during the study. The presence of *Listeria* spp. was 18% for both supermarkets and industry. Samples sliced by supermarkets and industry were 9% and 17% positive for *L. monocytogenes*, respectively. Prevalence of *L. monocytogenes* was higher in samples sliced by the supermarkets during the winter. The data suggest that both the supermarkets and the meat companies can contribute to post-cooking contamination with pathogens such as *L. monocytogenes*, which may be controlled with antimicrobials applied during product slicing.

P171 Ability of *Listeria monocytogenes* to Withstand Re-heating of Frankfurters

Anna C. S. Porto, Manuela Osoria, Peggy Williamson, Caitriona Byrne, Lisa Yoder, JEFFREY E. CALL, and John B. Luchansky, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Listeria monocytogenes (Lm) has been associated with some ready-to-eat meats, including frankfurters. This study was conducted to investigate the effect of temperature, time, and product formulation on the survival of Lm on frankfurters. Individual links containing 0 or 2% potassium lactate were inoculated

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with approximately 8.0 $\log_{10}$ CFU/package of a five-strain mixture of Lm, vacuum-packaged, and stored at 4°C for up to 3 days. Next, frankfurters were heated to a surface temperature of 60°, 70°, 80° or 90°C for up to eight min by submersing the packages in a water bath. Survivors were recovered and enumerated by rinsing each package with sterile peptone water and direct plating onto MOX selective agar. The data for each of two trials were averaged: D-values at 60°, 70°, 80° and 90°C were 2.5, 0.3, 0.2, and 0.06 min respectively for frankfurters containing 2% potassium lactate and 2.4, 0.3, 0.1, and 0.06 min respectively for frankfurters without added potassium lactate. Similar results were obtained using frankfurters inoculated with Lm and stored at 4°C for 15 days and at -18°C for 30 days prior to cooking. In general, statistical analyses revealed a significant ($P < 0.05$) decrease in D-values with an increase in temperature. Product formulation did not appreciably affect D-values for any of the parameters tested. These findings establish cooking guidelines that can be followed by consumers to ensure that frankfurters, which may be contaminated with low levels of Lm after unpackaging, are adequately re-heated before consumption.

P172 Use of PFGE to Determine the Persistence of a Five-strain Mixture of *Listeria monocytogenes* during Chilled Storage of Vacuum-sealed Packages of Frankfurters

Anna Porto, LAURA WONDERLING, Jeffrey Call, and John B. Luchansky, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Listeria monocytogenes can survive and often grow under conditions of high osmolarity, low pH, and low temperature such as found in some ready-to-eat meats. In a previous study, we monitored the viability of the following five-strain mixture of *L. monocytogenes* during refrigerated storage of frankfurters prepared with and without 3% potassium lactate (Klac): ScottA (serotype 4b), LM-101M (serotype 4b), F6854 (serotype 1/2), H7776 (serotype 4b), and MFS-2 (serotype 1/2c). Throughout a 90-day period, the starting inoculum of 20 CFU/ml remained relatively constant in the presence of Klac, but there was about a 2- $\log_{10}$ increase of the pathogen in the absence of Klac. To determine which of the 5 strains persisted under these conditions, randomly-selected colonies obtained after 28 and 90 days of refrigerated storage were analyzed by PFGE with the restriction enzyme SmaI to generate distinct banding patterns for each of these 5 strains. In the absence of Klac, 43% of the 58 isolates from day 28 were identified as ScottA, 12% as LM-101M, 22% as F6854, 10% as H7776, and 12% as strain MFS-2; however, by day 90, the majority (83%) of the 60 isolates were identified as strain MFS-2. In packages containing frankfurters prepared with Klac, all 5 strains were present (5-36%) among the 19 isolates tested; however, by day 90, strain MFS-2 comprised the majority (63%) of the 27 isolates tested. The results of this study indicate that strain MFS-2 was more persistent than strains ScottA, LM-101M, F6854, and H7776 during refrigerated storage of frankfurters.

P173 Evaluation of *Listeria monocytogenes* Survival in Inoculated Frankfurters Following Consumer Accessible Cooking Instructions

M. T. ORTEGA-VALENZUELA, R. K. Phebus, and H. Thippareddi, Kansas State University, 201 Call Hall, Manhattan, KS 66502, USA

Listeria monocytogenes outbreaks in ready-to-eat products has been linked to hot dogs and deli meats. USDA Food Safety and Inspection Service has zero tolerance for *Listeria monocytogenes* in ready-to-eat products and has advised the reassessment of HACCP plans and environmental testing in plants. Nevertheless, the last HACCP linkage is at "the table" and FSIS has recommended for preventing listeriosis to "Reheat [hotdogs] until steaming". Our objective was to evaluate *Listeria monocytogenes* survival in frankfurters processed using commercially available cooking. Franks were inoculated with fluorescent *Listeria monocytogenes*, to determine, through confocal laser scanning microscopy, depth of penetration after 24-h vacuum packaging. Franks were mist inoculated with a six-strain mix of *Listeria monocytogenes* (approximately 10^7 CFU/g). Eight inoculated and vacuum packaged frankfurters were cooked using boiling water, a conventional oven or a microwave oven. *Listeria monocytogenes* was recovered after plating on modified Oxford agar and triptose phosphate agar. Cross-sectional visualization showed *Listeria monocytogenes* fluorescence density at 200 mm. *Listeria monocytogenes* reductions were £3.2 $\log_{10}$ CFU/g in franks microwaved with or without water for 60 s or cooked in a conventional oven at 260°C for 2 or 5 min. Franks boiled in water for 30 and 60 s achieved 4.3 and 4.9 $\log_{10}$ CFU/g, respectively. Franks wrapped in a paper napkin and microwaved for 60 s accomplished 6.8 $\log_{10}$ CFU/g. Microwaving of franks wrapped in paper napkins for 60 s was the consumer instruction that resulted in an adequate level of safety and could help limit the presence of *L. monocytogenes*.

P174 Edible Zein Film Coatings Containing Nisin and EDTA to Control *Listeria monocytogenes* Inoculated onto the Surfaces of Turkey Franks

M. E. JANES, B. Lungu, and M. G. Johnson, University of Arkansas, Dept. of Food Science, 2650 N. Young Ave., Fayetteville, AR 72704, USA

We determined the inhibitory activities of nisin directly added to zein edible films coated onto the surfaces of turkey franks (irradiation at 3 kGy) for protection against *Listeria monocytogenes*. Turkey franks (95% fat free – 1 g portions) were immersed into 24 h broth cultures of *L. monocytogenes* strain V7, for 1 min, and allowed to drip free of excess inoculum. The meat samples were then dipped into an edible zein film dissolved in either propylene glycol (ZP) or ethanol (ZE), with and without added nisin (N) (1000 IU/g) and/or EDTA (Ethylenediamine tetraacetic, sodium salt) (1.6 mg/ml). Samples were

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placed into sterile Whirl pack bags, stored at 8°C. *L. monocytogenes* counts were determined by stomaching samples at 0, 7, 14, 21 and 28 days, spread plating on nutrient medium , then incubating at 37°C for 24 h. An initial 7 log CFU/g inoculum of *L. monocytogenes* on the surfaces of control turkey franks dropped to 6.5 log CFU/g at day 7 through 28. Zein films without N or EDTA did not significantly reduce *L. monocytogenes* counts from controls on the surfaces of turkey franks by 28 days. At 8°C N, N + EDTA, ZPN, ZPN + EDTA and ZEN + EDTA significantly reduced *L. monocytogenes* counts by 2.5 to 3.0 log CFU/g by 7 days. The most effective coating by 28 days was ZPN + EDTA, which reduced *L. monocytogenes* counts by 3 log CFU/g. Nisin was more inhibitory to *L. monocytogenes* at 8°C on the surfaces of turkey franks when zein films contained both propylene glycol and EDTA.

P175 Inactivation of *Listeria monocytogenes* on Ready-to-eat Hot Dogs Treated with Volatilized Acetic Acid

Nancy Jensen, Andrew Inglis, and PETER W. BODNARUK, Food Science Australia, P.O. Box 52, North Ryde, NSW 1670, Australia

The ICMSF has proposed a preventative scheme for managing microbiological hazards in foods. The scheme uses the concept of food safety objectives that allows equivalence of different control measures to be assessed. Control measures for *Listeria monocytogenes* contamination of ready-to-eat (RTE) hot dogs can encompass several strategies. These include prevention of contamination prior to packaging, growth inhibition in packaged product during storage using a preservative system, or a validated pathogen reduction step after packaging. This study evaluated a novel pathogen reduction step for RTE hot dogs in this context. The process involves the use of volatilized acetic acid partitioned in CO_2, creating an acetic acid vapor (AAV: Vaporex P/L™). A five-strain mixture of *L. monocytogenes* was inoculated onto the surface of RTE hot dogs. The inoculated hot dogs were dried for 20 min. and then treated with different concentrations of AAV. Inoculated and treated hot dogs were stored at 4° and 8°C for periods of up to 28 days. Hot dogs were sampled following the initial treatment with AAV, after 24 h and then every seven days. Samples were analyzed for *L. monocytogenes* by enrichment and enumeration. Studies showed that surface treatment of inoculated hot dogs with AAV resulted in a 2 to 6 D reduction after 24 h storage. An additional 2 D reduction was noted during extended storage, indicating the treatment had a residual effect. The data indicate that this process has potential to offer a validated pathogen reduction step as part of a risk management system.

P176 Effects of Pediocin and Post-packaging Thermal Pasteurization on *Listeria monocytogenes* on Frankfurters

CHIH-MING CHEN, Joseph G. Sebranek, James S. Dickson, and Aubrey F. Mendonca, Iowa State University, Animal Science, Meat Laboratory, 214 Meat Laboratory, Animal Science, Ames, IA 50011-3150, USA

Pediocin and post-packaging thermal pasteurization (PPTP) have each been reported to have significant benefits for control of the post-processing contamination of frankfurters by *Listeria monocytogenes*. The objectives of this study were to evaluate the inhibitory effectiveness of pediocin (in Alta™ 2341 in combination with PPTP on *L. monocytogenes* on frankfurters in different package arrangements. Frankfurters were surface-inoculated with 3.4 and 5.2 log CFU/g of a 5-strain *L. monocytogenes* mixture, vacuum packaged and stored at abuse and refrigerated temperatures of 25, 10, and 4°C. Packages consisted of either 10 links, 5 links or 1 link per package. Each package was treated with Alta™ 2341, equivalent to 3,000 or 6,000 AU per link. Thermal pasteurization was done in hot water at 71, 81, or 96°C for 30, 60 or 120 s. *L. monocytogenes* were reduced markedly by all treatments, but 81°C for 60 s was necessary to achieve a 50% reduction of originally inoculated *L. monocytogenes* numbers. Thermal pasteurization was most effective in single-link packages, followed by 5-link packages. Packages with 10 links per package resulted in greatest *L. monocytogenes* survival. The 6,000 AU pediocin, combined with PPTP at 80 or 96°C for at least 60 s, extended the lag phase of *L. monocytogenes* growth during storage at 25, 10, or 4°C for 1 to 8 days, 1.5 to 3.5 weeks or 7 to 12 weeks, respectively. Almost no *L. monocytogenes* were detected on frankfurters stored at 4°C, in single-link packages. Thus, pediocin, combined with PPTP, inhibited the growth of *L. monocytogenes* on frankfurters, especially during storage at 4°C.

P177 Effects of Pediocin and Post-packaging Irradiation on *Listeria monocytogenes* on Frankfurters

CHIH-MING CHEN, Joseph G. Sebranek, James S. Dickson, and Aubrey F. Mendonca, Iowa State University, Animal Science, Meat Laboratory, 214 Meat Laboratory, Animal Science, Ames, IA 50011-3150, USA

This study evaluated the potential of Alta™ 2341, combined with irradiation to control *Listeria monocytogenes* on frankfurters in different package arrangements. Frankfurters were surface-inoculated with 3.4 and 5.2 log CFU/g of a 5-strain *L. monocytogenes* mixture, vacuum packaged and stored at 25, 10, and 4°C. Packages were treated with Alta™ 2341, equivalent to 3,000 or 6,000 AU per link, prior to sealing and contained of either 10 links, 5 links or 1 link per package. The absorbed doses of irradiated

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samples for two dose levels were 1.2 and 2.3 kGy (1-link and 5- link packages), and 1.4 and 3.5 kGy (10-link packages), respectively. *L. monocytogenes* on frankfurters were reduced significantly by pediocin combined with irradiation at 2.3 kGy or greater. When 6,000 AU pediocin was combined with irradiation at 2.3 kGy or greater, the lag phase of *L. monocytogenes* growth during storage at 25, 10, or 4°C was 3 to 12 days, 3.5 to 12 weeks or 12 weeks, respectively. Almost no *L. monocytogenes* were detected on frankfurters stored at 4°C. Most of the samples stored at 10°C showed non-detectable *L. monocytogenes* levels until 3.5 weeks. Therefore, pediocin in combination with irradiation was very effective for inhibiting the growth of *L. monocytogenes* on frankfurters, especially during storage at refrigerated temperatures (10 or 4°C).

P178 DSC Hydrostatic Pressurization at 50°C in the Presence of Bacteriocin Completely Eliminated Contaminated *Listeria monocytogenes* in Processed Meat Products

SOMNATH BANDYOPADHYAY, Alex Wolf, Norsak Kalchayanand, and Bibek Ray, University of Wyoming, Animal Science Dept., P.O. Box 3684, Laramie, WY 82071-3684, USA

The effectiveness of 345 MPa hydrostatic pressure (HP) at 25°C or 50°C for 5 min with pediocin AcH and nisin A to destroy over 6 log *Listeria monocytogenes* cells in hotdogs was studied. Initial studies revealed 10 *L. monocytogenes* strains differed in resistance to HP, pediocin, nisin or a combination of HP and bacteriocins at 25°C; this difference is reduced by pressurization at 50°C. When five resistant strains were used as a cocktail their viability losses following pressurization at 25°C were (log cycles): 0.8 by HP, 1.2 by HP + pediocin, 4.1 by HP + nisin, and 4.26 by HP + pediocin + nisin. By pressurization at 50°C, the viability loss was 7.0 log cycles by HP alone, and no survivors among 7.7 to 8.0 log cycles were detected with HP + pediocin, HP + nisin, or HP + pediocin + nisin. In the product studies commercial hotdogs were packaged, inoculated with 7.6 log cells of five strains mixture with or without pediocin + nisin, pressurized at 50°C, and survivors were enumerated. While HP alone reduced viability by 4.2 log, no survivors were detected with HP + pediocin + nisin treatment. This, as well as storage studies, showed that pressurization at 345 MPa for 5 min at 50°C with the two bacteriocins could eliminate over 6 log *L. monocytogenes* in hotdogs.

P179 Effect of Packaging Materials on Inactivation of Pathogenic Microorganisms on Meat during Irradiation

Kathiravan Krishnamurthy, ALI DEMIRCI, Virendra M. Puri, and Catherine N. Cutter, Pennsylvania State University, 231 Agricultural Engineering Bldg., University Park, PA 16802, USA

Irradiation appears to be most promising method to eliminate pathogenic microorganisms. However, a low irradiation dose is preferred for economic and food quality reasons. Before irradiating the food products, they are packaged to avoid any cross-contamination after irradiation. The objective of this research is to compare the effect of packaging materials on inactivation of pathogens during low-dose irradiation. Meats were inoculated with *Escherichia coli* O157:H7 or *Salmonella* Typhimurium and vacuum packaged with polyethylene, polylactic acid (PLA), polynylon, polypropylene, or low-density-polyethylene (LDPE). Then the samples were subjected to various irradiation doses (0.5, 1.0 and 2.0 kGy) in a ^{60}Co irradiator. Triplicate samples were analyzed in terms of microbial reduction, immediately after irradiation and after 7-day refrigerated storage (4°C). For *S.* Typhimurium, both the effect of packaging material and storage were significant ($P < 0.05$). The storage samples showed higher log reduction than for immediate analysis at all tested doses. For example, average log reduction for all plastics at 1.0 kGy were 2.22 CFU/cm^2 for immediate analysis, and 3.70 CFU/cm^2 for 7-day storage. PLA demonstrated the highest log reduction for immediate and 7-day storage analysis; 1.91, 2.72, 4.73 CFU/cm^2 for immediate analysis and log 4.86, 5.18, 5.73 CFU/cm^2 for 7-day storage at 0.5, 1.0, and 2.0 kGy, respectively. On the other hand, polyethylene demonstrated log reductions of 0.59, 1.75, 3.88 CFU/cm^2 for immediate analysis and 1.65, 1.95, 4.98 CFU/cm^2 for 7-day storage at 0.5, 1.0, and 2.0 kGy, respectively. The other plastics were between PLA and polyethylene at all doses, in the order of PLA>LDPE>polypropylene> polynylon> polyethylene. However, the effect of plastics on *E. coli* O157:H7 reduction was not significant (at a level of $P < 0.05$) at all doses. In general, *E. coli* O157:H7 showed a higher log reduction than *S.* Typhimurium at the same dose. For example, average log reductions for all plastics at 1.0 kGy were 2.22 and 3.17 CFU/cm^2 for immediate analysis and 3.70 and 3.92 CFU/cm^2 for 7-day storage for *Salmonella* and for *E. coli* O157:H7, respectively. The mechanical strength of irradiated and unirradiated plastics did not differ significantly ($P > 0.05$).

P180 Effect of Acid Adaptation on Destruction of *Salmonella* during Drying (60°C) and Storage (25°C) of Beef Jerky Treated with Marinades

Mehmet Calicioglu, JOHN N. SOFOS, John N. Samelis, and Patricia A. Kendall, Colorado State University, Dept. of Animal Sciences, 14-B Animal Sciences Buliding, Ft. Collins, CO 80523-1171, USA

Salmonella may survive the jerky manufacturing process and may cause foodborne disease outbreaks. This study evaluated the influence of various pre-drying treatments on inactivation of acid-adapted or nonadapted *Salmonella* on beef jerky during preparation, drying and storage. The inoculated (five-strain composite, 6.0 $\log_{10}$ CFU/cm^2) slices were subjected to the following marinades (24 h, 4°C) prior to drying at 60°C for 10 h and aerobic storage at 25°C for 60 days: control (C), traditional marinade (TM), double amount of TM with added 1.2% sodium lactate, 9% acetic acid, and 68% soy sauce with 5% ethanol

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(MM), dipping into 5% acetic acid followed by TM (AATM), and dipping into 1% Tween 20 followed by 5% acetic acid, followed by the TM (TWTM). Bacterial survivors were determined on tryptic soy agar with 0.1% pyruvate and XLT-4 agar. Results indicated that drying reduced bacterial populations in the order of pre-drying treatments TWTM (4.8-6.0 $\log_{10}$ CFU/cm^2)≥ AATM≥MM>TM≥C (2.6-5.0 $\log_{10}$ CFU/ cm^2). Nonadapted *Salmonella* were significantly ($P < 0.05$) more resistant to drying than acid-adapted in all treatments. Bacterial populations decreased below the detection limit (-0.4 $\log_{10}$ CFU/ cm^2) as early as 7 h during drying or remained detectable even after 60 days of storage, depending on acid-adaptation, pre-drying treatment, and agar media. These results indicated that acid-adaptation may not cause increased resistance of *Salmonella* to the microbial hurdles involved in jerky processing and that use of modified marinades in jerky may improve the effectiveness of drying in inactivating *Salmonella*.

P181 Influence of Marinades on Survival during Storage at 25°C of Acid-adapted and Nonadapted *Listeria monocytogenes* or *Salmonella* Inoculated Post-drying on Beef Jerky

Mehmet Calicioglu, JOHN N. SOFOS, and Patricia A. Kendall, Colorado State University, Dept. of Animal Sciences, 14-B Animal Sciences Bldg., Ft. Collins, CO 80521-1171, USA

Post-processing contamination of food products with pathogenic bacteria may occur and be a health risk to consumers. This study evaluated survival of acid-adapted and nonadapted *Listeria monocytogenes* or *Salmonella* inoculated after drying on beef jerky that was treated with marinades before drying. Beef slices were subjected to the following marinades (24 h, 4°C) prior to drying at 60°C for 10 h: control (C), traditional marinade (TM), double amount of TM modified with added 1.2% sodium lactate, 9% acetic acid, and 68% soy sauce with 5% ethanol) (MM), dipping into 5% acetic acid and then TM (AATM), and dipping into 1% Tween 20 and then into 5% acetic acid followed by the TM (TWTM). Jerky slices were inoculated with a five-strain composite of acid-adapted or nonadapted *L. monocytogenes* or *Salmonella* (5.7 $\log_{10}$ CFU/cm^2) prior to aerobic storage at 25°C. Survivors were determined using tryptic soy agar with 0.1% pyruvate and PALCAM or XLT-4 agar. Results indicated bacterial decreases during storage following the order of TWTM≥AATM≥ MM≥C≥TM treatments. Acid-adapted *Salmonella* decreased faster than nonadapted in all treatments whereas no difference was found for *Listeria*. Bacterial populations decreased below the detection limit (-0.4 log CFU/cm^2) as early as 14 days (*Salmonella*) or 28 days (*L. monocytogenes*) or remained detectable at 60 days of storage, depending on acid-adaptation, treatment or agar media. These results indicated that use of modified marinades in jerky processing and low water activity provide antimicrobial effects against possible post-processing contamination with *L. monocytogenes* or *Salmonella*.

P182 Distribution of *Escherichia coli* O157:H7 in Ground Meat Resulting from a Laboratory-scale Grinder

ROLANDO A. FLORES, Tod Stewart, and Mark Tamplin, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Epidemiological reports link contaminated meat grinders to human illnesses caused by pathogens. This research was designed to study the distribution of *Escherichia coli* O157:H7 in ground beef when a contaminated beef trim was introduced into a batch of uncontaminated beef prior to processing in a laboratory grinder. Beef trim was inoculated with a rifampacin-resistant strain of *E. coli* O157:H7 at seven inoculum levels (10^6, 10^5, 10^4, 10^3, 10^2, 10^1, and 10^0 total CFU). The highest *E. coli* O157:H7 concentration in the ground beef corresponded to the inoculated ground trim or the point where the pathogenic organisms were inserted into the grinding batch. For all inoculum levels, the pathogen concentration in the ground beef was a function of the initial inoculum level. A decreasing distribution of the pathogen was observed in the ground beef following the grinding of the contaminated trim. The location and concentration of *E. coli* O157:H7 retained in the grinder was proportional to the inoculum level. To extrapolate the distribution patterns observed in this study to a plant level operation, large-scale trials need to be conducted. However, these results show that the amount of *E. coli* O157:H7 on a single trim is distributed over a quantity of ground beef larger than the original trim size, and that *E. coli* O157:H7 is retained in parts of the grinder. Consequently, sanitation schedules should consider the potential accumulation of pathogens in grinder parts, and the design of grinders should attempt to minimize accumulation and maximize sanitation.

P183 Origin of Ground Beef Contamination and Genetic Diversity of *Escherichia coli* in Beef Production Processes

MUEEN ASLAM, Frances Nattress, Gordon Greer, Colin Gill, and Lynn McMullen, Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C and E Trail, Lacombe, Alberta T4L 1W1, Canada

The objectives were to determine the origin of *Escherichia coli* contamination in ground beef and to evaluate the genetic diversity of *E. coli* populations in beef cattle using the RAPD (randomly amplified polymorphic DNA) technique and PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) analysis of the fliC gene. *E. coli* were recovered from the feces of 10 beef cattle at several times while they were grazing on pasture and while they were held in a feedlot (855 isolates) and from their hides (320 isolates), carcasses (153 isolates) and ground beef (75 isolates) during slaughter and processing in a research abattoir. The *E. coli* isolates were grouped into 121 distinct genotypes with the RAPD technique. The *E. coli* population changed over time in all cattle while the animals were grazing on

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pasture and in the feedlot. Although a few *E. coli* genotypes were shared among cattle at all sampling times, they were generally unique to individual animals. Some *E. coli* genotypes identified in cattle feces were also found on their hides and carcasses at the time of slaughter and in the ground meat after processing. *E. coli* isolates (120) from one animal were grouped into 8 different genotypes with PCR-RFLP analysis of fliC gene. Results with both the RAPD method and PCR-RFLP technique were comparable. This study provides evidence that the RAPD and PCR-RFLP can be used to determine the genetic diversity of *E. coli* strains harbored by beef cattle and to trace genetically identical strains throughout processing to ground beef.

P184 The Growth of *Escherichia coli* O157:H7 in Retail and Irradiated Ground Beef at 10°C

MARK L. TAMPLIN, USDA-ARS-ERRC, Microbial Food Safety Research Unit, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Estimates of the growth of *Escherichia coli* O157:H7 in ground beef have been done using microbial models developed in pure culture, broth-based systems. The lack of adequate validation studies in ground beef introduces uncertainties in risk assessments. In the present study, the growth of nine beef isolates of *E. coli* O157:H7 was measured in retail ground beef stored at 10°C. The growth parameters of *E. coli* O157:H7 in sterile, irradiated ground beef were similar to estimates of the US Department of Agriculture Pathogen Modeling Program (PMP) version 5.1 (maximum population density [MPD] of 9.32 $\log_{10}$ CFU/g, exponential growth rate [EGR] of 0.048 $\log_{10}$ CFU/h, and lag time of 48.5 h), except that no lag phase was observed. In contrast, the mean MPD and EGR in retail ground beef were 5.09 $\log_{10}$ CFU/g and 0.019 $\log_{10}$ CFU/h, respectively. There was low variation in the MPD and EGR values among the nine *E. coli* O157:H7 beef isolates. Individual isolates of competitive native flora were added to sterilized (irradiated) ground beef and the EGR and MPD decreased as the ratio of competitive flora to *E. coli* O157:H7 increased. For one strain, at ratios of 1:1, 10:1 and 100:1, the EGR and MPD were 0.033, 0.025, 0.018 and 6.09, 5.05, 4.73, respectively. These results demonstrate that existing broth-based models must be validated in food and that models are needed to estimate the effects of the food matrix, the competitive microflora, and strain variation.

P185 Influence of Composition and Packaging of Beef Patties on Gamma Radiation Inactivation of *Escherichia coli* O157:H7

Dory Worcman-Barninka, Bernadette D. G. M. Franco, Maria Teresa Destro, and MARIZA LANDGRAF, University of São Pãulo-Brazil, Av. Prof. Lineu Prestes, 580 B.14, São Pãulo 05508-900, Brazil

Food irradiation has been studied worldwide as a food preservation technique. The resistance of microorganisms to ionizing radiation is influenced by different factors such as food composition, especially fat content, temperature of irradiation, and packaging. The aim of this study was to evaluate the influence of composition and packaging of hamburgers on the radio-resistance of *Escherichia coli* O157:H7. Four loads of lean ground beef were individually homogenized with two levels of beef fat (10% and 20%) and spices (salt 2%, pepper 0.2%) or a commercial mix of condiments for raw meat products (1kg/25kg of meat). Each load was inoculated with a pool of three isolates of *Escherichia coli* O157:H7 (10^7 to 10^8 CFU/g), homogenized, subdivided into 100g portions, and molded into hamburgers. Half of the patties were vacuum packaged in barrier pouches and half were air packed. All samples were tempered to -18°C before exposure to gamma radiation (^{60}Co) doses of 0, 3.0kGy, 4.0kGy, 5.0kGy, 6.0kGy and 7.0kGy, and the patties were still frozen after irradiation. Non-inoculated controls were carried out for each dose. The studies were done three times in duplicate. *E. coli* O157:H7 was enumerated and non-inoculated samples submitted to sensory evaluation after irradiation. Doses of 4.0kGy reduced the number of bacteria by approximately 7 log with no changes in sensory attributes independent of formulation and packaging. The use of this irradiation dose is economically feasible and ensures the quality of the product.

P186 DSC PCR Characterization of Enterohemorrhagic *Escherichia coli* from Fecal, Hide and Ground Beef Samples

ADAM B. OLSON, Frances Nattress, Gordon Greer, Mueen Aslam, and Lynn M. McMullen, University of Alberta, 4-10 Agriculture Forestry Centre, Edmonton, Alberta T6G 2P5, Canada

Enterohemorrhagic *Escherichia coli* (EHEC) including *E. coli* O157:H7 have emerged as a significant risk to meat safety in recent years and the origin of organisms found on finished products needs to be clarified. Genotypic methods provide the ability to track organisms from the live animal to the finished product. Virulence factors [enterohaemolysin gene (*ehx*A), intimin gene (eaeA) and Shiga-like toxin genes 1 and 2 (*stx*-$_1$, *stx*-$_2$)] were used to differentiate *E. coli* strains isolated from fecal, hide and ground beef samples from two animals. Samples were enriched in tryptic soy broth supplemented with novobiocin and plated on vancomycin, cefsulodin and cefixime washed sheep blood agar. Colonies with small turbid zones of hemolysis were picked and DNA was isolated by boiling. Identification and differentiation of possible EHEC strains was done using PCR with primers for the different virulence factors. Isolates were confirmed as *E. coli* by the presence of the *E. coli* universal stress protein (uspA). The presence of the glucuronidase base shift (uidA) identified *E. coli* O157:H7. By use of this technique, hemolytic strains of *E. coli* were isolated from all samples. Four common virulence combinations were identified: (1) *ehx*A; (2) *eae*A, *ehx*A; (3) *stx*-$_2$, *ehx*A; and (4) *eae*A, *stx*-$_1$, *stx*-$_2$, *ehx*A, *uid*A. Strains with *ehx*A were isolated from samples at all three stages of production. Samples from the hide had the greatest number of isolates carrying multiple virulence factors. Virulence patterns observed in strains isolated from ground beef were detected at different stages in the handling process.

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P187 The Effect of a Mixture of Lactic Acid and Nisin on the Shelf Life of Retail and Vacuum Packaged Fresh Meat

ENRIQUE MARQUEZ SALAS, Kenna Ferrer, Yasmina Barboza de Martinez, and Jorge Ruíz Ramires, Universidad del Zulia, Facultad de Ciencias Veterinarias, Maracaibo, Zulia, Venezuela

The objective of this work was to determine the effect of a mixture of lactic acid (LA) and nisin (NIS) on the shelf life of both retail packaged (RP) and vacuum packaged (VP) fresh meat. Top round cuts were divided aseptically in portions. Some portions were not treated and kept as control. The others were treated with one of the following: LA (1.5%), NIS (500 IU/ml) or a mixture of LA-NIS. Samples were packaged (RP and VP) and stored up to 7 days for RP and up to 21 days for VP at 5 °C. Aerobic plate counts (APC), *Pseudomonas* spp., coliforms and *Escherichia coli* were determined for RP. Lactic acid bacteria were determined for VP samples. Results indicated that a mixture of LA-NIS had a synergistic effect in reducing bacterial counts. The storage life of RP was increased up to 6 days. In VP storage, the numbers of lactic acid bacteria were low at the end of the 21 day period. LA and NIS were less effective than the mixture LA-NIS. In conclusion, LA-NIS mixture is an alternative method to increase the shelf life of both retail packaged and vacuum packaged fresh meat.

P188 Outbreak Alert

CAROLINE SMITH DEWAAL, Center for Science in the Public Interest, 1875 Connecticut Ave. NW, Washington, D.C. USA

Contaminated foods are estimated to cause 5,000 deaths and 76 million illnesses in the US per year. The Center for Science in the Public Interest (CSPI) maintains a database of foodborne-illness outbreaks categorized by food vehicle. Linking food hazards to specific foods serves to alert consumers to food-safety hazards and gives policymakers and public-health officials better information to design risk-based hazard-control plans. CSPI's database was compiled from sources such as the Centers for Disease Control (CDC), state and local health departments, and medical and scientific journals. The database is updated yearly, and contains only those outbreaks with known etiology and food vehicles. CSPI found that the top five food categories linked to food-poisoning outbreaks, not including multi-ingredient foods, were seafood (340 outbreaks), eggs (271 outbreaks), produce (148 outbreaks), beef (134 outbreaks), and poultry (79 outbreaks). Multi-ingredient foods, such as salads, pizza, and sandwiches, caused a total of 337 outbreaks. Overall, 18% of food-poisoning outbreaks were attributed to meats such as beef, poultry, pork, luncheon meats, and game, while 77% of outbreaks were linked to other foods such as seafood, multi-ingredient foods, eggs, produce, dairy, breads, and beverages; 5% of outbreaks were linked to multiple foods. Historically, meats have been thought to pose greater risks than other foods because of possible contamination with pathogenic microorganisms. As this data emphasizes, all types of foods have the potential to carry hazards, and should be treated properly and handled safely to avoid food-poisoning outbreaks.

P189 Investigation of *Clostridium botulinum* (Botulism) Outbreak in Texas, 2001

STEVEN D. BENGTSON, Denise R. Lewis, Kristin G. Holt, and D. W. Chen, USDA-FSIS-OPHS-HHSD, 1400 Independence Ave., SW, Room 334 (Aerospace Bldg.), Washington, D.C. 20250-3700, USA

The Texas Department of Health (TDH), Centers for Disease Control and Prevention (CDC), and the Food Safety and Inspection Service (FSIS) investigated an outbreak of botulism, type A toxin, in August 2001. All but one case was associated with consumption of a chili dish served at a church supper in Sanger, Texas. Frozen chili products implicated in all cases were obtained from the same retail salvage store. Epidemiological, laboratory, environmental, and traceback investigations were conducted. In addition, TDH utilized multiple methods for additional case ascertainment. A total of 15 cases were identified; 10 were hospitalized, with six requiring ventilation. Cohort analysis demonstrated a positive association between consumption of any chili consumed either at the church supper or as leftovers from the supper, and illness. Toxin was confirmed in six patient stool specimens, and in chili products. Environmental investigation supported toxin formation occurring prior to preparation of products. Investigations of chili production facilities did not indicate existence of conditions favorable to formation of botulism toxin during the time the products were produced. Questionable practices and conditions at the retail salvage store suggested the plausibility that temperature abuse and botulinum toxin formation could have occurred at this facility.

P190 Microbiology of Flour Milling

AILSA D. HOCKING, Lana K. Berghofer, and Di Miskelly, Food Science Australia, P.O. Box 52, North Ryde, NSW 1670, Australia

A survey was undertaken to determine the distribution of microorganisms through the flour milling process and in end products. A total of 650 milling process and end product samples were obtained from nine Australian flour mills during two wheat seasons. Counts in wheat and flour were respectively: total aerobic plate count, 10^5 and 10^2 colony forming units/gram (CFU/g); coliforms, 10 and 1 most probable number/gram (MPN/g); total *Bacillus* spp., 10^4 and 10^2 CFU/g; *B. cereus*, 1 and 0.1 MPN/g; mesophilic aerobic spores, 10 and 1 CFU/g; rope spores, 10 and 1 CFU/g; thermophiles, both 10 CFU/g; and yeasts and molds, 10^3 and 10^2 CFU/g. *Bacillus* spp., coliforms, yeasts and molds were the most frequently detected microorganisms throughout the survey. The most common molds isolated were *Aspergillus*, *Penicillium*, *Cladosporium* and *Eurotium* spp. *Escherichia coli* and *B. cereus* were present at very low levels, a majority of samples being at the minimum level of detection (3 and 0.3 MPN/g

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respectively). Results indicate the microbiological quality of incoming wheat has a strong influence on the ultimate quality of milling end products. As wheat grain layers are separated, surface-adhering contaminants are concentrated in end product bran, wheat germ and pollard, which comprise the outer layers of the grain. Consequently, the inner endosperm fraction contains lower microbial counts, and flour is the cleanest end product of the milling process. Higher microbiological counts mid-stream in the milling process indicate that equipment contamination constitutes a significant source of microbiological contamination.

P191 Commodity-specific Food Safety Training Program Partnerships

INGEBORG SMALL, Michelle Smith, Sandra Bahn, and Jeff Farrar, California Dept. of Health Services, Food and Drug Branch, Food Safety Section, Industry Education and Training Unit, P.O. Box 942732, Sacramento, CA 94234-7320, USA

Public health officials are faced with numerous challenges in the area of food safety. One of the most pressing needs is the development of commodity-specific food safety training programs for regulators and food processors. Most of the commercially available food safety training programs regarding food processing are generic and do not adequately address the specific needs and risks for each commodity. The California Department of Health Services-Food and Drug Branch (CDHS-FDB) and US Food and Drug Administration-Center for Food Science and Applied Nutrition (FDA-CFSAN) have led the development of a successful state-federal-industry-academia partnership to produce commodity-specific training videos that can be used by line workers and by regulators. An FDB advisory committee, composed of representatives from industry and academia along with state and federal officials, provided guidance on the commodities to target for developing educational programs. Commodity-specific working groups were then convened to draft scripts for each video. Individuals with expertise in each area of the specific commodity of interest were invited to participate in the development of the script. Background video was captured to provide visual reinforcement of the most significant points in the production and handling of each commodity. A professional video production studio utilized professional actors to provide the narrative for each project. To date, food safety training videos have been completed for two commodities, sprouts and fresh-cut produce. An additional video for juice is in progress. This presentation will detail the specific approach used in this successful partnership effort and will describe future training efforts.

P192 Commercial Food Handlers' Knowledge, Attitudes and Implementation of Food Hygiene Practices

D. A. CLAYTON and C. J. Griffith, University of Wales Institute-Cardiff, Food Research and Consultancy Unit, School of Applied Sciences, Colchester Ave., Cardiff, Wales CF24 2PN, UK

It has been suggested that improper food handling practices contribute to 97% of foodborne illnesses. Previous research investigating food handlers' implementation of food hygiene practices has tended to rely on self-report data. It has been shown that food handlers tend to over-estimate the frequency with which they carry out food hygiene practices. This paper aims to evaluate the relationship between food handlers' knowledge, attitudes and implementation of food hygiene practices. One hundred and thirty-seven food handlers from 19 businesses took part in the research. Each food handler was observed for a total of 270 actions on three occassions. Participants completed questionnaires to ascertain their knowledge and attitudes towards food safety. Food handlers were knowledgeable about food hygiene practices and had positive attitudes towards carrying out these actions but had a false perception of the risks involved. Food handlers' knowledge and positive attitudes were not always reflected in their implementation of practices. All participants answered questions on handwashing correctly yet only 52% washed their hand adequately on all appropriate occasions. Ninety-seven percent of participants knew both when it was appropriate to clean and how best to clean, yet only 67% of participants carried out cleaning actions adequately at all times. Significant differences in the implementation of food hygiene practices were apparent between those who had received formal training and those who had only received informal training ($P < 0.05$). For example, 55% of formally trained food handlers but only 29% of informally trained food handlers adequately washed their hands at all times. Results suggest that training does not always ensure that food handlers implement good practices and training needs to be integrated with the provision of resources, systems and an appropriate organizational culture.

P193 Operational Risk Management—Food Safety and Security Training

INGEBORG SMALL, Jennifer Thomas, Sandra Bahn, and Jeff Farrar, California Dept. of Health Services, Food and Drug Branch, Food Safety Section, Industry Education and Training Unit, P.O. Box 942732, Sacramento, CA 94234-7320, USA

Food contamination from a bioterrorism event is a threat to state and national food sources that must be addressed through food safety and security efforts. The concern is centered on protecting the public and US agriculture's $200 billion assets. Because of the extensive distribution of food products, a bioterrorism event directed toward California's food supply could create a public health crisis. Operational Risk Management (ORM) is a program developed to help protect the food supply from a bioterrorism event. ORM is based upon six steps with the goal of stopping attacks and reducing the vulnerability of the food chain. The California Department of Health Services, Food and Drug Branch (CDHS-FDB) has

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developed a one-day training session to educate regulators and industry representatives on ORM application in the food industry. The training consists of a series of lectures, panel discussion, sample risk assessment worksheets, and classroom exercises providing an overview and opportunity for participants to become familiar with steps needed to reduce risk during growing, transporting, processing, distributing, and retailing of food. Discussion and exercises include (1) identifying hazards by conducting a preliminary hazard analysis, (2) assessing the risks and ranking them according to threat assessments, (3) conducting risk control and ranking the benefits of the control, (4) making control decisions for each hazard identified, (5) implementing the risk control actions, and (6) supervising and reviewing to ensure the effectiveness of the risk controls implemented. All providing oversight and support to firms benefit from the knowledge and skills obtained during the training.

P194 A Meta-Analysis of International Consumer Food Safety Studies

E. C. REDMOND and C. J. Griffith, University of Wales Institute-Cardiff, Food Research and Consultancy Unit, Colchester Ave., Cardiff, Wales CF23 9XR, UK

Illness resulting from foodborne disease has become one of the most widespread public health problems in the contemporary world. Internationally, between 12% and 62% of reported foodborne disease outbreaks are believed to be associated with food prepared in the home and this has prompted numerous studies evaluating consumer food safety perceptions, knowledge and behavior. Electronic searches of internet and library databases, personal communication with food safety professionals and attendance at international conferences has facilitated the collection of 85 studies carried out over 27 years. Analysis of when studies were published showed increased interest in consumer food safety has corresponded with increased incidence of foodborne disease. Most (54%) of the studies were published between 1995 and 1999, and a further 28% of studies were published between 2000 and 2002. The majority of published studies were carried out in the UK (46%) and North America (45%), as well as New Zealand, Australia, Italy and Eire. A variety of research methods were employed to collect information, including interviews (50%), self-completed questionnaires (26%), direct observations (16%) and focus groups (8%). Analysis of survey contents showed 92% of studies investigated self-reported practices, 73% assessed knowledge and 50% assessed attitudes / beliefs. Although consumers generally have a high level of concern for food safety issues, key areas where knowledge is commonly lacking, e.g., cooling foods, were identified. Substantial proportions of consumers failed to implement important food safety practices and findings showed that knowledge and self-reported practices of specific behaviours do not correlate with actual observed practices. Results will be discussed in the context of government targets and strategies for reducing foodborne disease and development of future consumer food safety initiatives.

P195 Efficacy of Alcohol Gel Instant Hand Sanitizer When Used in Conjunction with Normal Handwashing

BARRY MICHAELS, Maria Arenas, Ann Schultz, and Vidhya Gangar, Georgia-Pacific Corporation, P.O. Box 919, Palatka, FL 32178-0919, USA

Over the years, numerous studies on the hygienic efficiency of either handwashing or use of alcohol gel instant hand sanitizers (AGIHS) has been reported against normal microflora (NF) or transient microflora (TF), using marker bacteria or viruses. Most studies to date were meant to validate health care procedures. When AGIHS is used alone, application quantity is known to influence treatment effectiveness. Relevant to the food industry, little work has been done in order to understand the variables affecting efficacy when handwashing and AGIHS are combined. In this study, 3 subjects on each of 6 days contaminated hands with *Serratia marcescens* (TF marker) in Tryptone Soya Broth (TSB). One hand was sampled to establish NF and TF baseline counts using the glove juice technique. Hands were then washed with a mild antimicrobial soap (0.5% triclosan), followed by drying with paper towels and application of either 0.0ml, 1.5 ml, 3.0 ml or 6.0 ml AGIHS for each 6-day treatment period. Using the combined methods to enhance the hygiene process, 0.9, 1.5 and 1.4 $\log_{10}$ reductions of NF and 2.2, 3.2 and 3.9 $\log_{10}$ reductions of TF were achieved with 1.5 ml, 3.0 ml, and 6.0 ml of AGIHS respectively. While there were significant ($P < 0.05$) increases in efficacy in almost all instances, as quantity of AGIHS increased, a significant difference in efficacy over handwashing alone ($\log_{10}$ reductions of 0.3 NF & 2.3 TF) is seen only when larger quantities (3 ml & 6 ml) of AGIHS are employed.

P196 Characterization of the Acid Tolerance Response in *Salmonella* Species Induced by Acid Shock and Moderate pH

Malika Meemongkolkiat, David Benson, and CLAUDIA KOERTING, University of Connecticut, Nutritional Sciences, 3624 Horsebarb Hill Road Ext., Unit 4017, Storrs, CT 06269-4017, USA

Salmonella species demonstrate optimal growth at neutral pH and 37°C. However, *Salmonella* and other food pathogens have previously been demonstrated to possess an acid tolerance response system, which allows these organisms to survive very low pH for brief periods. We have chosen to characterize the acid response of 5 strains of *Salmonella* collected from field samples and obtained from ATCC. Adaptation was performed in moderate pH medium (pH 5.5 to 6.0) and acid shock pH (pH 4.5 or less) for one h, 37°C. The pH of the BHI medium was adjusted with

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HCl. *Salmonella* that survived were exposed to a subsequent acid-shock treatment conducted at pH 3 for 90 min at 37°C. There was a 10^6 to 10^5 reduction in non-adapted control organisms, whereas there was less than 1 log decrease in the preadapted organisms. Chloramphenicol had no effect on survival. We have found that sodium acetate pretreatment also leads to an induced acid tolerance response; however, it is a weaker response than observed for very low pH adaptation. As the adaptation pH dropped, there was an increase in the survival of acid shock treated *Salmonella*. Pre-adaptation temperatures inlfluenced the acid shock response. Lower temperature allowed for greater acid tolerance response to the acid shock treatment. Cells in mid-log phase were also more susceptible to acid shock than those in the stationary phase. The results were the same for all *Salmonella* strains studied. The results have important implications for food safety. The sequence and combination of treatments used to preserve foods must be carefully considered.

P197 Determining the Feasibility of Developing a Food Safety Virtual Reference Service on the World Wide Web

DANIEL HENROID, JR. and James Huss, Iowa State University, 1055 LeBaron, Ames, IA 50011, USA

Consumers and foodservice operators receive information about food safety from many sources, including the Internet. Though the Internet has made access to information easier than with traditional print and visual media, consumers must still judge the validity of the information available and be able to find the information they are seeking. To facilitate consumer access to food safety information, a food safety virtual reference web site is being developed to answer food safety questions. The goals of the project are: (1) Determine the existing US based sources of food safety information on the World Wide Web and the software/hardware used in communicating the information; (2) Evaluate the web-based delivery systems/technology being used to communicate with consumers/foodservice operators and recommend the most efficient model for information dissemination; (3) Develop the selected model for information delivery/questions and answers directly to consumers; (4) Convene multiple panels of experts to provide a consensus of up-to-the-minute research-based food safety answers that do not currently exist, which can be given directly to consumers and foodservice operators. To meet the first objective, the amount of food safety information available on the World Wide Web and the feasibility of developing a virtual reference desk for food safety are being investigated. Food safety web sites will be surveyed for methods used to delivered food safety information, quality and depth of information available, resources used to answer consumer questions, and cost to the consumer. Issues and challenges related to developing such a service will also be presented.

P198 Migration and Growth of *Salmonella* Enteritidis in Chicken Eggs as Influenced by Storage Time and Temperature and by Breakdown of Yolk Membrane

NUTAN MYTLE and Jinru Chen, CFS, University of Georgia, 1109 Experiment St., Griffin, GA 30223-1797, USA

Migration of *Salmonella*, from albumen to yolk, and their growth as a function of storage time and temperature, of antimicrobial agents in albumin, and of breakdown of yolk membrane were investigated. Fresh and processed eggs were surface-disinfected with ethanol and air-dried. A 5-strain mixture of *S.* Enteritidis in phosphate buffered saline containing a food grade dye was injected into egg albumin or onto yolk membrane with the aid of a fiberscope. After inoculation, injection holes on eggshells were sealed with wax. Contaminated eggs were stored at 22°C for 3 weeks or at 4° or 15°C for 6 weeks. Samples comprising 10 eggs were withdrawn from each treatment once a week and assayed for *Salmonella*. Following sampling, eggs in each sample group were cracked open and separated into albumin and yolk. The yolk was washed twice with sterilized water to remove residual albumin. Both the albumin and the yolk were appropriately diluted and plated on bismuth sulfite (BSA) and tryptic soy agar. The plates were incubated at 37°C for 24 h. Samples that did not yield typical *Salmonella* colonies on the BSA plates were subjected to enrichment. The integrity of yolk membrane was examined at each sampling day, using scanning electronic microscopy. Results suggest that migration and growth of *Salmonella* in eggs are temperature-dependent. Both antimicrobial agents in albumin and integrity of yolk membrane played a role in inhibiting the growth and migration of *Salmonella*. However, these inhibitory properties varied between eggs.

P199 Thermal Inactivation of *Salmonella* Senftenberg and *Listeria innocua* in Battered and Breaded Meat Product during Frying and Convection Cooking

RONG Y. MURPHY and E. R. Johnson, University of Arkansas, 203 Engineering Hall, Fayetteville, AR 72701, USA

Ground meat or poultry products are more likely to be contaminated with pathogens than whole muscle meat products. Due to battering and breading, fried meat products have a higher carbohydrate content than non-fried products. The presence of carbohydrates in foods could affect the thermal resistance of microorganisms. The objective of this study was to evaluate the thermal inactivation of *S.* Senftenberg and *L. innocua* in commercial meat products. Seven to eight log CFU of *S.* Senftenberg or *L. innocua* were uniformly mixed with ground beef or turkey meat. The inoculated meats were formed into the patties and then battered and breaded. The product was cooked in a fryer (177°C) and/or an air

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convection oven (288°C). Three cooking methods were used. (1) The patties were fried, (2) the patties were fried, and frying was immediately followed by cooking in an air convection oven, and (3) the patties were cooked in an air convection oven. For each cooking method, a model was developed to correlate the cooking time with survivors of *S.* Senftenberg or *L. innocua*. Frying, or frying combined with oven cooking, reduced the total cooking time by 76 or 57%. Seven $\log_{10}$ reductions could be achieved for *S.* Senftenberg or *L. innocua* by combining frying (31 to 47 s) with oven cooking (60 to 30 s). Thermal inactivation for *S.* Senftenberg or *L. innocua* in the beef/turkey blended patties was affected by cooking history. To validate thermal processes and determine thermal lethality of pathogens in cooked meat products, cooking history for the product must be known.

P200 Thermal Inactivation of *Salmonella* Senftenberg and *Listeria innocua* in Undercooked Meat Product during Impingement Cooking

RONG Y. MURPHY and E. R. Johnson, University of Arkansas, 203 Engineering Hall, Fayetteville, AR 72701, USA

During commercial cooking, meat product could be accidentally overlapped on a process conveyor belt. Because the dwell time for a commercial cooking process is determined based on a single-layer product placement, overlapping products would result in undercooking and limit the effectiveness of the cook step in eliminating bacterial contamination. The objective of this study was to evaluate the effect of product overlapping on thermal inactivation of *Salmonella* and *Listeria* in chicken breast patties during air/steam impingement cooking. Approximately 10^7 CFU/g of *Salmonella* and *Listeria innocua* were uniformly inoculated in the chicken patties. The patties were randomly placed on the conveyor belt in a single layer or double overlaps (about 50% of the surface areas) and cooked in a prototype air/steam impingement oven. Single and overlapped patties were sent side by side through the conveyor belt into the equilibrium chamber (99°C), stayed there for 1 min, and then moved into the cooking chamber for additional 2.5, 3.0, 3.5, and 4.0 min, respectively. The total dwell time for the equilibrium chamber and oven was 3.5, 4.0, 4.5, and 5.0 min, respectively. At a total dwell time of 3.5, 4.0, 4.5 and 5.0 min, the meat internal temperature in the single-layered patties was 53, 60, 65, and 68°C, respectively. The endpoint temperature in overlapped patties was about 50% lower than that in single-layered patties. The thermal destruction for *S.* Senftenberg and *L. innocua* was linearly related to dwell time. If the patties were overlapped during cooking, thermal kill for *S.* Senftenberg and *L. innocua* was about 6 logs lower than that in the patties cooked in a single layer. This information is useful for evaluating and controlling commercial meat cooking processes in order to avoid undercooking.

P201 Comparison of the Pulsed Field Gel Electrophoresis (PFGE) Patterns for *Salmonella* Enteritidis Isolates of Human Origin in Taiwan and Those from Poultry Origin in the USA

HAU-YANG TSEN and Jer-Sheng Lin, National Chung-Hsing University, Dept. of Food Science, Taichung, Taiwan, R.O.C.

Poultry products are one of the major US-imported food items in Taiwan. Because human infections by *Salmonella* Enteritidis have been increasing in Taiwan since 1980 and epidemiological studies have implicated the consumption of poultry, egg and egg products, it is of interest to trace the clonal relationship for *Salmonella* Enteritidis strains of human origin in Taiwan and of poultry origin in the US. To perform such a study, 77 *Salmonella* Enteritidis strains isolated from chickens in the US during 1995 were subjected to chromosomal DNA digestion using the restriction enzymes XbaI, SpeI and NotI followed by PFGE analysis. The PFGE patterns obtained from those US strains were then compared with those from the 63 human isolates obtained in Taiwan, mainly during 1995 to 1997. Results showed that although multiple pattern combinations, i.e., 14 and 30 PFGE pattern combinations, respectively, were found for 63 Taiwan and 77 US isolates, two major pattern combinations, pattern X3S3N3, shared by 47 Taiwan isolates, and pattern X5S4N4, shared by 63 US isolates, are the most common patterns shared by Taiwan and USA isolates. Such results seem to imply that stains of those two PFGE patterns may be the most epidemic and infectious strains of poultry and human origin.

P202 Survival of *Campylobacter jejuni* on Sterile Chicken Breast Burgers Stored at Refrigeration and Ambient Temperatures

KISUN YOON, Candace N. Burnette, and Thomas P. Oscar, University of Maryland Eastern Shore, 2103 Trigg Hall, Princess Anne, MD 21853, USA

Campylobacter is recognized as the most frequent cause of foodborne bacterial diarrhea in humans. Although usual food storage conditions are not associated with the growth of *Campylobacter*, it is very important to predict how well *Campylobacter* will survive at ambient and refrigerated temperatures because as few as 500 cells can cause illness. The objective of this study was to model the kinetics of *Campylobacter* survival and death on sterile chicken breast burgers incubated at refrigeration and ambient temperatures. Burgers were inoculated with 10^6 stationary phase cells of a single strain of *Campylobacter jejuni* (ATCC 43051) and stored at 4 to 30°C under aerobic conditions. The modeling results indicated that a two or three phase linear model fit the survival curves well ($R^2 = 0.97$ to 0.99) at all incubation temperatures. An initial lag phase was followed by a linear decrease in cell population, which varied by rate depending on the temperature, and in some cases by a bottom plateau of survival. Results indicated that *Campylobacter jejuni* can survive well at refrigerated temperatures under

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aerobic conditions, but shorter survival characterized by shorter lag time and faster death rates were observed on burgers stored at ambient temperatures. The data collected in this study will be used to develop a predictive model that will be added to the USDA Pathogen Modeling Program, which presently does not contain a survival model for *Campylobacter*.

P203 Reduction of *Salmonella* Typhimurium in Experimentally Challenged Broilers by Nitrate Adaptation and Chlorate Supplementation in Drinking Water

YONG SOO JUNG, Robin C. Anderson, James A. Byrd, Randle W. Moore, Todd R. Callaway, Thomas S. Edrington, and David J. Nisbet, USDA-ARS, Food and Feed Safety Research Unit, 2881 F and B Road, College Station, TX 77845, USA

Salmonella are recognized as a major foodborne pathogen in the poultry industry. Consequently, the elimination of *Salmonella* before harvest is desired. In the present study, the effect of administering sodium nitrate (SN) and an experimental sodium chlorate product (SCP) alone or in combination on reducing *Salmonella* Typhimurium (ST) in market-age broilers was determined. Broilers were orally challenged with 10^7 CFU of a novobiocin- and nalidixic acid-resistant strain of ST. Broilers were divided into four groups of 20 birds each: control, SN-treatment (574 mg $NaNO_3$/kg feed), SCP-treatment (15 mM $NaClO_3$ equivalents), and SCP in combination with SN-treatment. SN was administered via feed for 5 days immediately preslaughter and SCP was provided via ad libitum access to drinking water for the last 2 days before slaughter. Cecal contents were aseptically removed and subjected to bacterial analysis. Mean ± SD concentrations ($\log_{10}$ CFU/g) of ST were reduced ($P < 0.05$) in broilers receiving SCP in combination with SN when compared to untreated controls (0.19 ± 1.37 versus 2.07 ± 1.45, respectively). There was no significant effect of the SN only treatment on ST concentrations, suggesting that nitrate by itself did not alter ST populations. These results suggest that pretreatment with nitrate effectively enhanced the bactericidal effect of chlorate on ST in ceca by enriching the nitrate reductase activity of ST, consequently enhancing the reduction of chlorate to chlorite, which is cytotoxic to ST. Based on these results, pre-adaptation with SN followed by SCP supplementation immediately preharvest is a potential strategy to reduce ST in chickens.

P204 Water as a Possible Vehicle of Infection for *Campylobacter* in Broilers

I. D. OGDEN, M. MacRae, M. Johnston, and D. Newell, University of Aberdeen, Applied Food Microbiology Group, Dept. Medical Microbiology, Foresterhill, Aberdeen AB25 2ZD, UK

Thermophilic *Campylobacter* are a major cause of human food poisoning in the UK and the consumption of poultry meat is a commonly identified source of human Campylobacteriosis. The majority of broiler flocks are infected with *Campylobacter* despite rigorous disinfection of housing sheds between flocks. The route of entry of *Campylobacter* into this environment has been the subject of investigation in a number of studies. This report examined drinking water as the vehicle of infection. Samples were taken throughout the whole delivery system from source (mains, river or borehole) to nipple drinker and tested for the presence of *Campylobacter jejuni*. Isolates were typed by restriction fragment polymorphism of the PCR product of the flaA and flaB genes and pulsed field gel electrophoresis and compared with isolates from birds reared in the same shed. Where water was shown to be contaminated with *Campylobacter*, attempts were made to differentiate between drinking water infecting the birds or the birds themselves infecting the drinking water. Cost effective methods of eliminating *Campylobacter* from water are also discussed.

P205 Microbiological Assessment of Raw and Ready-to-eat Meat and Poultry Products Collected from the Retail Marketplace in Edmonton, Alberta, Canada

LYNN M. MCMULLEN, Michael E. Stiles, Valerie Bohaychuk, Gary Gensler, Robin King, Ole Sorensen, John Wu, and Ken Manninen, University of Alberta, Dept. of Agricultural, Food and Nutritional Science, 4-10 Agriculture Forestry Centre, Edmonton, AB T6G 2P5, Canada

Microbiological safety of meat and poultry products has assumed paramount importance among consumers and public health officials and yet little is known about the microbiological quality and apparent safety of meat and poultry products in the retail marketplace. The microbiological quality (total, lactic acid bacteria, coliform, *Escherichia coli*, enterococci counts) and the incidence of pathogens (*Salmonella* spp., *Listeria monocytogenes*, coagulase positive *Staphylococcus* spp., verotoxigenic *E. coli*, thermophillic *Campylobacter* spp.) were determined on raw (ground beef, pork chop and chicken leg) and ready-to-eat (beef weiners, chicken weiners, cooked turkey roll, roast beef and fermented sausage) products (100 samples of each product). Bacterial counts ranged from below detectable levels to greater than log 8 CFU/g for samples within the same product group. Verotoxigenic *E. coli* was detected in one raw ground beef sample. *Salmonella* spp. and *Campylobacter* spp. were only detected on chicken leg samples (30% and 60%, respectively). Using REVEAL®, *Listeria* spp. were detected on 78, 55, 66, 3, 5, 11, 10 and 9% of the ground beef, pork chop, chicken leg, roast beef, turkey breast, beef wieners, chicken wieners and fermented sausage samples, respectively. When culture methods were used, *L. monocytogenes* was detected on 52, 24, 34, 0, 3, 5, 3, and 4% of the same samples, respectively. This study showed that microbial contamination of meat products in the retail marketplace varies tremendously.

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P206 Antibiotic Resistant *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, and *Campylobacter jejuni* Isolated from Poultry Processing in Korea

WONKI BAE, Nam-Hoon Kwon, Ji-Yeun Lim, Jun-Man Kim, Kyoung-Min Roh, Jin Hur, Ji-Yeon Kim, So-Hyun Kim, and Yong-Ho Park, Seoul National University, 103 Seodun-Dong, Gwonsun-Gu, Dept. of Microbiology, College of Veterinary Medicine and School of Agricultural BioTech., Suwon, Gyounggyi 441-744, Korea

Foodborne diseases cause public health problems and the major etiology is contamination of livestock products. Antimicrobial agents are administered to food animals in an effort to control diseases and improve feed efficiency in the livestock industry. Development of resistance in foodborne bacteria constitutes a public health risk, primarily through the increased risk of failure of antibiotic therapy in human medicine. The disk diffusion method was used to measure the susceptibility to selected antimicrobial agents. The antimicrobial susceptibilities of 46 isolates of *Salmonella* spp., 72 *Listeria monocytogenes*, 170 *Staphylococcus aureus*, and 41 isolates of *Campylobacter jejuni* were measured. Ninteen and six-tenths (9/46) of *Salmonella* spp. isolates were multi-drug resistant (ampicillin, chloramphenicol, streptomycin, triple-sulfa, tetracycline). Eighty-six and one-tenth (62/72) and 50% (36/72) of *L. monocytogenes* were resistant to tetracycline and ciprofloxacin, quinolone agent, respectively. Ninety percent (153/170), 52.4% (89/170), and 4.1% (7/170) of *S. aureus* were resistant to tetracycline, penicillin, and vancomycin, respectively. One-hundred percent (41/41), 97.6% (40/41), 95.1% (39/41), and 97.6% (40/41) of *C. jejuni* were resistant to ciprofloxacin, erythromycin, tetracycline, and triple sulfa, respectively. High resistance to antimicrobial agents of *L. monocytogenes*, *S. aureus* and *C. jejuni* isolates in this study could be attributable to chlortetracycline, sulfathiazole, and enrofloxacin currently being used in poultry farming in Korea.

P207 Prevalance of *Salmonella* on Young Chicken Carcasses in the US: 1999–2000

DENISE R. EBLEN and L. Victor Cook, USDA-FSIS-OPHS, 1400 Independence Ave. SW, Washington, D.C. 20250, USA

The US Dept. of Agriculture Food Safety and Inspection Service recently completed a one-year prevalence study of *Salmonella* spp. on young chicken carcasses from November 1999 to October 2000. The prevalence of *Salmonella* was 8.7%, variables considered included plant size and geographical location. Data analysis of *C. jejuni*/coli test results undertaken as part of the same study indicated that limitations of current cultural methodology prevented the accurate assessment of prevalence and levels of this organism.

P208 Reduction of *Campylobacter jejuni* on Poultry by Low-temperature Treatment

TONG ZHAO, Gabriel O. I. Ezeike, Michael P. Doyle, Yen-Con Hung, Rhonda S. Howell, and Jim Ayres, University of Georgia, Center for Food Safety, Griffin, GA 30223, USA

Campylobacter jejuni has emerged as the leading cause of acute bacterial gastroenteritis, which exceeds the combined rates of salmonellosis and shigellosis in the United States. Studies were done to determine rates of *C. jejuni* inactivation on poultry exposed to different cooling and freezing temperatures. A 3-strain mixture of *C. jejuni* isolated from poultry was used to inoculate chicken wings. Representative samples were assayed for aerobic bacteria counts and *Campylobacter* counts before inoculation. The *C. jejuni* (0.1 ml) inoculum was sprayed onto the wings' surfaces and air-dried for 20 min in a laminar flow hood before freezing. Results revealed that holding wings at -20°C and -30°C for 72 h reduced the population of *C. jejuni* on wings by 1.3 and 1.8 $\log_{10}$ CFU/g, respectively. Results of long-term freezing revealed an ca. 3 $\log_{10}$ CFU/g reduction of *C. jejuni* on wings held at –20°C for 5 months, but only a 0.5 $\log_{10}$ CFU/g reduction of *C. jejuni* at –86°C for 5 months. Protocols were developed to superchill wings with liquid nitrogen at –80, –120, –160, and –196°C such that the internal portion of the chicken sample reached –3.3°C quickly but did not freeze. An insulated chamber designed for tests at cryogenic temperatures using liquid nitrogen was used for the study. Results of superchilling wings at different temperatures for the amount of time required for the wings to reach an internal temperature of –3.3°C (20 to 330 sec) revealed a 0.5 $\log_{10}$ CFU/g reduction of *C. jejuni* on the surface of wings at –80°C; a 0.8 $\log_{10}$ CFU/g reduction at –120°C; a 0.6- $\log_{10}$ CFU/g reduction at –160°C; and a 2.4 $\log_{10}$ CFU/g reduction at –196°C. Super chilling of chicken wings to quickly cool meat to –3.3°C (internal temperature) can substantially reduce *C. jejuni* populations, with the greatest inactivation occurring at –196°C.

P209 *Campylobacter* MPN Enumeration in Chicken Carcasses

G. PEZZOTTI, A. Serafin, A. Buratin, and C. Bacelle, Istituto Zooprofilattico Sperimentale delle Venezie, Via Romea 14/a, Legnaro, Padova 35020, Italy

The aim of the study was to evaluate the level of thermophilic *Campylobacter* contamination in chicken carcasses before distribution. One hundred and fifty-four chicken carcasses were sampled in several processing plants in north-eastern Italy and examined within 48 h of slaughter. *Campylobacter* was enumerated using the MPN technique; ten grams of breast muscle and skin were excised from the carcasses. Decimal dilutions were prepared using buffered peptone water and the enrichment procedure was adopted to detect *Campylobacter*. Each dilution (corresponding to 1, 0.1, 0.01 and 0.001 g of sample) was inoculated in triplicate in tubes containing Preston broth, and incubated at 42°C for 24 h in

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microaerophilic atmosphere. Following incubation, one loopful of culture was streaked onto plates of *Campylobacter* blood-free selective medium (CCDA-Preston) and incubated in modified atmosphere at 42°C for 48 h. Plates containing colonies of curved or spiral Gram negative rods, oxidase positive, presumptively identified as *Campylobacter*, were considered as positive. Results showed that 78.6% of the samples were contaminated by *Campylobacter*, and the number of germs ranged from 0.36 MPN/g to >1100 MPN/g. The median value observed was 22 MPN/g.

P210 Growth and Survival of *Salmonella* Typhimurium and *Campylobacter jejuni* on Sterile Ground Chicken Patties under Aerobic Conditions at Various Temperatures

CANDACE N. BURNETTE and Ki S. Yoon, University of Maryland Eastern Shore, Dept. of Human Ecology, 2103 Trigg Hall, Princess Anne, MD 21853, USA

Campylobacter and *Salmonella* are common foodborne pathogens related to poultry and poultry products. *Campylobacter* is now considered to be the most common cause of infectious diarrhea in the United States, significantly ahead of *Salmonella*. Although poultry and poultry products are major sources of *Salmonella* and *Campylobacter* infections in humans, the epidemiology of disease is quite different for these two organisms. The objective of this study was to compare the change in populations of *Salmonella* and *Campylobacter* grown on sterile chicken breast patties under aerobic conditions at 8, 10, 18, 24, 30, or 37°C. Each sterile patty was surface inoculated with 10^6 *Campylobacter jejuni* (ATCC 43051) and 10^6 *Salmonella* Typhimurium (ATCC 14028). Appropriate dilutions of the patty homogenate were made in 0.1% peptone water and spiral plated on Karmali for *Campylobacter* and on Trypticase soy agar for *Salmonella*. All plates were subsequently incubated aerobically at 37°C for 24 h for *Salmonella* and at 42°C for 48 h under micro-aerobic conditions for *Campylobacter*. Results indicate that the population of *C. jejuni* decreased during incubations at 8, 10, 18, 24, or 30°C, while the population of *Salmonella* increased at all tested temperatures. No population changes in *C. jejuni* and *S.* Typhimurium were observed at 37°C and 8°C, respectively. These results indicate that *C. jejuni* survived well in the presence of *Salmonella* on chicken patties under aerobic conditions at 8, 10, 18, 24, 30, or 37°C. This suggests that poultry and poultry products should be stored at 8°C or below in the retail markets to prevent the growth of *Salmonella* and *Campylobacter* and lessen the possibility of foodborne disease due to these two microorganisms.

P211 Variation in Genetic Clonality among Multi-drug Resistant *Salmonella enterica* Isolated from a Turkey Production Facility

RAJESH NAYAK, Rong-Fu Wang, and Carl E. Cerniglia, FDA, National Center for Toxicological Research, Div. of Microbiology, 3900 NCTR Road, Jefferson, AR 72079, USA

Bacterial typing methods are useful in epidemiological investigations of outbreaks and tracing zoonotic *Salmonella* among livestock and from livestock via food to humans. In this study, 3 *S.* Anatum, 22 *S.* Heidelberg, 2 *S.* Worthington, and 2 *S.* Muenster, isolated from different sources in a turkey production facility, were pheno- and geno-typically characterized. Isolates were resistant to bacitracin (100%), erythromycin (100%), novobiocin (100%), rifampin (100%), gentamycin (50%), spectinomycin (50%), streptomycin (64%), tetracycline (29%), sulfamethoxazole:trimethoprim (4%) and tobramycin (4%), while sensitive to ampicillin, ofloxacin, chloramphenicol, kanamycin, nalidixic acid, ciprofloxacin, norfloxacin and polymixin. Isolates showed moderate to high minimum inhibitory concentration (MIC) for tetracycline, spectinomycin, streptomycin, gentamycin or erythromycin (32 to > 512 ug/ml). PFGE analysis, with the restriction enzyme XbaI, resolved 6 different clusters ranging from 11 to 20 DNA fragments. Each serotype had a different PFGE pattern ranging from 6.5 to 870 kb. *S.* Heidelberg isolated from 9 turkey ceca and 3 drinker samples had identical antibiotic resistance profile and PFGE cluster, suggesting that transmission of *Salmonella* may have occurred from the bird to the drinkers. Isolates contained between 1 to 6 plasmids ranging from 1 to 10 kb. Each serotype had an identical plasmid profile. PCR analysis revealed the presence of a 275-bp invA gene in all isolates. This virulent gene is involved in attachment and invasion of the mammalian epithelial cell by *Salmonella*. A combination of genotypic and phenotypic markers is useful in studying genetic variation among natural salmonellae populations in turkey production to delineate possible transmission pathways.

P212 Molecular Typing of Guillian-Barré Syndrome Initiating Antibiotic-resistant *Campylobacter* Strains Isolated from Turkey Litter

R. Nayak, M. S. NAWAZ, R. F. Wang, S. A. Khan, and A. A. Khan, FDA, National Center for Toxicological Research, Div. of Microbiology, 3900 NCTR Road, Jefferson, AR 72079, USA

Contaminated raw and processed poultry products have been implicated in several cases of Campylobacteriosis in humans. Campylobacteriosis is an infectious disease caused by *Campylobacter jejuni* and *C. coli*. It is also a causative agent of Guillian-Barré Syndrome (GBS), an autoimmune muscular disorder in humans. In this study, several turkey farms in Arkansas were surveyed for the presence of *Campylobacter*. A total of 32 *Campylobacter* strains (21 *C. jejuni* and 11 *C. coli*) were isolated from turkey litter. Each isolate was resistant to multiple antibiotics, but all strains were sensitive to erythromycin, gentamicin and chloramphenicol. Isolates were tested for the presence or absence of the galE gene, responsible for GBS, using polymerase chain reaction (PCR) analysis. The PCR protocol amplified a 496-bp region of the galE gene from all 21 *C. jejuni* isolates but only one *C. coli* isolate, suggesting that GBS onset may be

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predominantly triggered by *C. jejuni* infection rather than *C. coli*. PCR assay could accurately detect the presence of the galE gene in a bacterial suspension containing as few as 5 cells/ml. The virulent galE gene was highly conserved in *Campylobacter* and the PCR method could not detect the gene in 11 *Escherichia coli*, 3 *Salmonella* or 5 *Helicobacter* ATCC cultures. Restriction analysis of chromosomal DNA was conducted on each isolate by pulsed-field gel electrophoresis (PFGE). PFGE analysis with restriction enzyme SalI or SmaI showed 17 and 19 gene clusters, respectively. A combination of SalI and SmaI resulted in 24 clusters, indicating a higher rate of clonal variability between the isolates.

P213 Quantitative Monitoring of Ciprofloxacin-Resistant and -Sensitive *Campylobacter* Populations on Pre- and Post-chilled Raw Broiler Carcasses from Poultry Processing

R. Nannapaneni, R. Story, K. Wiggins, and M. G. JOHNSON, University of Arkansas, Dept. of Food Science, 2650 N. Young Ave., Fayetteville, AR 72704, USA

The FDA is currently undertaking quantitative risk assessment for fluoroquinolone-resistant and sensitive *Campylobacter* on raw poultry products. Also, new data is needed for on-going efforts by USDA-FSIS to develop a scientific basis for performance standards regarding *Campylobacter* for raw poultry. Also, new testing technologies that are cost-effective and user-friendly for population surveillance of *Campylobacter* on raw poultry are needed. We developed a simple strategy for quantitative enumeration of Ciprofloxacin-sensitive and Ciprofloxacin-resistant *Campylobacter* populations by direct plating of concentrated rinses from broiler carcass on Ciprofloxacin-screening medium. This screening medium was constructed by adding Ciprofloxacin (a commonly prescribed fluoroquinolone for humans) to yield 10 or 20 µg/ml into Bolton agar medium containing other FDA-approved selective antibiotics for *Campylobacter* isolation. Over a 16-week sampling period in 2001, the total counts of *Campylobacter* in pre-chilled and post-chilled raw broilers were 0 to 38,144 and 0 to 9400 CFU/carcass. During this period, the counts of *Campylobacter* tolerating 10 or 20 µg/ml of Ciprofloxacin were 0 to 3600 CFU/carcass in pre-chilled broilers, and 0 to 1660 CFU/carcass in post-chilled broilers. In some rinse samples, *Campylobacter* populations tolerating 10 µg/ml of Ciprofloxacin (a level 2–3 times the blood levels commonly achieved with human prescriptions per dose) comprised up to 75% of the total *Campylobacter* levels both in pre-chilled and post-chilled raw broilers. Picked *Campylobacter* microcolonies tolerant of 10 to 20 µg/ml of Ciprofloxacin were purified and expressed stable resistance upon freezing and subculture.

P214 Antibiotic Resistance in Guatemalan Cattle

ERIC RUNDLETT, Eric Davis, and Ann Draughon, University of Tennessee Food Safety Center of Excellence, 2605 River Road, 105 Food Safety and Processing Bldg., Knoxville, TN 37996, USA

Antibiotic resistance of zoonotic pathogens is an increasing concern for public health. The objective of this study was to determine the incidence and antibiotic resistance of *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* in Guatemalan cattle populations. All documentation required by APHIS was provided and approved for importation of samples. Fifty samples were collected from three farms in Macanche. Fifteen samples were collected from one farm in El Caoba, Guatemala. Swab samples were taken rectally using commercial transport media and were shipped under refrigerated storage until assay. *Salmonella* and *Listeria* were isolated using FDA BAM enrichment methods. *E. coli* was isolated using VRB-MUG at 37°C for 48 h. All isolates were confirmed biochemically and/or serologically. Antibiotic resistance of isolates was determined using the Kirby-Bauer Agar Disk Diffusion Susceptibility Test. The highest incidence of *Salmonella* in cattle was 56% in one herd from Macanche. Two other herds in Macanche had a 15% and 16% incidence of *Salmonella*. The herd from El Caoba had a 20% incidence of *Salmonella*. No *Listeria monocytogenes* were isolated from cattle in any of the herds tested. Numerous *E. coli* (no O157:H7) were isolated for antibiotic resistance testing. All *Salmonella* and *E. coli* isolates were resistant to penicillin. A high percentage of *Salmonella* were also resistant to tetracycline (95%) and oxytetracycline (37%). No *Salmonella* isolates were resistant to Ciprofloxacin and only 5% were resistant to streptomycin. Two percent of the *E. coli* isolates were resistant to streptomycin and 5% were resistant to Ciprofloxacin. Frequently, antibiotics are dosed incorrectly and without the supervision of veterinarians in Guatemala. Quinalones are being used in animal populations in Guatemala, which may explain the 5% resistance of *E. coli* to Ciprofloxacin.

P215 Treatment of Wastewater in a Laboratory-scale Fluidized Bed Bioreactor

D. Lindsay, U. Meeta, S. Moodley, V. Gray, and A. VON HOLY, University of the Witwatersrand, School of Molecular and Cell Biology, Private Bag 3 WITS, 2050 Johannesburg, South Africa

Most research on denitrification of nitrate-containing wastewater has focussed on the use of suspended cultures, packed bed bioreactors or submerged biofilters, while fewer studies investigated the efficiency of fluidized bed bioreactors. This study compared the microbial populations associated with two laboratory-scale fluidized bed bioreactors used to treat nitrate-containing wastewater (A) or effluent from yeast processing (B). Microbial populations in the wastewater were observed by light microscopy, scanning electron microscopy and confocal scanning

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laser microscopy (CSLM) coupled to the dead/live Baclight Bacterial Viability Kit TM. In addition, numbers of planktonic cells and cells attached to glass slides suspended in the wastewater were determined by plate counts. Nitrate and nitrite concentrations (A) and chemical oxygen demand (COD) (B) were determined. Nitrate reduction and subsequent accumulation of nitrite was observed in bioreactor A. Similarly, COD decreased in bioreactor B over 30 days. Counts of planktonic cells in the wastewater of both bioreactors were ca. 7 log CFU/ml. Counts of attached cells were higher in bioreactor A (ca. 5 log CFU/cm^2) compared to bioreactor B (1 to 5 log CFU/cm^2). Light and scanning electron micrographs showed that mainly Gram-negative rods and yeast cells were associated with bioreactor A, while Gram-negative and -positive rods were associated with bioreactor B. CSLM images showed that the microbial populations associated with both bioreactors were viable (stained green). This study indicated that micobiological removal of both nitrate and other organic wastes from industrial effluents occurred in laboratory-scale fluidized bed bioreactors.

P216 A Predictive Model to Determine the Effects of Temperature, Sodium Pyrophosphate, and Sodium Chloride on Thermal Inactivation of Starved *Listeria monocytogenes* in Pork Slurry

MAKUBA LIHONO, Aubrey Mendonca, and James Dickson, Iowa State University, Dept. of Food Science and Human Nutrition, 2313 Food Sciences Bldg., Ames, IA 50011, USA

The effects and interactions of heating temperature (57.5 to 62.5°C), sodium pyrophosphate (SPP; 0 to 0.5%, wt/vol), and salt (NaCl; 0 to 6% wt/vol) on the thermal inactivation of starved *Listeria monocytogenes* ATCC 19116 in pork slurry were investigated. A split-split plot experimental design was used to compare 27 combinations of heating temperature, SPP, and NaCl levels. *L. monocytogenes* survivors were enumerated using tryptic soy agar supplemented with 0.6% yeast extract (TSAYE). Means of decimal reduction times (D-values) were modeled as a function of heating temperature, SPP, and NaCl levels. Increasing concentrations of SPP or NaCl in pork slurry protected starved *L. monocytogenes* from the destructive effect of heat. Combinations of 6.0% NaCl and SPP (0.25 or 0.5%) increased the thermal inactivation of the organism compared to 6% NaCl alone. All three variables interacted to affect thermal inactivation of *L. monocytogenes*. A mathematical model describing the combined effect of temperature, SPP, and NaCl levels on thermal inactivation of starved *L. monocytogenes* was developed. There was a high correlation ($R^2 = 0.97$) between D-values predicted by the model and those observed experimentally. The model can predict D-values for any combinations of temperature, SPP, and NaCl that fall within the range of those tested. This predictive model can be used to assist food processors to design thermal processes that include an adequate margin of safety for controlling *L. monocytogenes* in processed meats.

P217 Influence of Fingernail Length and Type on Removing *Escherichia coli* from the Nail Regions Using Different Hand Washing Interventions

C.-M. LIN, F.-M. Wu, M. P. Doyle, B. S. Michaels, and K. Williams, University of Georgia, CFSQE, 1109 Experiment St., Griffin, GA 30223-1797, USA

Inadequate hand washing has been responsible for outbreaks of foodborne illness and areas underneath fingernails harbor higher microbial populations than other areas of the hand. An evaluation of the efficacy of hand washing to remove microorganisms from different types (artificial vs. natural) and lengths of nails is needed. Volunteers with artificial fingernails or natural nails were recruited and their fingernail lengths were measured. Volunteers' nails were contaminated by ground beef inoculated with nonpathogenic *Escherichia coli* JM109 containing a fluorescent protein-labeled plasmid. They then washed their hands and nail regions with tap water, liquid soap, antibacterial liquid soap, alcohol gel, liquid soap plus alcohol gel, or liquid soap plus a nailbrush. Five nails on different hands were scrubbed by an electronic toothbrush presoaked in Butterfield's phosphate buffer (BPB) before washing to determine the baseline number. The other five nails were scrubbed after washing. BPB was decimally diluted and plated onto tryptic soy agar (TSA) plates containing 100 mg ampicillin/l. The greatest reduction (3 $\log_{10}$ CFU/volunteer) of *E. coli* was obtained by washing with soap plus a nailbrush. Other washing methods achieved approximately a 1- to 2- $\log_{10}$ CFU reduction. Fewer *E. coli* were removed from artificial than natural nails, and from long than short nails. However, there was no statistically significant difference ($P \geq 0.05$) in *E. coli* populations between types and lengths of nails, except for washing with soap plus a nailbrush. Therefore, physical elimination such as with nailbrushes was a critical step to remove microorganisms from areas underneath fingernails.

P218 Biocontrol of Zearalenone, an Estrogenic Mycotoxin: Interaction with Food Grade *Lactobacilli*

HANI EL-NEZAMI, Nektaria Polychronaki, Seppo Salminen, and Hannu Mykkanen, University of Kuopio, Dept. of Clinical Nutrition, P.O. Box 1627, Kuopio 70211, Finland

Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin produced by several species of *Fusarium* colonizing maize, and to a lesser extent barley, oats, wheat and sorghum. ZEN has estrogenic properties that are manifested in female swine, cattle and sheep as reproductive problems. A metabolite of ZEN, á-Zearalenol (á-ZOL) has about 10–20 times the estrogenic activity of ZEN. The interaction between these two *Fusarium* mycotoxins with two food grade strains of *Lactobacillus* was investigated. The mycotoxins (2 µg/ml) were incubated either with *Lactobacillus rhamnosus* strain GG (*L. rhamnosus* GG) or *Lactobacillus rhamnosus* strain LC705 (*L. rhamnosus* LC705). A considerable proportion (38–46%) of both toxins was

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recovered from the bacterial pellet, and no degradation products of ZEN and á-ZOL were detected in the high performance liquid chromatograms of the supernatant of the culture media and the methanol extract of the pellet. Both heat-treated and acid-treated bacteria were capable of binding the toxins, indicating that metabolism is not the mechanism by which the toxins are removed from the media. Binding of ZEN or á-ZOL by lyophilized *L. rhamnosus* GG and *L. rhamnosus* LC705 was a rapid reaction; approx. 55% of the toxins was bound instantly after mixing with the bacteria. Binding was dependent on the bacterial concentration, and co-incubation of ZEN with á-ZOL significantly affected the percentage of the toxin bound, indicating that these toxins may share the same binding site on the bacterial surface. These results can be exploited in developing a new approach for detoxification of mycotoxins from foods and feeds.

P219 The Influence of Food Microtopography on the Distribution of Bacteria in Two Food Spoilage Associations

GARY A. DYKES and Alexander von Holy, Saskatchewan Food Product Innovation Program, Dept. of Applied Microbiology and Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan S7N 5A8, Canada

The ecology of food spoilage and safety entail complex interactions between environmental factors and microorganisms of concern. One such interaction of increasing interest is the role played by food structure on bacterial growth kinetics. Practical constraints have meant that traditionally many experimental and mathematical modeling studies of bacterial growth and survival on foods assume a uniform distribution of microorganisms. To assess the importance of the influence of food microtopography on bacterial distribution we conducted a qualitative study of two spoilage associations by scanning electron microscopy (SEM). The "rope" spoilage of bread by *Bacillus subtilis* and the slimy, blown-pack type spoilage of vacuum-packaged Vienna sausages by lactic acid bacteria were chosen as model systems. Fresh product was obtained from commercial manufacturers and allowed to undergo spoilage in the laboratory. Samples of spoiled and spoiling product were taken at appropriate times, gently fixed in glutaraldehyde, dehydrated in alcohol and coated with gold for SEM. Results indicated an uneven distribution of bacteria associated with particular microtopographic features in both food types. Most notable was the strong association of bacteria with holes in the surface of sausages and the absence or sparse colonization of adjacent surface areas and the interior of this product. On bread-crumb, bacteria were more uniformly distributed than on sausage but starch granules appeared in many cases to be relatively or totally free of bacteria while the adjacent areas were extensively colonized. The importance of considering food structure in studies of bacterial growth kinetics on food was highlighted by this study.

P220 Ultraviolet Inactivation of Caliciviruses: First Study

SUPHACHAI NUANUALSUWAN, Sakchai Himathongkham, Hans Riemann, MingQi Deng, and Dean Cliver, University of California-Davis, Dept. of Population Health and Reproduction, School of Veterinary Medicine, One Shields Ave., Davis, CA 95616-8743, USA

Norwalk and Norwalk-like viruses (NLVs) are significant causes of food-related illness and death in the United States. There is no host cell line in which the NLVs can be tested for infectivity. Feline calicivirus (FCV) and NLVs both belong to the family Caliciviridae. Unlike NLVs, FCV has a Crandell Reese feline kidney (CRFK) cell line for infectivity assay so FCV has served as a surrogate for NLVs. Ultraviolet (UV) irradiation has been used to inactivate viruses of food contact surfaces and in water and wastewater. This study is the first report of UV inactivation of FCV, and also of using the plaque assay to determine FCV infectivity titer. UV radiation was generated by a low-pressure mercury-vapor discharge lamp that emits a monochromatic peak at 253.7 nm. The intensity of UV irradiation was measured by a digital UVX radiometer. The infectivity titers of UV inactivated FCV ($\log_{10}$ PFU/ml) were plotted as a function of UV dose and analyzed by regression analysis. These fitted straight-line curves (R^2 = 0.81–0.93) represent exponential inactivation, so UV inactivation can be said to show "one-hit kinetics." FCV requires a UV dose of 50.35 ± 12.61 mWs/cm^2 to inactivate 90% of PFU/ml (1 $\log_{10}$ inactivation). FCV appears to be more resistant to UV than is hepatitis A virus (37.18 ± 5.79 mWs/cm^2) or poliovirus 1 (24.25 ± 1.46 mWs/cm^2).

P221 Increased Thermotolerance of *Clostridium perfringens* Spores following Sublethal Heat Shock

VIJAY K. JUNEJA and John S. Novak, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Beef gravy samples inoculated with *Clostridium perfringens* spores were heat shocked at 75°C for 20 min, and then thermotolerance at 100°C was assessed using a submerged-coil heating apparatus. Survivors were enumerated on Shahidi-Ferguson *perfringens* agar. An association of heat resistance with the origin of the *C. perfringens* strains could not be established due to significant variations in the heat resistance among strains. Interestingly, deviations from classical logarithmic linear declines in the log numbers with time were not observed in either control or heat-shocked samples. D-values at 100°C for *C. perfringens* spores ranged from 15.5 to 21.4 min. Heat shocked spores of 9 of 10 strains had significantly higher ($P < 0.05$) D-values at 100°C than unstressed spores. Proteins with epitopic and size similarity to *Escherichia coli* GroEL and *Bacillus subtilis*

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small acid-soluble protein, SspC, were present in the spores. However, heat-shock treated spores did not appear to significantly increase expression of these proteins. Acquired thermotolerance is of substantial practical importance to food processors and should provide useful information for designing thermal treatments to eliminate *C. perfringens* spores in ready-to-eat foods.

P222 Screening of Lactic Acid Bacteria Strains as Potential Probiotics

CHENG-CHIH TSAI, Li-Fang Huang, Chia-Chan Lin, Wen-Hsin Lin, and Hau-Yan Tsen, National Chung Hsing University, Dept. of Food Science, 250 Kuokuang Road, Taichung 402, Taiwan, ROC

Lactic acid bacteria are widely used as human and animal food supplements. The basic requirements for lactic acid bacteria strains as probiotics are their capability to tolerate gastric juice and bile salts, their ability to inhibit pathogenic bacteria, such as *Salmonella* spp., and their adherence to host intestinal epithelium cells. In addition, the ability to adhere to human gastric carcinoma cell line may enable them to protect the host from gastric ulcer. In this study, we screened lactic acid bacteria strains isolated from porcine and poultry intestinal tracts and found that most of these lactic acid bacteria strains were tolerant to pH 3.2 gastric juice and 0.3% bile salts. However, only a few strains, such as *Lactobacillus acidophilus* LA5 and *Lactobacillus fermentum* LF33, were found to be strongly adhesive to intestinal epithelium cells isolated from mice (BALB/c), swine, and poultry. These strains also showed the ability to strongly adhere to the human intestinal cell lines Int-407 and Caco-2 cell lines as well as human gastric carcinoma cell line TSGH. These properties may enable them to be useful for animal feed and human food supplements.

P223 Growth Kinetics of Parent and Green Fluorescent Protein-producing Strains of *Salmonella*

T. P. OSCAR, USDA-ARS, 1124 Trigg Hall, UMES, Princess Anne, MD 21853, USA

The green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* can be expressed in and used to follow the fate of *Salmonella* spp in microbiologically complex ecosystems, such as food. As a first step in evaluating GFP as a tool for the development of predictive models for naturally contaminated food, the current study was undertaken to compare the growth kinetics of parent and GFP-producing strains of *Salmonella*. Growth kinetics of stationary phase cells of parent and GFP strains of *Salmonella* Enteritidis, *S.* Typhimurium and *S.* Dublin were compared using a previously established sterile chicken burger model system. Growth curves, which were conducted at constant temperatures from 10 to 48°C, were fit to a three-phase linear model to determine lag time, specific growth rate and maximum population density. Secondary models for the growth parameters as a function of temperature were generated and compared between the parent and GFP strain pairs. Effects of GFP on the three growth parameters were significant ($P < 0.05$) and were affected by serotype and incubation temperature. Expression of GFP reduced specific growth rate and maximum population density while having only a small effect on lag time of the three serotypes. The optimum specific growth rate was reduced by 0.2 log CFU per h, whereas the maximum population density was reduced by 1 to 1.5 log cycles in the GFP-producing strains. Results indicated that the GFP strains had different growth kinetics from the parent strains and thus may not be good marker strains for developing predictive models for naturally contaminated food.

P224 High Hydrostatic Pressure Inactivation of Calicivirus (SMSV-17) in Oysters

K. R. CALCI, W. Burkhardt III, and A. W. Smith, FDA, P.O. Box 158, Dauphin Island, AL 36528, USA

The consumption of raw or partially cooked shellfish resulted in more than 2,100 illnesses in the United States from 1991 to 1998. Norwalk-like viruses (NLV), which are non-culturable members of the Family Caliciviridae, were epidemically linked to ~1,250 illnesses. The majority of the implicated shellfish were traced back to growing areas in approved status which were thought to have become contaminated by illegal overboard discharges of human waste or failures of proximal wastewater treatment facilities. In this study, San-Miguel Sea Lion virus (SMSV-17) was used as a surrogate for NLV since it is also a member of the Family Caliciviridae and is quantifiable. SMSV-17 was subjected to HHPT within PBS, oyster homogenate, and whole shucked oysters. The virus was spiked into PBS and oyster homogenate, whereas the whole oysters to be shucked were allowed to accumulate the virus in a flow-through system. HHPT was used to inactivate Calicivirus during a 1-min interval at 200, 250, 275, or 300 MPa. These pressures are within ranges currently used in commercial processing of shellstock oysters for reducing *Vibrio* sp. and facilitating shucking. The greatest inactivation was observed in PBS with $\log_{10}$ reductions of 1.69, 3.43, 4.06, and >4.60 PFU /ml, at 200, 250, 275, and 300MPa, respectively. In oyster homogenate the virus were reduced by 0.04, 1.57, 3.35, and 3.97 $\log_{10}$ PFU/ml, at 200, 250, 275, and 300 MPa, respectively. The virus inactivation in the shucked whole oysters was >2.49 $\log_{10}$ PFU/ml at all pressures except 200 MPa, which produced a 1.59 $\log_{10}$ PFU /ml reduction.

P225 Comparison of Sample Preparation Methods for Recovering *Salmonella* Enteritidis in Eggs

K. H. SEO, R. E. Brackett, I. E. Valentin-Von, and P. S. Holt, FDA-CFSAN, 200 C St. SW, HFS-300, Washington, D.C. 20204, USA

Human salmonellosis outbreaks attributed to *Salmonella* Enteritidis (SE) have been mainly associated with raw eggs or foods containing undercooked

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eggs. Homogenizing shell eggs using a stomacher, an electric blender, and a hand massage have been adapted for detection of SE in eggs. However, no studies have attempted to determine the effect of sampling method on the recovery of SE from raw eggs. Two experiments were conducted using two inoculum levels, 10 or 100 cells/pool of ten eggs. Eggs were aseptically broken and the contents were separated and inoculated. Four different sampling methods, stomaching, electric blending, hand massaging, and whipping for 30 or 60s, were used to homogenize eggs. Egg were incubated at 37°C, and the SE were enumerated after 24 and 48 h incubation. After 48 h incubation, growth of SE was not observed in samples inoculated with less than 10 cells/pool and stomached or electrically blended whereas SE counts in eggs prepared by hand massaging or whipping reached approximately 1×10^9 CFU/ml and 3×10^8 CFU/ml, respectively. In trial 2, when eggs were inoculated with 100 cell/pool, significant differences in SE counts between eggs mixed with hand-massaging or whipping, and eggs mixed by stomaching or electric blender were observed after 24 h incubation. The homogenizing time did not affect the growth of SE in eggs. In conclusion, hand massaging and whipping methods could be recommended to improve the detection of low numbers of SE in shell eggs.

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Technical Abstracts

T01 Withdrawn

T02 Microbiological Risk Assessment on Raw Beef Carcasses in Ontario Abattoirs

PAT JOHNSON, Joseph Odumeru, Abdullahi Mahdi, and Tom Baker, Ontario Ministry of Agriculture, Food and Rural Affairs, 1 Stone Road W., Fifth Floor NW, Guelph, Ontario N1G 4Y2, Canada

The prevalence and levels of indicator and pathogenic bacteria of public health concern were monitored in a baseline study, with the main objective of assessing the microbiological risk associated with beef carcasses dressed in Ontario's provincially regulated abattoirs. The survey was designed to account for contaminations contributed by production volume (Strata), geographical location (GL), Season and other dressing variables. Within Stratum, abattoirs were randomly selected weekly using probabilities proportional to slaughter volume. Samples were collected through a carcass swabbing technique, using a sponge on 100 cm^2 area of the rump, flank and brisket of randomly selected carcasses. Swabs immersed in 25 ml sterile buffered peptone water were submitted for microbiological analysis. Samples from 1459 carcasses were analyzed for indicator organisms: aerobic colony count (ACC), total coliforms (TC) and *Escherichia coli* (Ec); and for pathogens: *Campylobacter* spp. (C), *Listeria monocytogenes* (Lm), *Salmonella* spp. (S) and VTEC. Overall, the bacterial contamination rates were 100.0%, 27.8%, 18.6%, 1.5%, 9.9%, 1.6% and 0.3% for ACC, TC, Ec, C, Lm, S and VTEC, respectively. GL and Season affected ($P < .001$) TC and Ec whereas TC, C and S were influenced ($P < .05$) by Strata. ACC, Lm and VTEC were not affected ($P > .05$) by Strata, GL and Season. Means ($\log_{10}$ CFU/cm^2) for TC (1.44, 1.66, 2.03) and Ec (1.22, 1.62, 2.06) varied significantly ($P < .001$) among the northeastern, south central and southwestern regions of Ontario, respectively. Data will be used to develop microbiological performance standards for beef processing in Ontario.

T03 Incidence of *Clostridium perfringens* in Commercially Produced Cured Raw-Meat-Product Mixtures and Behavior in These Products during Cooking, Chilling, and Refrigerated Storage

PETER J. TAORMINA, Gene W. Bartholomew, and Warren J. Dorsa, John Morrell and Co., 805 E. Kemper Road, Cincinnati, OH 45246-2515, USA

FSIS cooling (stabilization) guidelines have prompted supplementary research related to *Clostridium perfringens* in meat products containing sodium nitrite to demonstrate that the requirement of less than a 1 $\log_{10}$ multiplication of *C. perfringens* is met. Cured whole muscle, ground, and emulsified raw-meat-product mixtures acquired from industry sources were monitored over a five-month period for vegetative- and spore-forms of *C. perfringens*. Black colonies formed on Shadhi Ferguson Perfringens (SFP) agar after 24h at 37°C were assumed positive. Samples that were positive following a 15-min heat shock at 75°C were considered positive for spores. Of 149 samples, 22.8% were positive and 4.7% were positive for spores. Populations of vegetative cells did not exceed 2.54 $\log_{10}$ CFU/g and averaged 1.76 $\log_{10}$ CFU/g while spores did not exceed 2.00 $\log_{10}$ CFU/g and averaged 1.63 $\log_{10}$ CFU/g. Raw bologna (70% chicken), chunked ham, and large whole-muscle honey-ham product mixtures were inoculated with *C. perfringens* spores (ATCC 12916, ATCC 3624, FD1041, and two product isolates) to ca. 4.0 $\log_{10}$ CFU/g before being subjected to thermal processes mimicking cooking and cooling regimes determined by in-plant temperature probing. Populations of *C. perfringens* were recovered on SFP from each product at the peak cook temperatures, at 54.4, 26.7, and 7.2°C, and during 14 days of storage under vacuum at 4.4°C. In each product, populations remained relatively unchanged during cooling from 54.4 to 7.2°C, and declined during refrigerated storage. These findings indicate processed meat products cured with sodium nitrite are not at risk for growth of *C. perfringens* during extended chilling and cold storage.

T04 Microbiological Analysis of Ground Beef Treated with Hydrodynamic Pressure Processing

ANISHA WILLIAMS-CAMPBELL and Morse Solomon, USDA-ARS, Food Tech. and Safety Laboratory, 10300 Baltimore Ave., Bldg. 201, Room 2, Beltsville, MD 20705, USA

Hydrodynamic pressure processing (HDP) is an emerging non-thermal technology that has been shown to instantaneously reduce (2-3 log) spoilage microorganisms in/on fresh beef products. The objective of this study was to identify indigenous microorganisms in ground beef susceptible or resistant to HDP. Retail ground beef was mixed (2 min) for microbial uniformity (Day 0) and divided equally for control and HDP-treated samples. All meat was packaged in plastic wrap followed by a multilayer barrier bag that was vacuumized and sealed. Control samples were held at 4°C (30 min) until sampled. For HDP-treated samples, the packaged meat was placed in a shockwave container filled with water. An explosive (100 g) was placed in the water above the meat and detonated. Immediately following HDP, control and HDP-treated samples were spiral plated onto plate count agar (PCA) and incubated at 30°C

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for 48 h. After incubation, isolated bacterial colonies (n=60 controls; n=60 HDP-treated) were prepared for VITEK 32 identification (biochemical) by the manufacturer's instructions. Results showed that bacteria groups isolated most frequently in the control ground beef on Day 0 were *Serratia* (23%), *Pseudomonas* (5%), *Hafnia* (5%), *Yersinia* (5%), and *Streptococcus* (5%). For HDP-treated samples, the predominant group was *Streptococcus* (15%), while *Serratia*, *Pseudomonas*, *Hafnia*, and *Yersinia* combined were less than 2% of the bacterial population. These results suggest that Gram-negative bacteria may be more susceptible to HDP than Gram-positive bacteria. Inhibition of Gram-negative spoilage microorganisms could extend the shelflife of fresh meat products.

T05 High Efficiency Microbial Collection of Beef Carcasses with Wet-Vacuum Procedures

BRUCE J. BRADLEY, Filomena S. Saddler, and Joseph K. Hillers, Microbial Vac Systems, RMR Labs, Inc., 801 North Lincoln, Jerome, ID 83338-1583, USA

Improved carcass processing procedures have resulted in a reduction of surface microbes on meat carcasses and cutting surfaces. Low-level carcass pathogens may remain sheltered in minute surface hydrophobic crevices or channels thus avoiding sanitation and accurate sample collection. Collection efficiencies of sponge methods may be questionable where microbes are located slightly below the surface. The Microbial-Vac™ (M-Vac™) is a non-destructive wet-vacuum sampling device that allows surface sampling of diverse food surfaces with higher efficiencies than swabs using Liquid or Air-Assisted Microbial Detachment And Capture (LAMDAC™) techniques. Suspended microbes that have been detached by liquid and air turbulence are recovered within the M-Vac by use of vacuum pressure. Collected liquid aliquots may be analyzed or further concentrated within the M-Vac by filtration onto 0.2 – 0.45 μm membranes. Field trials were conducted at commercial packing facilities to compare Sponge (SP) and M-Vac sampling methods. Post-chill samples were collected and analyzed for aerobic plate counts (APC) and total coliforms using liquid aliquots on Petrifilm and direct culture of concentrated samples on the M-Vac's final filter on m-Endo media. A total of 241 observations from 68 beef carcasses demonstrated the M-Vac collected 12 times higher total coliform and 4 times higher APC levels than the SP ($P < .05$). Coliforms were detected from only 4 of 54 carcasses with the sponge compared to 26 of 54 with the M-Vac. These data indicate the M-Vac provides a more efficient bacterial sampling method for evaluation of meat and food surfaces for improved consumer safety and confidence.

T06 DSC Microbiological Profile of Air Chilled Chickens from Farm to Table

W. FLUCKEY, M. Sanchez, M. Brashears, S. McKee, and E. Pendleton, Texas Tech University, P.O. Box 42141, Lubbock, TX 79409, USA

The microbiological profile of an air chilling poultry process was investigated at the farm level and through the processing plant. In a one-year period, nine broiler flocks from four different farm sources were studied. Incidence and prevalence of total aerobes (APC), coliforms, psychrotrophs, generic *E. coli*, *Salmonella* spp. and *Campylobacter* spp. were determined for multiple sampling sites on the farm as well as in the processing plant. Farm samples were collected the day before the chickens were slaughtered at the plant. The same flock was then sampled at the plant, the day of slaughter. Sampling sites were located directly before evisceration (BE), after evisceration (AE) and after chilling (AC). Results indicated a significant positive correlation between ceca contamination with *Salmonella* on the farm and *Salmonella* levels observed at the plant, for all three sampling sites. For *Campylobacter* a slight positive trend was found between on-farm ceca contamination and in-plant levels observed; however, it was not significant. The in-plant trend for APC, coliforms and generic *E. coli* revealed a decrease in microbiological levels from the BE to the AC final product when taking into account flock variations. For coliforms the profile was 3.91, 3.27 and 2.59 and for Generic *E. coli* 3.74, 3.08 and 2.20, respectively. There were no reductions observed for *Campylobacter* or *Salmonella* during processing. These data suggest practical intervention strategies for lowering pathogen levels are important on a multilevel basis at the farm and in the plant.

T07 Association of *Campylobacter* spp. Levels in Poultry Production to Levels Found on Processed Product

NORMAN J. STERN and Michael C. Robach, USDA-ARS-RRC, Poultry Microbiological Safety, 950 College Station Road, Athens, GA 30604, USA

Within the United States, *Campylobacter* spp. is the most frequently reported bacterial agent of human gastroenteritis, and poultry is thought to be a significant source for human infection. We provide data to describe the relationship of *Campylobacter* spp. found in poultry production to the levels found on the fully processed product. During 2001, we sampled 10 commercial flocks by assaying fifty random fecal droppings per flock the day before processing. The following day, fifty random carcass rinses were obtained from the same flocks at the pre-chill and post-chill phases of processing. Sample dilutions were plated onto Campy-Cefex and enumerated as previously described. To obviate the influence of cross-contamination, the flocks were the first processed each day. One important aspect of poultry processing is to control pathogen spread and reduce pathogens on the final product. The level we found in production of fully grown broilers was ~105.5 CFU per gram of feces. During processing of these same commercial flocks, levels of *Campylobacter* spp. from post-evisceration, pre-chill operations were ~104.8 CFU per carcass and the fully processed samples had levels of ~103.5 CFU per carcass. Variation in levels of *Campylobacter* spp. found in each poultry operation was substantial and will be presented. Greater than

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90% of the birds tested in production yielded the organism. The levels of *Campylobacter* spp. on processed broilers were associated with the levels found in production. By understanding the contributions of the various poultry operations we hope to direct effective control interventions.

T08 *Salmonella* on Free-range Chickens

J. S. BAILEY and D. E. Cosby, USDA-ARS, P.O. Box 5677, Athens, GA 30606, USA

Many consumers assume that broiler chickens grown under traditional commercial conditions will have more *Salmonella* than free-range chickens, which are usually less crowded and have access to outside spaces during grow-out. However, because of the lack of published information about the microbiological status of free-range chickens, the object of the current study was to determine the prevalence of *Salmonella* on commercially grown free-range chickens. A total of 110 processed free-range chickens from 3 different commercial free-range chicken producers were sampled in lots of 10 per each of 11 replication for the presence of *Salmonella*. Carcasses were shipped by overnight freight on ice to our laboratory and traditional USDA cultural methods were used to sample for the presence of *Salmonella*. Overall, 6 of 11 (55%) lots and 27 of 110 (24.5%) of the carcasses were positive for *Salmonella*. No *Salmonella* were detected from 5 of the 11 lots and in one lot 100% all of the chickens were positive for *Salmonella*. Overall, the free-range chickens tested in this study had slightly higher levels of *Salmonella* than the 11% found in recent FSIS HACCP samples, but the trends seen were very similar to those found in commercial integrated operations. There was great variation from grower to grower and from lot to lot. Consumers should not assume that just because chickens are grown under free-range conditions that they will have less *Salmonella*.

T09 Comparison of *Salmonella* Prevalence Rates on Chicken Carcasses Before and After Processing

J. S. BAILEY and N. J. Stern, USDA-ARS, P.O. Box 5677, Athens, GA 30606, USA

To elucidate the relationship of poultry operations to the presence of *Salmonella* on processed chickens, the presence and levels of *Salmonella* in and on broiler chickens were determined as the birds entered and moved through the processing plant. Six large integrated companies from 6 different states participated in this study. Each company used a whole bird rinse procedures to sample carcasses immediately before the scald tank, after the inside/outside bird washer, and after the chill tank. An AOAC approved *Salmonella* screening procedure was used by each company to test for the presence of *Salmonella*. Selected positive samples were then subjected to an MPN procedure to determine the level of *Salmonella* present in the different samples. Each company examined a minimum of 5 carcasses per replication at each sample location from the same flock. For each company, a minimum of 6 replicate studies were completed with 3 replicates being the first flock of a processing shift and 3 replicates being the last flock of a processing shift for a total of 825 carcasses sampled (275 at each sample location). Overall, *Salmonella* were found on 69.5% of carcasses with feathers still on before the scald tank compared to 36% and 16% of carcasses after the inside/outside bird washer and chiller respectively. The number of *Salmonella* on positive carcasses was also reduced from 10 to 100 fold during processing. These data demonstrate that the production phase initially serves to contaminate poultry and antimicrobial treatments, washes, and chlorination of the chill tank significantly reduce *Salmonella* during processing.

T10 DSC Comparison of Shelf Life and Microbial Profile of Immersion-chilled and Air-chilled Broilers

NGAH-WAN (JENNIFER) PHOON, S. R. McKee, and M. Brashears, University of Nebraska-Lincoln, 356 Food Industry Complex, Lincoln, NE 68583-0919, USA

The purpose of this study was to evaluate and compare shelf life of immersion-chilled (IC) and air-chilled (AC) broilers. The number of psychrotrophs on broilers can be influenced by chilling method, thereby affecting the shelf life of birds. Spoilage of poultry occurs when psychrotroph levels reach 1×10^6 to 1×10^8 CFU/cm^2. In the current study, 150 birds (75 AC and 75 IC) were stored for 0, 7, 14, 21, and 28 days at 3°C to compare the shelf life of IC poultry to AC poultry. Shelf life and quality of broilers were assessed by evaluating color, slime, off-odors and numbers of psychrotrophs. Additionally, counts of total aerobes, generic *Escherichia coli*, coliforms, *Campylobacter* spp. and *Salmonella* spp. were evaluated. Combined subjective inspection, color measurement, and psychrotrophs levels indicated that IC broilers showed signs of spoilage faster than the AC broilers. Specifically, between 14 and 28 days of storage, psychrotroph levels were found to be lower in AC birds. IC broilers were found to spoil 3 to 5 days earlier than the AC broilers. Generic *E. coli* and coliforms did not differ considerably between IC and AC broilers on Day 0 and Day 7. However, these microorganisms were found to be lower in AC broilers on day 14, 21 and 28. Numbers of *Campylobacter* spp. and *Salmonella* spp. were found to be different based on day rather than chilling method. Air chilling may result in broilers that have a longer shelf life than those that are immersion chilled.

T11 DSC Inhibition of *Campylobacter jejuni* by Bacteria Isolated from Broiler Deboning Operations

TAM L. MAI and Donald E. Conner, Auburn University, Dept. of Poultry Science, 234 Upchurch Hall, Auburn, AL 36849, USA

Bacteria isolated from broiler deboning operations were investigated to determine their ability to inhibit *Campylobacter jejuni*. Isolates were tested for the inhibition effect by two methods. In the initial method, isolates were spot inoculated onto TSA plates and incubated at 35°C for 24 h. Bacterial

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growth was ended by exposing the plates to UV light for 2 h. The plates were then overlaid with 10 ml of Campy-cefex-agar containing 10^8 *C. jejuni* cells/mL and incubated microaerobically at 42°C for 48 h. Of 600 isolates tested, 62 isolates representing 26 different species produced inhibited *C. jejuni*. These 62 isolates were then confirmed for their inhibitory activity by a second procedure in which 1 ml of isolate culture containing 10^9 cells and 1 ml of *C. jejuni* containing 10^8 cells were grown together in 8 ml of double strength *Brucella* broth and incubated microaerobically at 42°C for 24 h. Growth of *C. jejuni* was measured by spiral plate technique using Campy-Line-agar incubated microaerobically at 42°C for 48 h. Of the 62 isolates tested, 26 isolates, representing 15 species, reduced *C. jejuni* growth by 1 to 2 $\log_{10}$ CFU/ml; 26 isolates, representing 16 species, reduced growth by 2 to 3 $\log_{10}$ CFU/ml; 10 isolates, representing 1 species, reduced growth by more than 3 $\log_{10}$ CFU/ml. *Pseudomonas aeruginosa* was the predominant isolate with clear inhibitory activity against *C. jejuni*. Different isolates of *P. aeruginosa* produced reduction in *C. jejuni* growth from 2.30 to 5.77 $\log_{10}$ CFU/ml. Results indicate that typical poultry-borne bacteria are antagonistic to growth and survival of *C. jejuni*.

T12 *Zygosaccharomyces bailii* and Other Yeasts Associated with Refrigerated Storage of Commercially Processed Broiler Carcasses

ARTHUR HINTON, JR., J. A. Cason, and Kimberly D. Ingram, USDA-RRC, 950 College Station Road, Athens, GA 30605, USA

Experiments were performed to examine changes in the size of yeast populations on broiler carcasses during refrigerated storage and to determine the relationship between *Zygosaccharomyces bailii* isolates recovered from the carcasses. Broiler carcasses were taken from the processing line of a local commercial poultry processing facility immediately after being chilled in an immersion chill tank. Whole carcass rinses were performed to recover yeasts from fresh carcasses and from carcasses stored at 4°C for up to 14 days. Yeasts in the carcass rinsates were enumerated on acidified Potato Dextrose Agar. Yeast isolates were identified with the MIDI Sherlock Microbial Identification System, and dendrograms of fatty acid profiles of *Z. bailii* isolates were prepared to determine the degree of relatedness of the isolates. Findings indicated that the yeast population of the carcasses may increase significantly ($P < 0.05$) during storage at 4°C. *Z. bailii* was isolated from fresh broiler carcasses in 1 of 3 trials and from refrigerated carcasses stored for either 7 or 14 days in 2 of 3 trials. Furthermore, the same strain of *Z. bailii* was recovered from carcasses that were processed together but stored for different periods of time, and the same strain of *Z. bailii* was isolated from carcasses processed on different days in the same processing facility. Monitoring the size and composition of the yeast population of processed poultry during refrigerated storage may provide information that can be used to control the proliferation of these microorganisms and to extend the shelf life of fresh poultry.

T13 A Non-selective/Differential Medium for Recovery of Stressed *Salmonella* from Cultured Dairy Products

YASHODHAR BURGULA and Sita Tatini, University of Minnesota, Dept. of Food Science and Nutrition, 1334 Eckles Ave., St. Paul, MN 55108, USA

Tryptic Soy agar containing 0.6% yeast extract, 0.08% ferric ammonium citrate, 0.68% sodium thiosulfate, and 0.1% sodium pyruvate (TASFC), upon which *Salmonella* formed black colonies, allowed enumeration of *Salmonella* in cultured buttermilk and aged Cheddar cheese. Lactic microflora from cultured buttermilk or Cheddar cheese did not interfere with the visualization of *Salmonella* as black colonies. While there was only a 2 to 3 fold lower count on XLD compared to TSAFC in freshly made cultured butter-milk from milk contaminated with *Salmonella* Typh-imurium or Enteritidis, there was a 50 fold higher count on TASFC than on the XLD in 4 to 6 week stored buttermilk or experimentally inoculated and aged Cheddar cheese. Diluent used to prepare dilutions for plating caused further damage to already stressed *Salmonella* and these were not recovered even on the TSAFC. Peptone water, Butterfield's Phosphate buffer, peptone salt water, and Lactose broth yielded 3 to 5 fold lower counts on both XLD and TSAFC as compared to milk or buttermilk as the diluent. Lactic flora interactions in Lactose broth in the Most Probable Number technique (MPN) did not allow recovery of all the stressed cells but diluting in milk and storing at 4°C for 7 days enhanced recovery of stressed *Salmonella* both by direct plating and by the MPN technique: 300,000/ml on TASFC, 6500/ml on XLD and an MPN of 73,000/ml on 0 day and after 7 day storage of milk diluted system the counts were 410,000/ml on TSAFC, 130,000/ml on XLD and 430,000/ml MPN. Use of milk diluent and TSAFC improves recovery of injured *Salmonella*.

T14 Comparison of Automated BAX for Screening System for *Listeria monocytogenes* and *Salmonella* with Culture Methods

EILEEN M. COLE, W. Mark Barbour, and George Tice, DuPont Qualicon, Bedford Bldg., 3531 Silverside Road, Wilmington, DE 19810, USA

The BAX® for Screening family of PCR assays for foodborne pathogens (DuPont Qualicon) integrates DNA amplification and detection in a single instrument to determine the presence or absence of a specific target. All primers, polymerase, and deoxynucleotides necessary for PCR as well as a positive control and an intercalating dye are incorporated into a single tablet. The BAX® for Screening *Listeria monocytogenes* assay was compared to one of three reference methods (AOAC Method 993.12, FDA-BAM, USDA/FSIS) based on food type. The BAX® for Screening *Salmonella* assay was compared to two culture methods (FDA-BAM and BS/ISO). In

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order to determine maximum sensitivity limits, samples of 17 foods representative of appropriate food types for each target were inoculated so as to generate fractional positives. In both studies, foods were inoculated at high (11 to 50 CFU/25 g), and low (1 to 10 CFU/25 g) levels with separate strains of *L. monocytogenes* (12) or *Salmonella* (5 serotypes). Each inoculation level was replicated 20 times along with five uninoculated replicates. The instrument conducted thermal cycling on the samples and then performed fluorescence-based melting curve analysis to determine the presence or absence of the target. The overall method agreement was >98% for both targets. Performance on the uninoculated samples was also comparable. The automated PCR assay demonstrated equivalent sensitivity to the reference methods for the tested foods as well as providing rapid, simple, and easy-to-use implementation.

T15 DSC Direct Microscopic Observation and Visualization of Viability Detection of *Campylobacter jejuni* on Chicken Skin

WALAIRUT CHANTARAPANONT, Mark Berrang, and Joseph F. Frank, University of Georgia, Dept. of Food Science and Tech., Athens, GA 30602, USA

The objective of this study was to develop a method to identify specific sites on chicken skin that allow survival of *Campylobacter jejuni*. This method employs confocal laser scanning laser microscopy (CSLM) visualization of *C. jejuni* transformed with Pcgfp plasmid (GFP-*Campylobacter*) exposed to 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), a redox dye which is taken up, reduced to CTC-formazan, and accumulated intracellularly in respiratory active cells. An Ar/Kr laser (excitation wavelength [l] = 488 nm) was used to excite both GFP-*Campylobacter* and CTC. The emission wavelength of 500-563 nm was assigned as green color for GFP-*Campylobacter* image, 600-680 nm as red color for CTC image, and 483-499 nm as grey color for chicken skin-reflected light. After 1 h inoculation with 2 ml of 10^8 to 10^9 CFU/ml GFP-*Campylobacter* suspension, 10^5 to 10^6 CFU of *C. jejuni* remained on 1 cm^2 of chicken breast skin after rinsing. Green fluorescence of all *C. jejuni* colonies as well as the CTC-formazan in viable *Campylobacter* was clearly visible on inoculated chicken skin. The data indicated that GFP-*Campylobacter* remaining on the chicken skin surface after rinsing were mostly located in crevices, entrapped inside feather follicles with water and also entrapped in the surface water layer. Most of viable cells were entrapped with water in the skin crevices and feather follicles. These sites apparently provide suitable microenvironments for GFP-*Campylobacter* to survive.

T16 DSC Characterization of Multiple Fluoroquinolone Resistance among Avian *Escherichia coli* Isolates from North Georgia

JUAN F. DE VILLENA, David D. White, Shaohua Zhao, John J. Maurer, and Jianghong Meng, University of Maryland-College Park, 3401 Marie Mount Hall, College Park, MD 20742, USA

Fluoroquinolones were introduced in 1995 for veterinary use in the United States and worldwide for treatment of avian colibacillosis caused by *Escherichia coli*. This study was undertaken to investigate the genetic determinants responsible for decreased susceptibility to fluoroquinolones among *E. coli* isolates from colisepticemia-diseased birds from North Georgia. Fluoroquinolones of human and veterinary significance were assayed, including: gatifloxacin, sarafloxacin, enrofloxacin, ciprofloxacin, difloxacin, danofloxacin, levofloxacin, orbifloxacin, and nalidixic acid. Antimicrobial minimum inhibitory concentrations (MICs) of 100 avian *E. coli* isolates were determined via broth micro-dilution, and interpreted according to the National Committee for Clinical Laboratory Standards. Fifty-nine percent of the isolates were resistant to nalidixic acid and sarafloxacin, while 34% displayed resistance to difloxacin. Decreased susceptibility to enrofloxacin (9%), ciprofloxacin (3%), and levofloxacin (1%) was observed. Amplification of the quinolone-resistance determining region (QRDR) by PCR, followed by DNA sequencing, revealed point mutations within the DNA sequences of the gyrA and gyrB genes, which code for DNA gyrase, and parC and parE genes coding for topoisomerase IV. Potential expression of efflux pump mechanisms was investigated by the use of a preliminary organic solvent tolerance test on the fluoroquinolone-resistant isolates. Twelve fluoroquinolone-resistant *E. coli* isolates were tolerant to cyclohexane. We report the emergence of fluoroquinolone resistance in *E. coli* from cases of avian colibacillosis. Detection of fluoroquinolone resistance in these birds stresses the need for judicious use of antimicrobials to prevent the development of resistance in other bacterial pathogens associated with poultry and introduction of antimicrobial-resistant bacteria into the nation's food supply.

T17 DSC Development of a Selection Method for Detection of Shiga Toxin-producing *Escherichia coli* Based on Glutamate-dependent Acid Resistance

GEUNWOO PARK and Francisco Diez-Gonzalez, University of Minnesota, Dept. of Food Science and Nutrition, 1334 Eckles Ave., St. Paul, MN 55414, USA

Antibiotics are typically used during the enrichment and selection steps to detect shiga toxin-producing *Escherichia coli* (STEC). However, little is known about antibiotic susceptibility of STEC populations, and other coliforms can be antibiotic resistant. Glutamate decarboxylase is produced by most *E. coli* strains and has been linked to increased acid resistance. This project was undertaken to investigate the use of a glutamate-based acid shock as an enrichment procedure to reduce the reliance on antibiotics and their potential negative effect on

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detection. Cultures of STEC and other fecal coliforms were grown overnight in TSB, washed in saline solution and subjected to acid shock (1 h at pH 1.5 to 3.0) in saline solutions containing 6.2 mM glutamate. Acid-shocked cells were serially diluted into TSB media or plated onto SMAC containing cefixime and potassium tellurite. The survivor numbers were determined by MPN or by plate count after incubation for 24 h at 37°C. The viable count of all cultures was reduced more than 7 log CFU/ml by acid shock at pH 2 without glutamate. Glutamate addition had little effect on the survival of *Proteus, Klebsiella, Pseudomonas* and *Citrobacter*, but increased the survival rate of *Enterobacter* up to 1%, and STEC strains to higher than 10%. With glutamate at pH 1.5, only STEC survived acid shocks and *Enterobacter* was completely killed. Recovery in CT-SMAC provided an additional difference between STEC and *Enterobacter*. These results clearly indicate that a glutamate-based acid shock could be a viable strategy to enhance the recovery of STEC from environmental samples.

T18 DSC Spinal Cord Tissue Detection in Comminuted Beef: Comparison of Two Immunological Methods

MAHA HAJMEER, Dean O. Cliver, and Roger Provost, University of California-Davis, School of Veterinary Medicine, 1028A Haring Hall, One Shields Ave., Davis, CA 95616, USA

Beef contamination with central nervous system tissue (CNST) due to slaughter or processing practices raises food safety and human health risk concerns associated with bovine spongiform encephalopathy. Two commercial immunological kits, ScheBo® Brainostic™ and Ridascreen®Risk Material, were compared for CNST detection in beef. Brainostic™ kit is based on immunological detection of CNS-specific antigen, and Ridascreen® on enzyme immunoassay (semi-quantitative analysis) of CNST in meat. Three quality grades (choice, select, utility) of fresh beef shoulder clod were ground, divided into batches, and mixed with minced spinal cord (SC) to yield concentrations of 0.0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6% SC in meat. Each batch was homogenized and divided into two equal portions; one was sampled 30 min following refrigeration, and the other frozen for 24 h, thawed, and then sampled. The tests (5 replicates per SC concentration) were performed per manufacturer's recommendations. Sensitivity and specificity in detecting SC in fresh and frozen meat were determined. Both Brainostic® and Ridascreen® kits detected SC at claimed levels: 0.25% and 0.11%, respectively. Although samples containing <0.1% SC were positive using Ridascreen® test, they consistently fell outside the quantifiable range. Also, this assay detected SC at 0.025%, a level lower than its claimed 0.11% designed for brain and SC combined. Detecting SC at 0.025% renders the Ridascreen® test ~10x more sensitive than Brainostic®. The Ridascreen® assay was easier and faster to run and less expensive than Brainostic® test. Quality grade had no impact on SC detection of either fresh or frozen meat.

T19 DSC Comparison of Recovery of Airborne Microorganisms in a Dairy Cattle Facility Using Selective Agar and Thin Agar Layer (TAL) Resuscitation Media

BETH ANN CROZIER-DODSON and Daniel Y. C. Fung, Kansas State University, Dept. of AS and I, 1600 Midcampus Drive, Call Hall 202, Manhattan, KS 66506-1600, USA

Thin Agar Layer (TAL) medium was developed at Kansas State University to improve resuscitation of injured cells, and has been shown to result in higher recovery than selective media alone for cold-, heat-, salt-, or acid-injured cells. This experiment was designed to determine the effectiveness of the TAL method for the recovery of organisms in air. Eleven agar media were used: Tryptic Soy agar (TSA), MacConkey Sorbitol agar (MSA), TAL-MSA, Baird-Parker agar (BP), TAL-BP, Modified Oxford agar (MOX), TAL-MOX, Xylose Lysine Sodium Desoxycholate agar (XLD), TAL-XLD, *Yersinia* Selective agar (CIN), and TAL-CIN. The TAL plates were prepared by pipetting 6 ml of a selective agar into a BBL Rodac™ plate (65 mm × 15 mm). Plates were allowed to solidify, and then overlaid with 6 ml of TSA. Selective agar plates were prepared by pipetting 12 ml of agar into BBL Rodac™ plates and allowing it to solidify. Samples (60 L/plate) were taken at an indoor cattle facility in 5 locations using a BioScience SAS air sampling instrument. Three replications of the experiment were performed. After incubation of plates, total CFU/m^3 of each location were obtained. Data indicated that in all locations, the TAL method resulted in higher counts of microorganisms on all media tested. In addition, 175 isolates were randomly selected and identified to test the selectivity of TAL and selective media for target organisms. This data has shown that the TAL resuscitation method is a very effective and appropriate procedure for the enhanced recovery of organisms in air.

T20 DSC A Simple and Inexpensive Method to Concentrate Bacteria from Produce for Detection Using Cultural or Molecular Techniques

LYNETTE KLEMAN and Lee-Ann Jaykus, North Carolina State University, Dept. of Food Science, Box 7624, Raleigh, NC 27695-7624, USA

Low levels of contamination and the need to test large sample sizes hinder the detection of pathogenic organisms in food. Bacterial concentration prior to detection addresses these issues but most methods to date are pathogen specific and expensive. The purpose of this study was to develop a simple, inexpensive, non-specific method to concentrate bacteria from fresh produce. The method consisted of the sequential steps of filtration and centrifugation prior to cultural and/or molecular biological detection. Samples of (25 g) of representative produce items were diluted, and homogenized in Whirlpak filter bags, and the resulting filtrate was collected. There was no statistically significant difference

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($P < 0.05$) between aerobic plate counts (APC) and total coliform counts (TCC) of homogenates compared to filtrates, regardless of produce type. After centrifugation, the resulting pellet (<0.5 g) represented a 50-fold sample concentration with bacterial recovery (APC, TCC) 67%-110% and minimal (<5%) loss to discarded supernatants. In order to ascertain that the method was effective in the concentration of pathogens of concern in fresh produce, antibiotic resistant strains of *Listeria monocytogenes, Salmonella,* and *Escherichia coli* O157:H7 were seeded into fresh alfalfa sprouts. The samples were processed for bacterial concentration and enumerated by plating on antibiotic supplemented media. Recoveries ranged from 68%-116% for all pathogens, with loss to supernatant again minimal (<2%). Concentrated pathogens could be detected by molecular methods such as PCR. This study represents significant progress towards the development of simple, inexpensive and non-specific methods to separate and concentrate microorganisms from foods prior to detection.

T21 Studies to Select an Appropriate Non-Pathogenic Surrogate *Escherichia coli* Strain for Use in Place of *Escherichia coli* O157:H7 in a Pilot Plant Environment

B. A. ANNOUS, D. C. R. Riordan, and G. M. Sapers, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Often, in the interests of operator safety, it is necessary to use surrogate organisms in place of pathogens in large-scale food challenge trials. The behavior of potential surrogates should closely mimic the behavior of the target pathogen under the challenge conditions. In this study the survival characteristics of sixteen non-pathogenic *Escherichia coli* strains were compared to two *E. coli* O157:H7 strains and two *Salmonella* strains; all pathogenic strains were previously associated with foodborne disease outbreaks in produce or apple cider. Growth characteristics (generation time, lag phase duration and maximum population), pH at stationary phase, heat resistance at 60°C, and membrane fatty acid composition were determined for each strain following growth in five different media. In addition, the level of attachment to apple surfaces and resistance to hydrogen peroxide decontamination treatments were assessed for each strain. Few differences in growth characteristics or pH at stationary phase were evident between the non-pathogenic strains and *E. coli* O157:H7, though higher final pH values were seen with *Salmonella* strains ($P < 0.05$). *E. coli* O157:H7 and *Salmonella* strains were not significantly different for any of the growth characteristics measured, or in the levels of attachment to apple surfaces and resistances to hydrogen peroxide; however, *E. coli* O157:H7 was significantly more resistant to heat than *Salmonella* ($P < 0.05$). *E. coli* O55:97.0152 appears to be a good surrogate candidate, with survival characteristics which are similar to *E. coli* O157:H7 under these challenge conditions. A less heat resistant strain of *E. coli*, perhaps *E. coli* NRRL 766, must be used when attempting to reproduce the heat resistance of *Salmonella*. These data will be useful for those interested in validating the efficacy of intervention steps in reducing *E. coli* O157:H7 and *Salmonella* populations in processing environments.

T22 *Pediococcus* Species NRRL B02354 as a Thermal Surrogate in Place of Salmonellae and *Listeria monocytogenes*

JEFFREY KORNACKI and Joshua Gurtler, University of Georgia, CFS, Dept. of Food Science, 1109 Experiment St., Griffin, GA 30223-1797, USA

The usefulness of selective agar underlay to recover injured cells and the potential usefulness of *Pediococcus* sp. NRRL B-2354 as a thermal surrogate for in-factory validation studies was evaluated. Inoculated (1 million CFU/g) and uninoculated samples were aseptically spread in pre-sterilized, heat-sealed plastic bags to a total thickness of about 0.1 cm to ensure rapid temperature adjustment, as monitored with thermocouples, when bags were added to a 62°C water bath. Typically, one uninoculated and three inoculated samples were treated for 0, 0.75, 5, 15, and 25 min before removal from the water bath, immediately cooled, and tested. Spiral plating of samples was performed on Trypticase Soy Agar with 1% Yeast Extract (TSAYE) with a selective agar underlay. Sample enrichments were done with Universal Pre-enrichment broth (UPB). Presence of *Listeria* and *Salmonella* spp. were assayed by enzyme-linked fluorescent immunoassay (ELFA) assay. Selective media were streaked for isolation of Pediococci from UPB enrichments, as were all ELFA presumptive positives. Typical isolates were screened biochemically. A rapid decrease in Salmonellae and *Listeria* counts, determined by spiral plating, occurred within 5 minutes to undetectable levels. Survival of challenge organisms, determined by enrichment, was longer. *Pediococcus, S.* Senftenberg 775W, *L. monocytogenes,* and *S.* Typhimurium DT104 survived 25 (3/3), 15 (3/3), 15 (1/3 samples), and less than 0.75 minutes of heating, respectively.

T23 Development of a Spatially Valid Sampling Technique for the Enumeration of *Salmonella* in the Swine Abattoir Holding Pen

Annette O'Connor, JARED K. GAILEY, and H. Scott Hurd, 207 Science I, Iowa State University, Ames, IA 50011, USA

Recent research indicates that the holding pen may be an important source of infection for swine entering the food chain at the abattoir. Currently, however, no standardized method for the quantification of *Salmonella* at the pen level exists. The objective of this research was to determine a sampling technique that would effectively quantify the amount of *Salmonella* in holding pens at the swine abattoir. Additionally, this study provides preliminary data about the levels of *Salmonella* in the abattoir holding pen. The extent and spatial pattern of contamination

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were examined using standard methods of spatial statistical analysis, such as variogram estimation. Contamination was also modeled using a Markov random field approach in which several definitions of neighborhood structure were examined for their ability to reflect observed dependence in measured CFU and CFU/ml values. Using this model, a spatially valid sampling technique was derived for the enumeration of *Salmonella* in the abattoir holding pen. The enumeration of fluids found on the abattoir holding pen floor found an average of about 10^3 CFU/ml of *Salmonella*, with a range of 0 to 10^5 CFU/ml.

T24 Development and Testing of a Method for the Detection of Molds Using the MicroFoss System

N. Beales, R. P. BETTS, S. McDougal, and R. Firstenberg-Eden, Campden and Chorleywood Food Research Association, Chipping Campden, Station Road, Gloucestershire GL55 6LD, UK

Rapid testing of food for molds has always provided the food industry with problems due to the very slow growth rate of these organisms. Most mold detection methods require a week or more of incubation, before a result can be achieved. Recently, a procedure has been developed using the Biosys MicroFoss instrument, which allows the detection of foodborne molds relatively quickly; this procedure has been evaluated at Campden & Chorleywood Food Research Association. The mold detection system has been assessed for its ability to indicate growth of a wide range of food spoilage molds against two reference methods using RBCA (BAM) and PCA + inhibitors (USA). Initially the system was assessed against five strains of *Penicillium* and *Aspergillus* using various broths. All broths examined showed the potential for use in this system, but YEB, YEDB and PDB had shorter detection times. These three broths were further analysed using fifteen other *Penicillium* and *Aspergillus* species and five strains of *Rhizopus, Mucor, Fusarium, Alternaria, Eurotium*, and *Wallemia*. The system detected the molds examined at inoculum levels of 1×10^3 to 1×10^4 and at 1×10^1 to 1×10^2 CFU/ml; detection times ranged from 28 to 63 h, and from 35 to 92 h, respectively. The mold detection system was uncomplicated, and its fully automated detection system made testing on larger numbers of samples relatively easy. This work is continuing with the assessment of this mold detection system using naturally and artificially contaminated food materials and comparing them against recognized reference methods.

T25 The Impact of Biotechnology on the Foodservice Industry

SHEILA COHN, National Restaurant Association, 1200 17th St., NW, Washington, D.C. 20036, USA

Biotechnology is one of the most exciting and promising advances in modern science today. It has also become one of the most controversial. With this technology, the potential for providing an abundant, nutritious and high quality food supply for worldwide consumers and foodservice operations can be a reality. However, concerns here and abroad threaten the advancement of this innovative technology. This paper is concerned with the latest advances as well as the truth behind some of the myths surrounding Biotechnology.

Scientists are using new technologies in selective breeding to improve food crops, making them insect/disease resistant, more nutritious, abundant and affordable. In 1992, the Food and Drug Administration (FDA) determined through sound science that biotech-enhanced foods are as safe as traditional foods and should be regulated the same as any other foods. Today, over 40 varieties of biotech food crops have been approved, and over 65% of processed foods contain biotech ingredients.

Foods produced through biotechnology have been FDA-endorsed as safe and are an integral, promising part of the food supply. The National Restaurant Association opposes *mandatory* labeling of biotech foods because labels are reserved for essential information and a mandatory designation that foods have been genetically modified (GM) would be an implied warning. The Association is educating Congress about the food industry's support for FDA's current science-based approach that ensures these foods are safe and labeled with meaningful information. FDA should establish science-based criteria for what constitutes a biotech food product and criteria for *voluntary* "GM Free" and "non-GM ingredient" labeling to ensure consistent claims in the marketplace.

T26 What Can We Learn about Biotechnology from the Retail Food Industry Experiences?

SUSAN HARLANDER, BIOrational Consultants, Inc., 3215 Linden Drive, New Brighton, MN 55112, USA

Forty-seven genetically modified crops have been reviewed by the US Food and Drug Administration and are making their way through commodity food channels into a variety of foods offered for sale through retail and food service outlets. In the US, no special labeling is required since these crops are considered "substantially equivalent" to their conventionally bred counterparts. This is not the case in other parts of the world where products containing genetically modified ingredients must be labeled if their content exceeds a specified threshold level. This dichotomy creates challenges for the retail and food service industries that must comply with labeling guidelines in every country where they do business. This presentation will focus on the challenges associated with identity preservation and segregation of genetically modified crops through a food supply chain geared for maximum efficiency and least cost. It will address the need for accurate, specific, reliable, standardized and validated testing methods to ensure compliance with guidelines. Various consumer interest groups are calling for labeling of all products containing genetically modified ingredients. The retail food industry has been tracking consumer response to genetically modified foods and at the present time

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this does not appear to be a major concern to most consumers of processed foods. The implications of labeling of retail products containing genetically modified ingredients will also be discussed. Finally, the lessons learned from the retail food industry will be applied to the food service industry.

T27 A Farm-to-Fork Case Study in Risk Communication—The Model Farm Project, Year 2

KATIJA A. BLAINE, Douglas A. Powell, and Jeffrey Wilson, University of Guelph, Guelph, ON N1G 2W1, Canada

Genetically engineered Bt sweet corn and Bt potatoes were grown on a 250 acre fruit and vegetable farm in Orton, Ontario, Canada, alongside conventional counterparts in 2000 and 2001. This paper describes year two of a continuing case study in risk communication related to a specific food production technology. This farm-to-fork research was conducted in a transparent manner both years, with information posters, letters pamphlets, and press conferences produced to provide the community and others with information on the project and the technology. This paper discusses the various communications strategies employed and evaluation of these strategies including the consumer response. The Bt sweet corn out-sold the conventional sweet corn by a margin of 3:2 the first year, and 5:2 in year two. In 2001, it was observed that consumers were more familiar with agricultural Biotechnology — at least in this specific farm market — and less concerned about Bt crops. There were fewer questions in total; however, the questions that were asked were more technical in nature. Another change in year two was that a large number of consumers wanted to buy Bt sweet corn and Bt potato seeds to grow in their home gardens. Media converage was significantly reduced in the second year. The results may be applicable to the introduction and consumer support for other food production and food safety technologies, and demonstrate that consumers can integrate information about risks and benefits when making food purchasing decisions.

T28 DSC Needs Assessment for a Proposed Biotechnology Education Initiative for Ontario High School Biology Students

LIZ GOMES and Douglas Powell, University of Guelph, Food Safety Network, Crop Science Bldg., Guelph, ON N1G 2W1, Canada

Numerous biotechnology education initiatives have been implemented throughout North America and the world to accompany the introduction of genetically engineered food. However, there has been little published work about how to best develop such a food safety educational initiative — even fewer have included a needs assessment. This paper outlines the results of a needs assessment conducted during the development of an agricultural biotechnology education initiative for Ontario high school biology students. A total of 37 participants were part of 4 focus groups (students and teachers from urban and rural areas) using the Nominal Group Technique as the primary data collection method. Responses identified preferred types of activities, information sources, issues for discussion, and supplemental resource characteristics along with the possibility of delivering the proposed initiative via CD-ROM. Students and teachers preferred interactive activities, varied information sources ranging from farmers to government, and issues ranging from scientific to wider social and ethical implications on local and global scales. Also, respondents responded positively towards a CD-ROM initiative overall, but expressed concern over the lack of computers in their respective schools. It was concluded that CD-ROM delivery was not feasible due to lack of computers for science students; however, a printed booklet/videotape pack was more realistic. Furthermore, it was concluded that an initiative would be well received if as many of the participant responses as possible were incorporated into the resulting document. These results are useful to anyone in the developmental stages of a food safety educational initiative in their locale.

T29 Public Attitudes toward Genetically Modified Foods

CHRISTINE BRUHN, University of California-Davis, Center for Consumer Research, One Shields Ave., Davis, CA 95616, USA

Public attitudes toward innovation from biotechnology continue to be positive. During the Star Link fiasco more than half of US consumers reported hearing of recalls related to a biotechnology modified product, but none changed buying habits as a result of this information. The majority of consumers continue to express interest in purchasing products modified by biotechnology for environmental or health reasons. A significant minority of consumers, 15% or more, are opposed to agricultural applications of biotechnology. These consumers may be attracted by food service operations which feature no products modified by contemporary biotechnology. "No GMO" claims would be challenging to achieve under a policy of zero tolerance, especially for mixed dishes containing soy, corn, dairy, or certain produce items. Ardent opponents of biotechnology consider meat, milk, or egg products from animals who have consumed biotech modified feed to be themselves products of biotechnology. Declaration of specific ingredients as not genetically modified may be difficult to communicate since consumers find labeling individual ingredients complex and confusing. At this time, food service operations that feature no biotechnology modified products fac~ exceptional challenges to meet and communicate this policy.

T30 Response to an Outbreak of Salmonellosis Associated with California Almonds

S. ISAACS, J. Aramini, B. Ciebin, J. Farrar, R. Ahmed, D. Middleton, E. Chan, S. Pichette, K. Campbell, P. Mead, L. Lior, M. Pearce, C. Clark, F. Rogers, F. Jamieson, I. Brophy, and A. Ellis, Health Canada, Population and Public Health Branch, 1 Stone Road W., Guelph, Ontario N1G 4Y2, Canada

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An outbreak of Salmonellosis due to a rare strain of *Salmonella* Enteritidis (SE PT30) was detected in Canada during the winter of 2000/2001. Epidemiological and laboratory based associations of human illness to almonds prompted traceback and environmental investigations involving food regulatory agencies and public health in both Canada and the United States. Enhanced laboratory surveillance (phage typing; PFGE analysis) identified cases. A case-control study involving 16 matched case-control pairs was conducted to test the hypothesis, derived from case food histories, that raw, whole almonds was the vehicle of transmission. Samples of almonds were collected from case homes, retail sources, and the implicated processor identified through traceback investigations. Environmental samples were collected from processing equipment and associated farmers' fields. All samples were submitted for laboratory analyses. One hundred sixty-two human cases were identified between October 2000 and July 2001. Cases were more likely than their matched controls to have eaten raw, whole almonds (matched OR= 21.14; 95% CI: 3.59 – infinity). Almonds consumed by cases were traced to the same processor. Laboratory analyses of raw almonds, equipment, and soil samples found SE PT30. The identification of raw almonds as the source of the outbreak eventually led to an international recall of almonds, and to a review of current industry standards. The outcome of this investigation demonstrates the value of good, fundamental food science and public health practice for expanding our knowledge and understanding of previously unrecognized health risks.

T31 Overcoming Barriers When Implementing an On-farm Food Safety Program: A Case Study of the Ontario Greenhouse Vegetable Growers

AMBER LUEDTKE, Benjamin Chapman, Douglas Powell, and all of the Food Safety Network, University of Guelph, Dept. of Plant Agriculture, Guelph, Ontario N1G 2W1, Canada

In 1998 the Food Safety Network (FSN) at the University of Guelph and the Ontario Greenhouse Vegetable Growers began the implementation of a proactive, on-farm food safety program. This initiative was designed to reduce the occurrence of microbial contamination of greenhouse vegetables by working with the 220 members of the grower group directly. The HACCP-based program focuses on the grower/packer/shipper area of the farm-to-fork food production chain. A manual that contained a variety of checklists that growers were to follow was produced and individual visits conducted. Initially the program was met with resistance from producers and was found to be inefficiently executed. Producer attitude towards the entire program and the lack of response to the manual were addressed by adjusting the program accordingly in response to grower input. Alterations included the addition of wall checklists, hand washing signs and a greater focus on individual farm issues. By comparing total coliform, *Escherichia coli* and *Salmonella* spp. sample testing results pre- and post- project reassessment, as well as general food safety perceptions through grower surveys, early indications point towards an increase in compliance with the program objectives. The information gathered through the reassessment of the OGVG on-farm food safety program provides a model for other researchers to follow if similar success problems are encountered.

T32 DSC Evaluation of the Use of Lactic Acid Bacteria to Control Pathogens on Alfalfa Sprouts

MARSHA R. HARRIS, Mindy M. Brashears, and Durward A. Smith, University of Nebraska-Lincoln, 143 Filley Hall, Lincoln, NE 68583-0919, USA

Many studies have investigated the control of pathogens on alfalfa sprouts and some treatments have been shown to be effective in pathogen reduction. However, control methods investigated thus far only provide pathogen control at a given point in the sprouting process. Competitive inhibition of pathogens with lactic acid bacteria (LAB) may provide pathogen control throughout the sprouting process and up to consumption. The purpose of this study was to evaluate the use of LAB to control pathogens on alfalfa sprouts. Agar spot tests and inhibition in laboratory media indicated that LAB significantly inhibit *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enterica*. Alfalfa seeds inoculated with 10^2 CFU/g of seed were subjected to 10^7 CFU/ml LAB in seed soak water. One isolate, identified as *Lactococcus lactis* subsp. *lactis*, was shown to significantly reduce the numbers of *L. monocytogenes* on the resulting alfalfa sprouts. Competitive inhibition shows promise as an intervention in the sprouting process.

T33 A Survey of Sprout Growers in California

JENNIFER THOMAS, Mary Palumbo, Dean Cliver, Jeff Farrar, and Thomas Farver, California Dept. of Health Services, Food and Drug Branch, Emergency Response Unit, 601 N. 7th St. MS#357, Sacramento, CA 94234, USA

Since 1995, nine *Salmonella* and two *E. coli* O157:H7 foodborne outbreaks have implicated raw vegetable sprouts as the source of the infection. To reduce the numbers of sprout-related outbreaks, the US FDA published Guidance for Industry: Reducing Microbial Food Safety Hazards for Sprouting Seeds, in 1999. Between October 2000 and April 2001, 61.5% (16/26) of the commercial sprout firms in California were enrolled in a survey to evaluate the industry practices of California sprouting operations and to determine compliance with FDA guidelines. A standardized questionnaire was used to collect data on firm demographics and seed disinfection practices. Additionally, free chlorine levels of the seed disinfection solution were measured and 48-h spent irrigation water samples were collected from each firm. The irrigation water was screened for *Salmonella* and *E. coli* O157:H7 using FDA recommended test kits. Free chlorine levels in the treatment solution ranged from 50 ppm to 35,000 ppm with a median of 14,000 ppm. Free chlorine levels were higher in firms producing alfalfa sprouts compared to those pro-ducing only mung beans or soybeans ($P = 0.03$). Firms using calcium hypochlorite tended to have higher median levels of free chlorine compared to firms using sodium

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hypochlorite as the treatment solution ($P = 0.067$). Irrigation water samples screened for *Salmonella* were negative (32/32). Of the irrigation water tested for *E. coli*, 75% (24/32) were negative and 25% (8/32) of the tests resulted in a presumptive positive. The eight presumptive positives were negative after further testing using BAM procedures.

T34 DSC Proteolytic Activity of Fungi Isolated from Decayed and Damaged Tomatoes

WENDY N. WADE and Larry R. Beuchat, University of Georgia, CFS, Dept. of Food Science and Tech., 1109 Experiment St., Griffin, GA 30223-1797, USA

The potential increase in pH of tissues of raw fruits and vegetables as a result of fungal growth may enhance the potential for growth of foodborne pathogens. A study was done to examine 77 decayed and 138 damaged raw, ripe tomatoes for the presence of yeasts and molds that produce proteolytic enzymes that can potentially increase the pH of pulp tissue. The pH of decayed and sound tissues from decayed tomatoes ranged from 4.7 to 7.8 and 4.3 to 5.8, respectively, whereas the pH of damaged and sound tissues from damaged tomatoes ranged from 4.2 to 7.8 and 4.0 to 8.0, respectively. Fu1gal isolates (n = 387) recovered on dichloran rose bengal chloramphenicol agar were examined for proteolytic activity on gelatin agar and standard methods caseinate agar. One hundred twenty-eight (33.1%) of the isolates exhibited proteolytic activity on one or both differential media. The pH at the edge of colonies increased on both media. Colonies of proteolytic isolates grown on tomato juice agar (pH 4.3) and on the surface of tomato juice (pH 4.1) caused an increase in mean pH at the colony/media interface to 6.4 and 5.8, respectively. Results show that some fungi capable of infecting raw tomatoes, as well as the mycoflora incident on tomato surfaces, can increase the pH of pulp and juice to levels favorable for growth of several foodborne pathogenic bacteria. Failure to remove decayed or damaged tissues during sorting and minimal processing may increase the risk of human infections associated with raw tomatoes.

T35 DSC The Use of Oxidation to Control *Cryptosporidium* Infectivity

K. KNIEL, S. S. Sumner, D. S. Lindsay, C. R. Hackney, M. D. Pierson, A. Zajac, and D. A. Golden, Virginia Tech., Food Science and Tech. (0418), Blacksburg, VA 24061, USA

Cryptosporidium parvum is a protozoan parasite associated with both waterborne and foodborne outbreaks. Cryptosporidiosis has been associated with consumption of contaminated apple cider and other foods. The infective life-stage is a small environmentally resistant oocyst that can be transmitted through water and food. Oocysts have a relatively thick membrane, which allows them to be resistant to chlorine and most environmental pressures. In this study oxidation through treatment with hydrogen peroxide or ozone was used to inhibit *C. parvum* oocyst viability. Oocysts excyst within the small intestine and infect the microvilli of host cells. Infectivity was monitored using a human illeocecal (HCT-8) cell culture system. Treating apple cider, orange juice, and grape juice with hydrogen peroxide (> 0.03% (v/v) for > 15 min) or ozone (0.9 g/l flow rate) inhibited *C. parvum* viability to > 90% reduction as monitored in the cell culture assay. The percent reduction was determined relative to control untreated oocysts. It is hypothesized that cysteine proteases, which are necessary for infection, is oxidized by these treatments. Assaying treated *C. parvum* homogenate in protease assays using a variety of cysteine and serine proteases (Aprotinin, Leupeptin, PMSF, Bestatin, Pepstatin, EDTA, E-64) supported this hypothesis. Treatment with oxidizing agents resulted in a significant decrease in protease activity (P value < 0.05). Additionally, oocysts treated with oxidative agents show signs of membrane damage in scanning electron micrographs. Both oxidizing treatments tested may be used as alternatives to pasteurization to treat fruit juices to successfully inhibit *C. parvum* viability.

T36 Proximity to Dairy Operations Influences the Presence of a Fecal Indicator on Peaches, Plums, and Nectarines

Shantana Goerge, Lorena Fernandez, and TREVOR SUSLOW, University of California-Davis, Dept. of Vegetable Crops, One Shields Ave., Davis, CA 95616-8631, USA

Various external influences in land-use have increased the regional concentration of animal production facilities and high value horticultural commodities in California. A three-year study was undertaken to assess the impact of proximity of tree fruit production operations to dairy operations on the preharvest and postharvest populations of nonpathogenic *E. coli* recovered from the phyllosphere. Commercial peach, plum, and nectarine orchards were selected to span a proximal and distal relational and cardinal range relative to concentrated dairy operations. Recovery was based on at least five replicate samples of five fruit per tree using 225 ml of phosphate buffered peptone (pH 7.0) in a shake-rub-shake-rub wash sequence for 5 min. Enumeration was conducted by membrane-capture, passing washate through a pre-wetted 0.45mm filter and aspetically placing the inverted membrane on ECC ChromAgar. Plates were incubated at 42.5°C to reduce interference from other coliform bacteria that limit outgrowth of *E. coli* at 35-37°C. Recoverable *E. coli* populations on fruit were most frequent and most numerous on trees directly adjacent to and downwind or crosswind from a dairy operation. Detectable populations of *E. coli* did not exceed log 2.0 CFU/fruit and were more typically less than log 1.27 CFU/fruit or undetectable. In most cases, typical postharvest operations, monitored at three commercial operations, were effective in removing recoverable *E. coli* from fruit prior to packing. No blue colonies, presumptive *E. coli*, were found to yield PCR products

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when evaluated with primers to VT1 and VT2, which amplified the predicted product in positive control DNA from *E. coli* O157:H7.

T37 Environmental Health Specialists Network (EHS-Net) – Understanding the Causes of Foodborne Illness and Improving the Practice of Environmental Health

ROBIN LEE, Craig Hedberg, Daniela Niutta, Carol Selman, and the EHS-Net Working Group, CDC-NCEH, Environmental Health Services Branch, Mailstop F-28, G338 4770 Buford Hwy., NE, Atlanta, GA 30341, USA

The Environmental Health Specialists Network (EHS-Net) is composed of environmental health specialists and epidemiologists located at the federal, state and local levels. Based on a systems approach, this collaboration is designed to improve the understanding of the underlying causes and interactions of factors that lead to foodborne illness and to use the knowledge gained to better prevent future cases of such illness. The EHS-Net is a combined effort among the CDC, FDA, and eight states. The EHS-Net projects will provide insights to understanding the environmental causes of foodborne illness. Current EHS-Net activities describe food safety systems in restaurants and other establishments where food is eaten outside the home. Survey tools have been designed and piloted by EHS-Net to collect data in outbreak and non-outbreak settings. Data collection encompasses the entire food preparation process from delivery of ingredients, through preparation and cooking, to the actual service of the food item. Both univariate and multivariate analyses are used to assess potential risks present in food establishments. By documenting the entire food preparation process, such as bare-hand contact with food, preparation of raw meats and poultry, and egg handling practices, we will be able to analyze the role of food handling and preparation practices in foodborne illness. On the basis of this information, data may support existing food handling guidelines as well as suggest revision of current guidelines and policy where necessary. Thus, EHS-Net provides an approach to understanding foodborne-related illnesses occurring outside the home and ways to prevent them from occurring.

T38 Staphylococcal Food Poisoning: Phenotypic and Genotypic Characterization of Isolates from Food and Human Samples

Viviane Colombari, Mariana D. B. Mayer, Zaira M. Laicini, Elza M. Mamizuka, Bernadette D. G. M. Franco, MARIA T. DESTRO, and Mariza Landgraf, University of São Pãulo - Brazil, Av. Prof. Lineu Prestes 580 BL 14, São Pãulo, 05508-900, Brazil

The occurrence of staphylococcal food poisoning (SFP) is very common worldwide. In Brazil it is ranked as the second most frequent foodborne disease usually linked to the consumption of fresh white cheese and cream filled cakes. The methods currently used in SFP epidemiological studies, enterotoxin typing and phage typing, have limitations. The use of simple and rapid molecular techniques can be a good tool in these studies. The aim of the present research was to identify the source of a staphylococcal outbreak involving 180 people in the city of Brodowski, São Pãulo State, Brazil. Sixteen isolates from foodstuffs served during a party and 42 from clinical specimens of food handlers were characterized. The tests carried out were: antibiotic resistance profile (15 different antibiotics) using standard methodology; enterotoxin typing by reversed-passive latex agglutination assay (Denka Seiken); phage typing using the International Set of Phages; and genotyping characterization with RAPD (primers OPA7 and OPE20). Strains from three foodstuffs (cooked vegetables in mayonnaise sauce, pasta with tomato sauce, and broiled chicken) and those from four food handlers were staphylococcal enterotoxin type A producers and shared the same phenotypic and genotypic profiles. It could be confirmed that those food handlers were responsible for the dissemination of enterotoxigenic *Staphylococcus aureus* to the food that caused the outbreak.

T39 Epidemiological Typing of *Campylobacter* Clinical and Food Isolates Using Pulsed-Field Gel Electrophoresis (PFGE)

Diane Medeiros, JEFF FARBER, and Syed Sattar, Bureau of Microbial Hazards, Food Directorate, Health Products and Food Branch, Sir F. G. Banting Research Centre, Ross Avenue, Tunneys Pasture, PL 2204A2, Ottawa, Ontario K1A 0L2, Canada

This study was done in an attempt to gain a better understanding of the epidemiology of Campylobacteriosis in the Ottawa-Carleton region. A variety of raw and ready-to-eat foods were tested for the presence of *Campylobacter* spp. From the 300 samples analyzed, *Campylobacter* spp. were detected in four samples, one raw beef liver sample and three raw chicken samples. An isolation rate of 9.7% was observed among the raw chicken samples tested, a significantly-reduced percentage, as compared to a 1981 Canadian survey. The prevalence of *Campylobacter* spp. in a poultry foodservice operation in Ottawa was also determined. No *Campylobacter* spp. were detected in the 125 samples tested. *Campylobacter* clinical and food isolates were characterized using PFGE with two restriction enzymes, *Sma*I and *Kpn*I. *Kpn*I produced more complex banding patterns than *Sma*I, and proved to be more discriminatory. Among the 154 isolates assigned to clusters by *Sma*I, only 42% gave concordant results with *Kpn*I. In contrast, among the 53 isolates assigned to clusters by *Kpn*I, 87% gave concordant results with *Sma*I. Five of these concordant clusters represented isolates obtained from the same person, suggesting that some of these individuals may have become re-infected. Spot map analysis revealed a significant clustering of campylobacteriosis cases in the former city of Ottawa, most of which did not belong to the same postal code. In contrast, very few cases were observed in outlying regions; however, most of these cases belonged to the same postal code, suggesting the possible presence of local outbreaks.

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T40 Dose Response Modelling of *Escherichia coli* O157 Incorporating Data from Foodborne and Environmental Outbreaks

N. J. C. STRACHAN and I. D. Ogden, University of Aberdeen, Dept. of Plant and Soil Science, Cruickshank Bldg., St. Machar Drive, Aberdeen AB24 3UU, UK

While it is generally accepted that ingestion of low doses of *Escherichia coli* O157 can lead to human illness there are few data to support this. Dose response models derived from human feeding studies would allow prediction of risk to humans following exposure to *E. coli* O157 from either foodborne or environmental vectors. However, due to the severe nature of the disease, human dose response studies have not been performed. Surrogate models from *Shigella* spp. (Crockett) and inoculated rabbits (Haas) have been utilised. However, these two Beta-Poisson dose response models are considerably different, with the Haas model requiring approximately 500 times as many organisms to infect 50% of humans exposed compared with the Crockett model. A more realistic way of either generating or validating a dose response model is to use data obtained from actual human outbreaks. To achieve this the attack rate (number of people becoming ill divided by total number exposed) and the actual numbers of organisms ingested must be known. In human outbreaks, however, either insufficient food is available for pathogen enumeration or bacterial numbers in the food are likely to have changed due to conditions of storage. An investigation into specific foodborne and environmental outbreaks of *E. coli* O157 worldwide has revealed a number of outbreaks where data are available. These are incorporated, for the first time, into a dose response model for *E. coli* O157 which can be used as a basis for quantitative microbial risk assessment.

T41 DSC An Analysis of US Cross-connection Incidents in the Food Industry: 1901–2000

PAULA MARIE TANNER and P. J. E. Quintana, Jack-in-the-Box Corporate Headquarters, 9330 Balboa Ave., San Diego, CA 92123-1516, USA

Cross-connection events are critical to the food industry. This study represents the first comprehensive data and risk-factor analysis conducted on cross-connections. A comprehensive journal, government publication and newspaper review documented a total of 838 incidents in the US—124 (16%) food-industry associated. The first was a 1914 meat-plant dysentery outbreak. The last was a county fair *E. coli* O157:H7 outbreak. The acme event occurred in 1985, the Chicago-based milk-related salmonellosis incident. There were 16,284 identified victims and two deaths. Comparatively, in the century's first quartile, 104 potable water cross-connections caused 15,000 typhoid cases and 500 deaths. Of the 124 events, 97 (78.2%) were pump and head back-pressure, of which a subset of 74 involved CO_2/copper backpressure retail incidents. Twenty-three (18.5%) were caused by backsiphonage. Thirty-seven events (31 manufacturing plants, 6 farms) proved a tremendous illness, injury, death and financial burden on the public and on industry. One event caused a $2-million pork impound, and another caused a $35-million crabmeat impound. There were 190,000 illnesses from typhoid, *Shigella*, *Salmonella*, *E. coli* O157:H7, and Malathion, with at least two deaths. Preventative measures include properly installed devices, scheduled testing, adequate employee training, and GMPs. Mandatory reporting of all incidents is not presently required. An important tool is the field survey. A survey of six milk plants identified 210 plumbing defects, 135 back-siphonage and 43 backpressure potential sites. This research determined that surveys represented one of the most pro-active health-related prevention activities for the entire century.

T42 Implications of Flies, Pathogens and Public Health Risks

JERRY BUTLER, James Maruniak, and Frank Meek, University of Florida, Entomology and Nematology Dept., Bldg. 970, Natural Area Drive, P.O. Box 110620, Gainesville, FL 32611-0620, USA

Food processing and foodservice settings provide perfect environments for flies to be attracted to and thrive: unlimited harborage, food, water and a never-ending stream of traffic. A 2001 study conducted by University of Florida professors Jerry F. Butler and James Maruniak confirms that flies are a more serious threat to food safety than many people think. In fact, three new pathogens were discovered in and on flies in the study, including one linked to meningitis. For the study, flies were collected from around the rear entrances and dumpsters of four Florida restaurants. The study's results, which were confirmed by two independent labs, probably apply to flies across the country. The study found bacteria in or on common house flies are linked to meningitis, pneumonia, upper respiratory diseases, pulmonary disease in infants, food poisoning, intestinal infections, diarrhea, typhoid fever, peritonitis and bloodstream infections. These organisms are also prime potential biological weapons for terrorists, according to a recent CDC Health Alert. This presentation will address commonsense operational as well as control measures that food industry operators can implement to limit the potential public health and liability risks posed by flies.

T43 Food Safety and Security: Operational Risk Management Systems Approach

LARRY BARRETT, US Air Force and California Dept. of Health Services, P.O. Box 942732, MS 419, Sacramento, CA 94234-7320, USA

Deliberate food contamination by terrorists is a real threat and should be assessed and handled accordingly. The Center for Counterproliferation Research reported that over 1/3 of the 16 confirmed cases of bioterrorism and 31 confirmed biocrimes

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committed in the United States (up to 1998) involved food or other agricultural products. The nation currently has a food supply that is safe from incapacitating agents. Action must now be taken to develop food safety and security from intentional contamination with lethal agents. Food can be contaminated at any point from the farm to consumption with microbial, chemical, radiologic, and physical agents. The United States must develop a national strategy to prevent or eliminate vulnerability to attack and diminish the impact of any potential attack against the nation's food supply. A guide using operational risk management (ORM) as a tool to conduct risk assessment and risk management of potential food safety and security hazards from farm to fork has been developed by the United States Air Force. Due to the Air Force's support for national food safety and security and homeland defense, they allowed their document to be used as a model by the US Food and Drug Administration for development of national guidelines. ORM is an organized process that can be used to prioritize and manage risk in food safety and security and can become an integral component of the nation's counterterrorism strategy to both stop and reduce threats before they occur in the food supply. This presentation will review threats and the ORM process.

T44 DSC The Food Safety Network: A Model for Scientific Risk Management and Public Engagement

BENJAMIN CHAPMAN and Douglas Powell, University of Guelph, Dept. of Plant Agriculture, Guelph, Ontario N1G 2W1, Canada

The Food Safety Network (FSN), at the University of Guelph, is a group dedicated to the development and implementation of scientific and publicly credible policies and programs to enhance the safety of the food supply, including the provision of information about risks associated with food and communicating them in a compelling manner. The objectives of this research were to examine the FSN and produce a model for food safety risk management and communication that could be used as a tool when addressing food risks. Case studies, media analysis, surveys and interviews were used during the construction process of the FSN model. Both the proactive and reactive nature of FSN activities was incorporated into the model design, including the rapid electronic communication listservs, development of on-farm food safety programs, and a model farm agriculture education project. The model has been established as a set of generalized flow charts that can be adapted to specific issues by risk managers to produce action plans. The construction and evaluation of the FSN model will help risk managers and decision makers engage a variety of audiences regarding food safety risks and new technologies.

T45 Comparison of the Linear and Nonlinear Models of Thermal Inactivation of Milkborne Microorganisms

C. R. LOSS and J. H. Hotchkiss, Cornell University, Dept. of Food Science, 160 Stocking Hall, Ithaca, NY 14853-7201, USA

Conventional characterization of thermal inactivation of food borne microorganisms is based on a log linear model. *D*-values derived from this model have been used to specify time-temperature treatments to inactivate pathogens. However, model system work suggests that thermal inactivation may be nonlinear (i.e. inactivation rate changes over time), and not accurately represented by a log linear function. The Weibull function is an exponential nonlinear model that has been used to predict complex system failure. The Weibull and other nonlinear models may more accurately characterize thermal inactivation. We have compared log linear and nonlinear models of thermal inactivation for a common milk spoilage organism (*Pseudomonas fluorescens*) and a milk borne, thermoduric, spore forming pathogen (*Bacillus cereus*). The R^2 value for the log linear model was lower (0.83) than for the nonlinear (Weibull) model (0.93) of inactivation of *P.* fluorescens at 50°C. Both models, when used to characterize inactivation of *B. cereus* spores (89°C), fit the data equally well ($R^2 = 0.97$). A lack of difference in fit for the two models for spores as compared to the difference in fit for vegetative cells may reflect different inactivation mechanisms (single-hit versus multi-hit kinetics). Thermal inactivation (63°C), of mixed cultures found in raw milk, fit the Weibull model better ($R^2 = 0.92$) than the log linear model ($R^2 = 0.42$). These results support reports from model systems suggesting that linear thermal inactivation models should be reinterpreted and that a nonlinear model, such as the Weibull, may more accurately describe thermal inactivation.

T46 A Quantitative Microbial Risk Assessment Model for Processed Postchill Broilers

IRA ZAKARIADZE and Yanbin Li, University of Arkansas, Dept. of Food Science, O-413 Poultry Science Bldg., Fayetteville, AR 72701, USA

Salmonella Typhimurium has been identified as one of the major pathogenic bacteria found in poultry. It is estimated that there is an annual 1.4 billion dollar *Salmonella*-related cost in human productivity lost and medical expenses in the US. This model is for predicting microbial hazard during postchill broiler processing and providing poultry companies with advanced tools to assist them in development of HACCP programs. The objectives of this study were to construct a quantitative microbial risk assessment and a computer simulation model of *S.* Typhimurium for postchill broiler processing and generate statistical results and distribution of the risk associated with the different aspects of broiler

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processing. The model contains the following nodes: Whole Bird, Cutup, Deboning, Skinning, Cooking, Packaging, Storing. Different data sources were searched to improve the predictive models (Food MicroModel, USDA Pathogen Modeling Program). The model linked different node operations to estimate the overall impact of *S.* Typhimurium on processed broiler products. As a result of primary modeling and simulation, changes in microbial concentration and prevalence were evaluated during the defined processing operation. The simulation results showed that probability frequency distribution for *S.* Typhimurium prevalence during Cutup and Deboning increased ($P = 0.674$ and 0.784). Cumulative distribution of *S.* Typhimurium on broilers after Cooking fell in the lowest range of 0.197, but significantly increased during Packaging ($P = 0.769$) due to cross contamination. The model was able to predict probability of microbial hazards occurring during processing and assist in development of HACCP programs at the broiler processing plants.

T47 DSC Food Safety and Control Standards in Food Manufacturing Premises in Wales

GORDON HAYBURN, Chris Griffith, and Adrian Peters, University of Wales Institute-Cardiff, Food Research and Consultancy Unit, School of Applied Sciences, Colchester Ave., Cardiff, Wales CF23 9XR, UK

In 2001, the United Kingdom Retail Food Sector had an approximate value of £80 billion, three-quarters of which was controlled by five "multiple retailers". Many manufacturers regard the supply, particularly of "retailer branded" products, a key business priority. A drawback of this supply is that legal liability lies with the retailer. The "British Retail Consortium Technical Standard and Protocol for Companies Supplying Retailer Branded Food Products" was developed by a co-operative of the major retail multiples and other parties, as a minimum food safety and control level, with auditors having no vested interest in the outcome. A random sample of food manufacturers, based in Wales, were audited against the BRC Technical Standard. Initial results indicate a range of standards, with approximately 25% of companies already achieving the standard, with others falling short in developing required controls. 80% of failures were due to inadequate HACCP plans or Quality Management Systems. Almost 90% of companies had satisfactory standards of premises and equipment. While visual hygiene standards appeared acceptable during the audits, monitoring and recording of cleaning standards were not effective in almost 20% of factories. Training was significant, a key finding being that the higher the level of Technically trained staff, the greater the level of compliance. Results will be discussed in relation to the influence of the multiple retailers, their impact on international companies supplying the UK market, and the ongoing developments of the UK Food Standards Agency in implementing higher levels of food safety controls in the food manufacturing sector.

T48 The Perceived and Actual Cost of Quality Failures in the Welsh Food Manufacturing Sector and Links with Food Safety Management

DAVID LLOYD and Chris Griffith, University of Wales Institute-Cardiff, Food Research and Consultancy Unit, Colchester Ave., Cardiff, Wales CF23 9XR, UK

For economic reasons, food manufacturers have adopted standardised systems for quality and safety management. Product quality failure represents major financial losses for food manufacturers. The aim of the research was to evaluate and quantify the scale of these losses in a representative sample of Welsh food manufacturing companies and to evaluate potential links with the efficacy of their food safety management systems. 610 food companies in Wales were sent a questionnaire to establish perceived quality failures and likely costs to the business, as well as HACCP efficiency. The quality losses, final customer complaints, Overall Operating Efficiency (OEE) and HACCP plans of twenty selected companies were audited to evaluate any links between operational and food safety management efficiency. 91% of manufacturers under-estimated their losses through quality failures and the impact on their business' profits. 66% of companies had an 'actual' cost of failure greater than 100% above the perceived figure. The highest area of losses were in internal failures, most significantly processing waste (35% of financial losses), fill weights of products (26% of losses), and reject product (17%). Customer complaint costs were significantly higher than perceived costs; in 15% of companies these were over 30 times the value anticipated. Results from the HACCP audit (number of corrective actions required and failure to adhere to monitoring frequency) indicated that 88% of companies would fail to mount an effective 'due diligence' defence. Of the five companies performing most poorly in the 'quality' audit, four appeared in the worst five companies for HACCP performance. The research links quality, production efficiency and food safety performance within the companies studied.

T49 Microbiological Levels in Warewash Machines Used in Foodservice Establishments

EVA STÅHL WERNERSSON, Håkan Håkanson, and Inger Lindvall, Granuldisk AB, Jägershillgatan 15, Malmö 213 75, Sweden

An evaluation of four warewash machines, at four different foodservice establishments, was conducted by means of microbiological methods. Aerobic Plate Count (APC) and analysis of *Bacillus cereus* and Enterobacteriaceae examined the natural microflora,

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after one day of washing. The growth of microorganisms in the dish solutions was examined after 24 and 48 h, using model experiments. These results were compared to the natural microflora and model experiments for tap water used for rinsing the wares. pH, conductivity and filtration determined the physical state of the dish solutions. The type of warewash machine examined, GD90, utilizes granules together with water and detergent as cleaning media. Granules are kept in the machines 24 h per day even when the machines are not running. There could be an increased microbiological risk to this type of machines due to granules. To evaluate this risk, granules were put in different dish and tap water solutions and the levels of microorganisms were determined. The level of APC in dish solutions, tap water and model samples was less or equal to levels required for microbiological quality of foodstuffs; $<10^5$ to 10^6 CFU/g. *B. cereus* were found at levels $>10^3$ CFU/ml, mainly in dish solutions samples. According to APC, *B. cereus* and Enterobacteriaceae analysis, there was no growth of microorganisms among the granules that had been in contact with dish water solutions after 24 or 48 h. Tap water samples showed presence of Enterobacteriaceae among the granules, verifying the influence of pH on slowing down microbiological processes in dishwashers and warewashers in general.

T50 Hygienic Practices Evaluation at Homes in Xalapa City, Veracruz, Mexico

PAOLA SABINA CONTRERAS ROMO, Laboratorio de Alta Tecnología de Xalapa, S.C., Calle Médicos no. 5 Col. Unidad del Bosque, Xalapa, Veracruz 91020, Mexico

The World Health Organization establishes illness resulting from contaminated food as one of the most widespread health problems; whereas several organizations in the US have long observed the role of unsafe practices in the home, in developing countries, attention to these kinds of practices is just beginning. Generally speaking, most of the steps and habits in food preparation are transmitted by oral tradition; however, their efficiency and safety is a matter for study. Therefore, it became of interest to us to compare FDA safety guidelines and the opinions of food safety specialists with common practices in our town. In different locations in Xalapa City (population: 270,000), 300 randomly assigned written questionnaires explored main practices in cleaning of installations, cooking procedures, and hygienic practices. Results showed generally safe practices in food preparation. Nevertheless, we found deficiencies in cleaning habits, especially in the frequency of kitchen cleaning (46.7% clean it only before cooking); bathroom cleaning (38.5% do it twice a week); dish sponge changing (59.1% wait one month or until the sponge wears out); kitchen hand towel laundry (37.9% wash them from one to three times a week); cutting boards (74.7% use wood surfaces); time invested for hand washing (48.2% invest one min); and use of lesser-contaminating cleaning products (83.4% use detergent, 78.1% use chlorine and 56.6% use synthetic pine products). It is concluded that a lack of information about food safety in the home leads to a need to identify other hazardous practices and the proposal to modify cultural habits towards safety practices.

T51 Bacterial Populations Associated with Water in Vending Machines

JAYNE DRAKE, Adrian Peters, Louise Fielding, and Mike Saltmarsh, University of Wales Institute-Cardiff, Food Research and Consultancy Unit, Colchester Ave., Cardiff, Wales CF23 9XR, UK

Chilled drink vending systems take drinking water, pass it through small bore tubing and chill it to around 4°C. Water may be dispensed alone or mixed with syrups or powders to form a soft drink. Bacteria from the drinking water supply will attach to tubing surfaces and contribute to the bacteriological quality of the dispensed drink. Vending system operators following codes of Good Practice have a duty to assess potential hazards and control measures. There is a lack of authoritative data on the levels and types of bacteria in vended chilled water and this hampers the risk assessment process and the development of realistic microbiological criteria for the industry. This paper examines the populations of bacteria associated with vended chilled drinks. Water and swab samples were taken from 50 vending installations and analysed for aerobic heterotrophs at 22 and 37°C; coliforms, *Escherichia coli* and *Pseudomonas* spp. isolates were identified by biochemical characterization. Levels of aerobic heterotrophs and pseudomonads in water from vending systems were often higher than those normally reported from mains drinking water. Most organisms isolated were gram negative, oxidase positive rods, with a significant number of pseudomonads. Coliforms and *Escherichia coli* were not commonly isolated from water samples but were sometimes detected from swab samples. While there is no evidence that bacterial levels in vended drinks poses a health risk to the consumer, there is a need for realistic microbiological criteria. This paper assists the industry in implementing HACCP-based food safety control and identifies the need for further research into the hygienic design and operation of vending machines.

T52 DSC Food Safety Education Using a Cross-disciplinary Approach and Web-based Teaching Materials

M. A. DAVIS, D. E. Conner, and W. F. Gale, Auburn University, 246 Ann Upchurch Hall, Auburn, AL 36849, USA

As part of a USDA Higher Education Challenge Grant, a cross-disciplinary, web-based course was developed. The focus of the course was food safety, and the course objective was to teach engineering principles to students in life sciences and biological principles to engineering students. Instructional material consisted of three module sites connected from a central web page. Each of these sites focused on one of three topics: the basics of agricultural

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production, introduction to engineering principles and their relation to food safety, and microbiological aspects of food safety. Course material was largely self-paced, with weekly interaction between students and teachers using e-mail, face-to-face interaction, or video conferencing. Laboratory exercises were also conducted during the first half of the semester on a weekly basis. These lab experiences, along with course material and directed homework assignments, prepared the students for a final project during the last half of the semester. This project combined students from different disciplinary backgrounds into a team to develop a safe food cooking process. Evaluation of student progress was done via graded examinations, laboratory reports, homework assignments, and group project presentations. Initially students expressed some apprehension with web-based delivery of instructional materials; however, all students (11) performed well. Moreover, the capstone team project was successfully completed. This course achieved its goal of educating students from diverse backgrounds such that they were able to use a systems approach to solve a food safety problem.

T53 External Review of an Evidence-based Web Site Containing Messages Related to Food and Water Safety for Consumers

BONNIE LACROIX and Douglas Powell, University of Guelph, Dept. of Plant Agriculture, Guelph, Ontario N1G 2W1, Canada

An external review of a test web site concerning safe food handling practices was undertaken to assess the site utility. Two focus groups, consisting of a total of nine local food and health professionals (from media, dietetics, food science and government) and trained volunteers, who work with consumers from the Guelph Community Food Advisors, a food safety and nutrition peer education program in Ontario, attended the sessions. The moderator's guide developed for the focus groups employed 24 questions in a variety of formats (both open-ended and closed questions) and incorporated several topics: food safety; the current content and format of the web site; potential users of the site; and ideas for future directions. Comments about the web site and project methodology were summarized. Main issues articulated by participants to be considered when developing a web site for consumers include accessibility, credibility, readability, and the ability to search the web site. All participants felt that the web site must contain basic information, be clearly written, and be taken from Canadian sources where possible. Using a web site with information in a central location was considered to be an effective approach to providing information necessary to reduce the physical and economic burden of food and water-borne disease. Results of the focus groups would be most useful to food professionals developing a web site for consumers.

T54 Inactivation of Foodborne Viruses by High Pressure Processing

ALVIN LEE, Michelle Bull, Cindy Stewart, Lisa Szabo, Jason Wan, John Coventry, and Martin Cole, Food Science Australia, 671 Sneydes Road, Werribee, Victoria 3030, Australia

Minimally processed foods, including bivalve molluscs and horticultural products which are typically eaten raw, are at risk to viral contamination. The aquaculture industry, for example, is one of Australia's fastest growing primary industries, equalling 25% of Australia's total value in fisheries production. The role of viruses in foodborne diseases is substantial and has been attributed to about 1/3 of the cases of non-specific gastroenteritis, and specifically 2-3 outbreaks of gastroenteritis per year have been associated with viral contamination of bivalve molluscs. High pressure processing (HPP) is a non-thermal technology that has been demonstrated to inactivate spoilage and pathogenic bacteria without significantly changing the appearance, flavor, texture or nutritional qualities of raw or fresh foods. Currently there is little published data on viral inactivation by HPP, and even fewer published studies in food systems. In this work, the efficacy of HPP for inactivation of viruses was studied by using feline calicivirus (FCV), a surrogate for non-culturable Norwalk-like viruses (NLVs). The studies were designed to produce inactivation regimes for determining appropriate process criteria for shellfish production. Pressures ranging from 200 to 600 MPa and processing times of 60 to 300 s at ambient temperature were used in buffer and oyster matrices. FCV was inactivated (5-$\log_{10}$ reduction) when a minimum pressure of 265 MPa for 90 s was applied.

T55 Extension of Produce Shelf Life following Acidified Sodium Chlorite Treatment during Processing

G. KERE KEMP, C. Cayce Warf, Chris Hawk, and Scott Musgrave, Alcide Corporation, 8561 154th Ave. SE, Redmond, WA 98052, USA

Acidified sodium chlorite (ASC) is an antimicrobial intervention, approved by FDA and EPA for application to intact fruits and vegetables prior to further processing. In a series of studies conducted in commercial processing plants, ASC was used as a replacement for chlorine or sodium benzoate in the treatment of whole fresh produce during washing. Tests were conducted on a variety of produce types including tomatoes (sliced and diced), spinach, broccoli and "spring-mix" lettuce. Evaluations of the microbial load on the produce were conducted at multiple locations on each processing line during typical operation and then again with ASC as the replacement antimicrobial. Plates were evaluated for

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total aerobic counts (APC) during processing. At the end of processing, produce from each treatment was packaged and placed in storage and then evaluated for APC plus yeasts and molds (Y&M) to assess shelf life and spoilage control. Significantly greater reductions in APC were seen following ASC treatment compared to the use of either chlorine or sodium benzoate during processing. These reductions resulted in lower APC of the final product when packaged. Evaluations of APC and Y&M during storage demonstrated a marked impact of ASC treatment on the recovery of these populations compared to those of chlorine or sodium benzoate treated produce. As a result, shelf life of the ASC treated produce was extended by several days.

T56 Application of Natural Antimicrobial Systems to RTE Food for Control of *Clostridium botulinum*

XINTIAN MING, Jeff Lambeseder, Jan Payne, and Bill King, Rhodia Foods, 2802 Walton Commons W., Madison, WI 53718, USA

The objective of this study was to develop a natural antimicrobial system for reducing the risk of *Clostridium botulinum* in Ready-to-Eat (RTE) food products. Based on in vitro demonstrations of synergistic interactions between Nisin, Lysozyme and organic acids, a novel food grade antimicrobial system was developed for RTE food such as Alfredo sauce, chicken piccata and fresh soups. The efficacy of this system was evaluated by a *C. botulinum* challenge study. The RTE foods were inoculated with a 10-strain cocktail of proteolytic *C. botulinum* spores. Aliquot samples were vacuum packed and stored at 15°C and 27°C for periodic sampling. The standard mouse bioassay was used for detecting the presence of botulinal toxin. The presence of toxin was confirmed by using ABE trivalent botulinal antitoxin. Significant antibotulinal effect of the antimicrobial system in RTE foods was observed from this study. Data demonstrated that the system significantly delayed the toxin production of the inoculated *C. botulinum* spores when incubated at elevated temperature as compared to controls. Alfredo sauce treated with the system remained toxin free for >61 days at 27°C while the control sauce became toxic on day 15. Data presented in this study indicates that natural antimicrobial systems may constitute an effective, multiple hurdle strategy for control of *C. botulinum* in RTE foods.

T57 Assessment of the Antibacterial Properties of Ozone on Aerosolized *Micrococcus luteus* Using a Bioaerosol Test Chamber

R. A. BAILEY, A. Young, L. Fielding, and C. Griffith, University of Wales Institute-Cardiff, Food Research and Consultancy Unit, UWIC, Colchester Ave., Cardiff, Wales CF23 9XR, UK

The adoption of the Biocidal Products Directive (BPD) (98/8/EC) within the European Union may lead to a reduction in the choice of commercial disinfectants. Ozone has been used for many years in the treatment of drinking water and is one of the few disinfectants that's considered not to be covered by the directive. This, along with concerns about cross contamination via bioaerosols, has led to a resurgence in interest in the use of ozone as a more general biocide. The effect of 0.05 ppm, 0.1 ppm and 2 ppm of gaseous ozone on aerosolised *Micrococcus luteus* was investigated using a purpose built bioaerosol test chamber. A stationary phase culture of *M. luteus* was aerosolised inside the bioaerosol test chamber using a collison nebulizer. Samples of the chamber atmosphere were taken at 2, 5, 10, 20 and 30 min after nebulization using an All Glass Impinger (AGI) containing 20 ml of Phosphate Buffered Saline (PBS). The AGI fluid was enumerated using a spread plate technique. Control experiments were carried out in the absence of ozone. Exposure to 2 ppm of ozone for 1 h caused the *M. luteus* aerosol concentration to be reduced to below detectable levels (< 2 CFU/ml). This indicates that there was at least a 3.9 net log reduction in the bacterial content of the aerosol ($P < 0.05$). Exposure to 0.1 ppm of ozone resulted in a 2.7 net log reduction in the aerosol bacterial concentration over 1 h ($P < 0.05$). While exposure to 0.005 ppm ozone still gave a 1.2 net log reduction after 1 h exposure ($P < 0.05$). The results indicate that ozone may be used continuously at low concentrations or in very high concentrations for short periods of time to reduce the level of aerosolized bacteria in food production areas.

T58 Evaluation of a Foaming High Retention Peracetic Acid System

CRYSTAL A. NESBITT, Kenneth E. Kellar, Joseph C. Richards, and Mark A. Weiss, FMC Corporation, Box 8, Route 1 and Plainsboro Road, Princeton, NJ 08543, USA

For open plant sanitization, quaternary ammonium compounds (quats) are commonly used sanitizers that are also capable of generating foam. Foam is advantageous because it provides a visual application marker and increases surface retention time for enhancing biocidal activity. However, foam generated by quats dissipates very rapidly, and quats generally do not perform well in the presence of organic loads. Furthermore, surfactants added to other sanitizers with improved tolerance to organic loads, such as peracetic acid (PAA), generate foams that readily break apart and do not provide extended, uniform coverage. A foaming system incorporating PAA was developed to provide high retention foam and to reduce the odor and irritation associated with PAA. This high retention system (HRS) was evaluated in a commercial food plant against both quats and liquid PAA. Total aerobic plate counts and selective *Listeria* testing were performed on samples taken from various locations within the plant. The HRS outperformed quats in twelve of the fourteen tested locations, and outperformed liquid PAA in twelve of seventeen tested locations. Additionally, even at 300 ppm PAA, the odor and irritation was virtually

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eliminated by the HRS. This will allow PAA, a much more effective and environmentally friendly biocide than quats, to be used for open plant sanitization.

T59 Decontamination in the Food Industry Using Ozone

L. M. FIELDING, L. Creed, R. A. Bailey, C. G. Griffith and A. C. Peters, University of Wales Institute-Cardiff, Food Research and Consultancy Unit, School of Applied Sciences, Colchester Ave., Cardiff, Wales CF23 9XR, UK

All food processing areas are susceptible to surface biofilm formation. These surfaces may be food contact surfaces, such as equipment, or environmental surfaces such as the walls and floors of a processing plant. Biofilms pose problems for the food processor in terms of cleaning and disinfection. Food contact equipment tends to be the focus of cleaning and environmental surfaces are often overlooked, although they form the first stage in protocols for elimination of *Listeria*. The use of gaseous ozone as a surface disinfectant was assessed in a bioaerosol test chamber and in a cheese production plant. A bioaerosol test chamber constructed at UWIC included an ozone generator capable of emitting variable, controlled concentrations of ozone. The effect of 0.1 and 2 ppm of ozone against *Micrococcus luteus* attached to stainless steel coupons was investigated. There was a significant reduction in the number of organisms on the coupons at 2 ppm but not 0.1 ppm. The orientation of the coupons (horizontal, vertical or inverted) made little difference to the efficacy of ozone. Ozone was used in situ in a cheese-making plant over four months, at levels of 2 ppm emitted overnight. The levels of Enterobacteriaceae and, to a lesser extent, the aerobic plate count showed a downward trend with the continued use of ozone. When use was discontinued the levels of Enterobacteriaceae and the aerobic plate count in both the environment and the food product increased almost immediately. The results will be discussed in terms of the potential applications of ozone.

T60 Efficacy of an Acidified Sodium Chlorite In-home Antimicrobial Spray on Produce

G. KERE KEMP and Keith Schneider, Alcide Corporation, 8561 154th Ave. SE, Redmond, WA 98052, USA

While several effective technology options exist today to assist in the control of pathogens and spoilage organisms on produce (raw agricultural commodities) in the processing industry, no similar such options have been available to the consumer in the home. An acidified sodium chlorite (ASC) process was recently developed for just such a use. As a part of this development, evaluations of the efficacy of the new system were conducted versus pathogens on various produce types. In one example of this testing an artificial inoculation model was utilized in which tomatoes and apples were contaminated with a 5-strain cocktail of *Salmonella* spp., which were allowed a period of attachment and then treated either with DI water or with ASC at three different doses (500, 850 & 1200 ppm). Treatments were applied via a hand-held trigger-activated spray bottle. Following treatment, a standardized rinse and recovery process was followed. After DI water rinsing, residual populations of 5.50 $\log_{10}$ and 5.30 $\log_{10}$ were recovered from the surfaces of the tomatoes and apples respectively. In comparison, treatment with ASC achieved maximum reductions of 4.27 $\log_{10}$ (tomatoes) and 4.29 $\log_{10}$ (apples). No discernible differences in reduction were noted between the three doses tested. During the same test series, a commercially available "produce rinse" was also evaluated using the same inoculation model. Compared to ASC, this rinse process was relatively ineffective, achieving reductions of only 1.97 $\log_{10}$ and 1.61 $\log_{10}$ for tomatoes and apples respectively.

T61 DSC Comparison of Intervention Technologies for Reducing *Escherichia coli* O157:H7 on Beef Cuts and Trimmings

J. R. RANSOM, K. E. Belk, J. N. Sofos, J. A. Scanga, and G. C. Smith, Colorado State University, Dept. of Animal Sciences, Room 6, Ft. Collins, CO 80523, USA

This study evaluated the decontamination efficacy of 0.5% cetylpyridinium chloride (CPC), 2% lactic acid (LA; 55°C), 0.02% acidified sodium chlorite (ASC), 2% acetic acid (AA), 0.02 peroxyacetic acid (PAA), 1% lactoferricin B (LB), 0.001% acidified chlorine (AC) and water (W; 25°C) applied to fresh beef carcass tissue (BCT) surfaces (5 × 2.5 × 1 cm) and lean tissue pieces (LTP; 300 g) that were subsequently ground and analyzed for microbial contamination. Samples were inoculated with a five-strain composite of *Escherichia coli* O157:H7 and then immersed in the solutions for 30 s. Viable cell counts were enumerated by plating on sorbitol MacConkey agar. Overall, CPC was most effective ($P < 0.05$) and reduced bacterial populations by 4.8 log CFU/cm^2 and 2.1 log CFU/g on BCT and LTP, respectively. Of the treatments commonly used by industry, LA was the most efficacious ($P < 0.05$), as it reduced populations by 3.3 CFU/cm^2 and 1.8 log CFU/g, on BCT and LTP, respectively. Additionally, ASC, AA, PAA, LB, AC and W reduced inoculated populations by 1.9, 1.6, 1.4, 0.7, 0.4 and 1.2 log CFU/cm^2 when applied to BCT, while corresponding reductions following treatment applications to LTP were 1.8, 1.1, 1.0, 0.4, 0.5 and 0.3 log CFU/g, respectively. Results from this study indicated that LA and ASC were the most effective decontamination solutions currently approved for commercial use. Information regarding the antibacterial efficacy of decontamination solu-

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tions should prove beneficial to industry personnel as a means of improving microbiological quality of beef carcass and potentially non-intact beef tissue.

T62 DSC Effects of Dried Plum Purées on Suppression of Growth of Foodborne Pathogens in Uncooked Pork Sausage

LESLIE K. THOMPSON and Daniel Y. C. Fung, Kansas State University, 1600 Mid Campus Drive, Manhattan, KS 66506, USA

One approach to the control and prevention of foodborne pathogens is using naturally occurring food ingredients. Commercial dried plums (*Prunus domestica* cv. French) contain phenolics, such as hydroxycinnamates, especially neochlorogenic acid and chlorogenic acid, which can inhibit the oxidation of low-density lipoprotein. Studies at Kansas State University have provided information on the killing effects of phenolic antioxidants on a large group of pathogens. The objective of this study was to evaluate the efficacy of various concentrations and various contact times of dried plum mixtures in controlling *Salmonella enterica* serovar Typhimurium, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, and *Salmonella aureus* in uncooked pork sausage. The dried plum mixtures tested were dried plum purée and light bake powder (3% and 6% w/w) obtained from the California Prune Board. A cocktail solution of *S.* Typhimurium, *L. monocytogenes*, *E. coli* O157:H7, *Y. enterocolitica*, and *S. aureus* was added to uncooked pork sausage with dried plum at an initial inoculation of 4 log CFU/g. The dried plum/beef mixtures were stored at 4°C. Microbial analysis was performed on days 0, 1, 3, and 5. The experiment was repeated three times. The uncooked pork (29.26 to 31.26% fat) with 6% light bake powder or dried plum purée had a 0.5 to 2.5 log suppression of total aerobic count, *L. monocytogenes*, *E. coli* O157:H7, and *Y. enterocolitica* compared to the control after 5 days. With *S.* Typhimurium and *S. aureus*, there was no significant difference between the control and treatments. The growth of foodborne pathogens can be suppressed by the addition of dried plums.

T63 Sources of *Listeria monocytogenes* Contamination in a Salmon Smokehouse and Comparison of Two Sanitizing Procedures

BIRTE FONNESBECH VOGEL, Dorthe Bagge, Kelna Gardshodn, and Lone Gram, Danish Institute for Fisheries Research, Søltofts plads, Build. 221, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark

Recent research points to contamination during processing as the primary source of *Listeria monocytogenes* in many ready-to-eat food products, including cold-smoked salmon. Some investigations have also shown that a reservoir of *L. monocytogenes* can be established in the processing plant environment. Using molecular typing methods, we have found that a particular *L. monocytogenes* strain persisted over a four year period in one salmon smokehouse. This particular strain (an in-house strain) was typically associated with the slicing machines and area. However the original reservoir was not identified. Other *L. monocytogenes* types occurred frequently, but could not be traced to specific processing steps. In 2001 we have evaluated: (1) if the distribution of *L. monocytogenes* strains detected was unchanged, (2) if the in-house strain persisted and if a reservoir could be identified, (3) if other sources of contamination could be identified and (4) if changes could be introduced to reduce or eliminate contamination of the processing plant. Environmental samples were taken with sponges before and during processing, and strains of *L. monocytogenes* were isolated and characterized by Random Amplified Polymorphic DNA (RAPD) typing. The cold-smoked salmon were mostly contaminated with RAPD types not found in 1995 to 1999. These types were found during production and in the processing environment. Contamination with *L. monocytogenes* probably occurred by transfer from the immediate processing environment to direct product contact surfaces. The in-house-strain was, however, found in the slicing processing environment, and drains constituted its reservoir. The effect of fog disinfection on the general level of hygiene and on *L. monocytogenes* was assessed in one of the slicing areas at the smokehouse. By fog disinfection the general level of hygiene (CFU/sampling site) was improved as compared to foam disinfection. However, the prevalence of *L. monocytogenes* was unchanged, but *L. monocytogenes* was only found in areas of poor cleaning.

T64 The Evaluation and Control of Biofilm of Significance to the Food Industry

ADRIAN PETERS, Karen Elvers, and Chris Griffith, University of Wales Institute-Cardiff, Food Safety Research Group, School of Applied Sciences, Colchester Ave., Cardiff, Wales CF23 9XR, UK

Bacteria readily attach to surfaces and, given suitable conditions, will grow to form a biofilm community. Biofilms develop where there are suitable surfaces, nutrient stream, and temperatures, as well as sufficient time, and the food industry provides numerous examples of such sites. Attached bacteria adapt physiologically and morphologically, giving them possible advantages over free-living bacteria. A number of in-situ investigations and laboratory studies have demonstrated the potential for biofilm formation in the food industry and have shown that pathogens can become established in biofilms. Although there is no direct evidence for biofilms compromising the safety of food products, the potential for contamination is clear. The present study examined the significance of biofilm formation in the food industry with an emphasis on the presence of foodborne pathogens within biofilm communities. High-risk processing plants were surveyed for evidence of biofilm and pathogen populations

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characterized. Sample sites were selected on the basis of their ability to support biofilm populations and sampling was carried out during a production run and following cleaning. The results suggest that proper cleaning limits the time available for bacterial attachment and growth but inefficient cleaning may allow the survival, on food contact and environmental surfaces, of biofilm populations that continue to develop over a longer period. *Listeria* sp. were isolated from 16% of food contact surfaces sampled and 33% of environmental sites. There was evidence that cleaning processes were poorly understood and implemented. Of particular concern are areas where cleaning is difficult or less frequent. Failure to validate or comply with cleaning programs can result in biofilm persistence.

T65 Comparison of Multiple Antimicrobial Resistance among *Salmonella* Isolates of Animal Origin

P. J. FEDORKA-CRAY, M. L. Headrick, B. Salamone, N. Anandaraman, B. Rose, J. T. Gray, and D. A. Dargatz, USDA-ARS, Antimicrobial Resistance Research Unit, 950 College Station Road, Athens, GA 30605, USA

Antimicrobial resistance is now a global problem. Of greatest concern is the development of multiple resistance among food borne and zoonotic pathogens. The animal arm of the National Antimicrobial Resistance Monitoring System — Enteric Bacteria (NARMS) monitors for developing resistance among *Salmonella* isolates of animal origin, particularly from cattle, chicken, swine and turkey. *Salmonella* isolates were collected from federally inspected slaughter and processing plants and veterinary diagnostic laboratory submissions. Antimicrobial susceptibility testing was conducted using the Sensititre System (Trek Diagnostics). Seventeen antimicrobials were tested. Data were analyzed for multiple resistance, defined as resistance greater than or equal to 2 antimicrobials. Overall, slaughter isolates were more susceptible than diagnostic isolates with the exception of isolates collected from chickens. Multiple resistance was observed most often among diagnostic isolates. The top ten serotypes varied widely by year between slaughter and diagnostic isolates for all sources (cattle, chicken, swine and turkey). Additionally, resistance, especially multiple resistance, varied widely among serotypes. Regardless of source, *S.* Typhimurium was both more resistant and the most multiple resistant serotype. Molecular analysis of multiple resistant cassettes will assist in further defining these isolates. These data indicate that multiple resistance is common among some serotypes of *Salmonella*.

T66 Withdrawn

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Symposium Abstracts

S01 Antibiotic Resistance in Humans and Feed Animals

THOMAS F. O'BRIEN, Brigham and Women's Hospital, Microbioloy Laboratory, Boston, MA, 02115 USA; Amita Gupta, CDC, 1600 Clifton Road, Atlanta, GA 30333, USA; Paula J. Fedorka-Cray, USDA-ARS-RRC, 950 College Station Road, Athens, GA 30605, USA; E. John Threlfall, Central Public Health Laboratory, Laboratory of Enteric Pathogens, 61 Colindale Ave., London NW9 5HT, UK; Susan F. Kortaski, Pharmacia Animal Health, Routing 7922-190-038, 700 Portage Road, Kalamazoo, MI 49001, USA; Hanne-Dorthe Emborg, Danish Veterinary Institute, Bulowsvej 27, Copenhagen V, DK-1790, Denmark

Few issues evoke as much discussion and disagreement as the use or misuse of antibiotics in feed animal production systems. Increased concern over the development of antimicrobial resistance in human bacterial pathogens that are also carried by feed animals has led many public health and medical professionals to advocate the elimination of sub-therapeutic antibiotics from food animal production. However, many veterinarians and animal production specialists are concerned that eliminating too many antibiotics will lead to poor animal health and increased bacterial pathogen loads in feed animals, and will adversely impact human health.

In this symposium, experts from the United States and Europe describe trends and changing perceptions of the issues surrounding antimicrobial resistance; discuss the possibility that *Salmonella* Newport may emerge as a resistant strain; examine data from the national monitoring system on trends and implications of multiple drug resistance; review antibiotic resistance trends in Europe; provide an update on the use of third-generation cephalosporins in animal medicine; and take a critical look at the consequence of removing subtherapeutic antibiotics from European farms.

S02 Viruses in Foods

Craig W. Hedberg, University of Minnesota, School of Public Health, 420 Delaware St. SE, Minneapolis, MN 55455, USA; Paul Allwood, Minnesota Dept. of Health, Metro Square Bldg., St. Paul, MN 55104, USA; Steve Monroe, CDC, Division of Viral and Respiratory Diseases, Mail Stop G-04, 1600 Clifton Road, Atlanta, GA 30333, USA; Lee-Ann Jaykus, North Carolina State University, Dept. of Food Science, Box 7624, Raleigh, NC 27695-7624, USA; Sagar M. Goyal, University of Minnesota, Dept. of Veterinary Diagnostic Medicine, 1333 Gortner Ave., St. Paul, MN 55108, USA; Dean O. Cliver, University of California-Davis, School of Veterinary Medicine, Dept. of Population Health and Reproduction, Davis, CA 95616-8743, USA

Human enteric viruses are the leading causes of foodborne outbreaks. In fact, human caliciviruses such as Norwalk virus (NV) and Norwalk-like viruses (NLV) are estimated to cause as many as 60% of all foodborne outbreaks. The significance of viruses in food contamination stems from the fact that the infectious dose of these viruses is fairly low and that they can survive in contaminated food for prolonged periods of time. There are two major sources of viral contamination of food: fecally contaminated water (irrigation water, shellfish growing water) that comes in contact with food and poor personal hygiene practiced by infected food handlers. Uncooked, ready-to-eat foods (fresh produce, shellfish) are more likely to be virally contaminated. Fecal indicator bacteria are useful in indicating bacterial contamination of food and water but are inadequate in determining virological safety. Although methods for bacterial detection in food are available and routinely used, methods for detection of viruses in food, with the exception of shellfish, are not available. It is important, therefore, to develop methods for direct examination of food for viruses and to explore alternate indicators that can accurately reflect the virological quality of food. This symposium will address these issues along with strategies for the prevention and control of food contamination.

S03 Development in Intervention Technologies to Enhance Produce Safety

Brian C. Hampson, California Polytechnic State University, Food Science and Nutrition Dept., San Luis Obispo, CA 93407, USA; Howard Q. Zhang, Ohio State University, 2015 Fyffe Road, Columbus, OH 43210, USA; Brendan A. Niemira, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; Dallas G. Hoover, University of Delaware, 017 Townsend Hall, Newark, DE 19176, USA; Kay Cooksey, Clemson University, 228 Poole Center, Box 340370, Clemson, SC 29634, USA; Bassam A. Annous, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Since 1991 there have been several outbreaks of foodborne illnesses associated with the presence of *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* on fresh fruits and vegetables and in unpasteurized juices. These outbreaks have led the FDA to mandate that fresh produce and juice products be treated with a process to yield a 5 log reduction in the most resistant organisms of public concern. In addition to microbiological safety, consumers are demanding that the fresh produce and juice products be processed in manner that would not alter their sensory characteristics. As a result of the FDA ruling and the consumer concerns,

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there have been considerable research effort for the development of intervention methods by which to achieve the target 5 log reduction during the processing of fresh and fresh-cut produce, and juice products, without the adverse changes to sensory characteristics. Among potential intervention technologies are: wash treatments using ozone and other antimicrobial agents, antimicrobial food packaging, irradiation, and microbial inactivation by high hydrostatic pressure and pulsed electric field.

This symposium will discuss the use of the above mentioned intervention technologies for the control of the human pathogens on fresh and fresh-cut fruits and vegetables, and/or fresh juices. The discussion will include the effects of each intervention technology on product sensory qualities and shelf-life, and factors which determine the applicability and limitations of such technology. Also, this symposium will discuss the development of novel Biosafety Level two (BSL-2) pilot plant facility at the USDA-ARS Eastern Regional Research Center. This BSL-2 facility will be used to validate intervention technologies with commercial scale processing equipment using produce inoculated with human pathogens.

S04 Safety of Latin-Style High Moisture Fresh Cheese

John E. Rushing, North Carolina State University, Box 7624, Raleigh, NC 27695-7624, USA; Susan Grayson, State of North Carolina, NCDENR/ Environmental Health Services, 1632 Mail Service Center, Raleigh, NC 27699-1632, USA; Kathryn J. Boor, Cornell University, Dept. of Food Science, 413 Stocking Hall, Ithaca, NY 14853-7201, USA; Eva Rodriguez, Tropical Cheese Industries, 450 Fayette St., Perth Amboy, NJ 08861, USA; Jack Guzewich, FDA, HFS-600, CFSAN, 5100 Paint Branch Pkwy., Room 3B-069, College Park, MD 20740-3835, USA

Foodborne illness from fresh Latin-style cheese is not new, one of the most deadly outbreaks in recent history occurred in these soft cheeses. Among several recent outbreaks, one in North Carolina in November 2000 resulted in five stillborn babies. There are several possible causes for these outbreaks. One factor is that the cheeses are often produced without pasteurization. They are produced as a cottage industry in the US and in Mexico, Central and South America. These cheeses are produced in North America both commercially and in an underground Cottage Industry. The State of Washington, in response to an outbreak, initiated a program called the *Abuela* influential project to teach members of the Latin community to make cheese using pasteurized milk. Pasteurization is not necessarily the complete answer. Many soft cheeses lack the traditional hurdles to the growth of foodborne illness organisms. Pasteurization eliminates competitive microflora.

S05 Enhancing Agricultural Security

Ed Mather, National Food Safety and Toxicology Center, Michigan State University, 165 Food Safety and Technology Bldg., East Lansing, MI, 48824, USA; Jeff Goldburg, Core Processes, Inc., 1800 N. Beauregard St., Suite 350, Alexandria, VA, 22311, USA; Paul Jonmaire, Ecology and Environment, Inc., 368 Pleasant View Drive, Lancaster, NY, 14086, USA; C. Neal Stewart, Jr., University of North Carolina, Dept. of Biology, 333 Eberhart Bldg., Greensboro, NC 27412, USA

The agricultural industry accounts for 13% of the US Gross Domestic Product or over $1 trillion worth of business annually and one out of every eight American workers is associated with agriculture or food products. A bioterrorist attack against animals, crops or food products is considered quite plausible by top US government officials since the agents for such attacks are readily available and the economic consequences quite devastating. At least 21 attacks against agriculture have been documented in the last 100 years. Most of these agents are readily available since many are naturally occurring at the farm and others have been developed as offensive weapons. Equally problematic is that developments in the farm-to-table logistics chain have increased the number of potential "entry points" for chemical or biological contamination of agricultural products. Animal diseases such as brucellosis, West Nile virus, Japanese encephalitis and Food and Mouth Disease create not only an economic and social upheaval but also endanger the lives of humans. Reaction to an agricultural bioterrorist event depends on the rapidity with which the disease is discovered and handled at the local, regional, state and federal level. Methodology for detection of agents of destruction is a rapidly evolving science with interdisciplinary expertise required from molecular biologists, food microbiologists and engineers. The ability to conduct research with pathogenic and toxic agents in a secure and responsible manner is essential and laboratory controls will continue to become stricter.

S06 Minimizing the Risk of *Salmonella* Enteritidis in Shell Eggs

Robert E. Brackett, FDA-CFSAN, Room 3B-004, HFS-32, 5100 Paint Branch Pkwy., College Park, MD 20740, USA; Richard K. Gast, USDA, Southeast Poultry Research Laboratory, 934 College Station Road, Athens, GA 30605, USA; Mark Walderhaug, FDA-CFSAN, Office of Plant and Dairy Foods and Beverages, HFS-517, 200 C St. SW, Washington, D.C. 20204, USA; Robert R. H. Davies, Veterinary Laboratories Agency, Dept. of Bacterial Diseases, Weybridge, New Haw, Addlestone, Surrey, KT15 3NB, UK; Patricia A. Curtis, Auburn University, Dept. of Poultry Science, Poultry Product Safety and Quality Program, 236 Ann Upchurch Hall, Auburn University, AL 36849-5416, USA; Brian Sheldon, North Carolina State University, Dept. of Poultry Science, Box 7608, 234D Scott Hall, Raleigh, NC 27695, USA; Shelly McKee, University of Nebraska, Dept. of Food Science and Technology, 143 Filley Hall, P.O. Box 830919, Lincoln, NE 68583, USA

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Shell eggs are remarkably resistant to bacterial invasion due to their structure and inherent antimicrobial properties; however, human infections caused by *Salmonella enterica* serotype Enteritidis (SE) are often associated with consumption of eggs or egg-containing products. Therefore, SE contamination of eggs continues to be a major food safety issue associated with egg production and processing in both the US and Europe. Minimizing the risk of SE in shell eggs entails control measures from production through processing and distribution. SE has the ability to infect hens and subsequently be transovarianly transferred to developing eggs such that an egg may be internally contaminated with SE at time of lay. Initially, SE contamination of eggs apparently arises from the egg production environment; therefore, risk factors associated with shell egg production must be considered. Once contaminated, egg handling can greatly influence the risk of SE illness to the consumer. Control measures for SE in eggs must also include temperature and process control during packing and distribution. Because temperature abuse of shell eggs can increase SE risk, rapid post-process cooling and subsequent refrigeration during distribution are recognized as potential control measures. New technologies and procedures to rapidly cool commercial shell eggs are emerging. In addition, in-shell pasteurization, which can eliminate internal SE while maintaining egg quality, has been researched and developed for commercial application. Understanding the risk factors for SE contamination and potential control measures, egg producers and processors can develop and implement effective HACCP programs to minimize SE risk to the consumer.

S07 Microbiological Food Safety at Retail

Shelly Huddle, CDC, Outbreak Response and Surveillance Unit, Foodborne and Diarrheal Diseases Branch, 1600 Clifton Road, MS A38, Atlanta, GA 30333, USA; Frank Busta, University of Minnesota, Institute Food Technology, 1354 Eckles Ave., St. Paul, MN 55108, USA; Tim Freier, Cargill, 15407 McGinty Road West, Wayzata, MN 55391-2399, USA; Sheila Cohn, National Restaurant Assn., 1200 17th St. NW, Washington, D.C. 20036, USA; Susan Sumner, Virginia Tech, Food Science and Technology (0418), Duckpond Drive, Blacksburg, VA 24061, USA; David Theno, Jack-in-the-Box, Inc., 9330 Balboa Ave., San Diego, CA 92123-1516, USA

Although the United States food supply is among the world's safest, an estimated 76 million cases of foodborne disease occur each year in the United States. While the majority of these cases are mild and cause symptoms for only a day or two, some cases are more serious. The CDC estimates that there are 325,000 hospitalizations and 5,000 deaths related to foodborne diseases each year. There has been extensive data analysis, sorting each illness by vehicle and mode of transmission and type of setting where the food was consumed (private residence, delicatessen, cafeteria, restaurant, school, picnic, church, etc.).

In previous years, a good number of symposia and technical sessions have focused on the microbial risks that food processors are challenged with. Most are aware of the burden and responsibility placed on the food processor. This symposium will take a hard look at the microbiological safety of food at retail and foodservice operations and the food safety risks that must be managed at that point. Specifically, speakers will be challenged to identify the percentage of foodborne outbreaks associated at retail/foodservice, and discuss and define "potentially hazardous foods." The production and sale of safe food is a shared responsibility, accordingly, speakers representing both suppliers as well as retail/foodservice will discuss measures that are taken to control and manage microbial risks. The symposium will focus on best practices, discussion of the Food Code, and incidence and control of pathogens and viruses at retail/foodservice operations.

S08 Extended Shelf Life Meat Products — Issues and Interventions

Bruce Tompkin, ConAgra Foods, Inc., 3131 Woodcreek Drive, Downers Grove, IL 60515, USA; Dorota M. Broda, AgResearch Limited, Food Safety Group, Ruakura MIRINZ Centre, East St., Private Bag 3123, Hamilton, New Zealand; Richard A. Holley, University of Manitoba, Dept. of Food Science, Winnipeg, Manitoba R3T 2N2, Canada; John Luchansky, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038-8598, USA; Robin Kalinowski, ConAgra Foods, Inc., 3131 Woodcreek Drive, Downers Grove, IL 60515, USA; John Sofos, Colorado State University, Dept. of Animal Sciences, Ft. Collins, CO 80523-1171, USA

Advances in extending the shelf life of refrigerated perishable products has demonstrated yet again that Mother Nature abhors a vacuum and will fill it with challenges. This symposium will offer the experience and findings of industrial, academic, government, international microbiologists in identifying, characterizing, and controlling several psychrotrophic spoilage bacteria. Among these are recently characterized *Clostridium* spp. and heterotrophic lactic acid bacteria. If released by meeting time, data on psychrotrophic pathogens from an ongoing project will be presented.

S09 Cooperating to Improve Foodborne Outbreak Investigations

Jack Guzewich, FDA, HFS-600, Center Food Safety & Applied Nutrition, 5100 Paint Branch Pkwy., Room 3B-069, College Park, MD 20740-3835, USA; Rob V. Tauxe, CDC, Foodborne and Diarrheal Diseases Branch, Atlanta, GA 30333, USA; Jeff Farrar, California Dept. of Health Services, Food and Drug Branch, 601 North 17th St. - MS 357, P.O. Box 942732, Sacramento, CA 94234-7320, USA; Mary Palumbo, California Dept. of Health Services, Food and Drug Branch, 601 North 17th St. - MS 357, P.O. Box 942732, Sacramento, CA 94234-7320, USA; Mindy Brashears, Texas Tech University, Animal Science and Food Technology, 204 E. Food Technology, Lubbock, TX 79409-2141, USA

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Investigating a foodborne outbreak involves a myriad of food safety professionals including, but in no way limited to, epidemiologists and food microbiologists. The scientists in these fields work together to identify and resolve an outbreak. The entire process from the initial identification of an outbreak through the final assessment involves an unspoken union between groups who perform various duties with a single goal. The goal is to promote and protect public health and the food supply, using science, reasoning, and practical common sense. While the scientists involved work towards a common end, they do so isolated from one another. Several successful collaborative projects exist, such as PulseNet and FoodNet; however, for the most part food safety experts in government agencies and research institutions have limited knowledge and appreciation for each other's work. Before any action can be taken or treatment provided, an initial hypothesis is formed and then tests are performed on the food in question. Epidemiological, environmental, and laboratory data are all important to an investigation, and examples of these related to recent outbreaks will be discussed in this symposium. It is important that food safety professionals work together in an attempt to understand the various roles of students and professionals involved in research and epidemiology in the investigation of an outbreak.

S10 Integrated Approaches for the Study and Control of Foodborne Pathogens in Meat and Poultry

Norman Stern, USDA-ARS, Poultry Microbiological Safety Research Unit, Russell Research Center, 950 College Station Road, Athens, GA 30605-2720, USA; Pascal Michel, Health Canada, Laboratory for Foodborne Zoonoses, FMV, Universite de Montreal, CP 5000, St-Hyacinthe, Quebec J2S 7C6, Canada; Greg Paoli, Decisionalysis Risk Consultants, Inc., 1831 Yale Ave., Ottawa, Ontario K1H 6S3, Canada; Roger L. Cook, Ministry of Agriculture and Forestry, Food Assurance Authority, ASB House, 101-103 The Terrace, P.O. Box 2526, Wellington, New Zealand; Dale Hancock, Washington State University, Dept. of Veterinary Clinical Sciences, Pullman, WA 99164-6610, USA; Vibeke Møgelmose, Danish Bacon and Meat Council, Axelborg, Axeltorv 3, DK-1609, Copenhagen V, Denmark

The integration of farm-to-plate approaches to assure food safety, based on food safety research investigating all possible routes of transmission, is becoming recognized as the only viable means of reducing foodborne illness. The need to extend the focus of intervention efforts to on-farm sources of human pathogens to food animals is clear. The challenge is how to design studies to determine important risk factors for sources of enteric zoonotic pathogens during primary production, with significance to ultimate human health burden.

This symposium has been designed to highlight how studies in Iceland, New Zealand, Denmark and the United States have approached the challenge of integrating the application of multiple disciplines to extend the study of sources and risk factors for foodborne pathogens back through the entire primary production sector (on-farm, hatchery, etc.), carrying through to consumer exposure.

This symposium has been designed to highlight how research into enteric zoonotic diseases is being coordinated, integrated and focused to enable rapid progression towards reducing the disease burden of enteric zoonoses in the human population.

S11 *Listeria* Research Update

Jeffrey Farber, Health Canada, Microbiology Research Division, Food Directorate, Banting Research Centre, Bldg. 22, Postal Locator 2204A2, Ottawa, Ontario K1A 0L2, Canada; Sophia Kathariou, North Carolina State University, Food Science Dept., 339 Schaub Hall, Raleigh, NC 27695, USA; Martin Wiedmann, Cornell University, Dept. of Food Science, 413 Stocking Hall, Ithaca, NY 14853, USA; Byron Brehm-Stecher, University of Wisconsin-Madison, Food Research Institute, 1925 Willow Drive, Madison, WI 53706, USA; Bala Swaninathan, CDC, Foodborne and Diarrheal Diseases Laboratory Section, National Center for Infectious Diseases, 1600 Clifton Road, Mail Stop C03, Atlanta, GA 30333, USA

A primary objective of the ILSI North America Technical Committee on Food Microbiology is to sponsor research targeted at improving understanding of microbial food safety hazards. In the wake of the 1998 and 2000 outbreaks of listeriosis in the United States, the committee intensified its efforts to address *the Listeria monocytogenes* problem, and, through a grant from the Grocery Manufacturers of America, funded four projects designed to improve our ability to identify *L. monocytogenes* subtypes that represent important human health hazards. In this symposium, the researchers who received this funding will report the results to date of their studies on molecular typing, detection, and enumeration of this important pathogen.

S12 Current Issues in Seafood Safety

Lee-Ann Jaykus, North Carolina State University, Food Science Dept., Box 7624, Raleigh, NC 27695-7624, USA; Marianne Miliotis, FDA-CFSAN-DVA-VMB, 200 C St. SW, HFS-327, Washington, D.C. 20204, USA; Linda S. Andrews, Mississippi State University, Experimental Seafood Laboratory, Coastal Research and Extension Center, 2710 Beach Blvd., Suite 1-E, Biloxi, MS 39531, USA; Lisbeth Truelstrup Hansen, Canadian Institute of Fisheries Technology, Dalhousie University, P.O. Box 1000, Halifax, NS B3J 2X4, Canada; Rita Schoeny, US Environmental Protection Agency, Health and Ecological Criteria Division, ML 4304, 1200 Pennsylvania Ave., NW, Washington, D.C. 20460, USA; Robert W. Dickey, FDA, Gulf Coast Seafood Laboratory, Chemical Hazards Research Unit, Dauphin Island, AL 36528-0158, USA

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Seafoods have historically been one of the leading vehicles of foodborne outbreaks among all food categories. As a diverse food category, seafoods can harbor biological, chemical, and physical hazards that may be detrimental to the consumer. Among these hazards, bacteria such as marine *vibrios* and *Listeria monocytogenes* have received much of the current interest. In addition, both naturally occurring and human-derived toxins are receiving much attention. This symposium will provide an overview of the current issues related to identification of seafoodborne hazards and methods for their control.

S13 Controlling *Clostridium perfringens* Hazards during Cooling

John S. Novak, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; O. Peter Snyder, Hospitality Institute of Technology and Management, 670 Transfer Road, Suite 21-A, St. Paul, MN 55114, USA; H. Thippareddi, Kansas State University, Dept. of Animal Sciences and Industry, 210 Call Hall, Manhattan, KS 66506-1600, USA; Vijay K. Juneja, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; Aamir Fazil, Health Canada, 110 Stone Road West, Guelph, Ontario N1G 3W4, Canada

Clostridium perfringens is a continuing concern to the food service industry. The abilities of this pathogen to form heat-resistant spores and to grow at a very rapid rate at relatively high temperatures are the major contributing factors leading to food poisoning. Spores of *C. perfringens*, which have a decimal reduction value at 99°C of 26 to 31 min, can be expected to survive the pasteurization temperatures and times used to cook or prepare foods in food-service operations. While the temperature range for growth of *C. perfringens* is 6°C to 52.3°C, rapid growth occurs between 35 and 48.9°C. The short generation time of the organism, 7.1 to 20 min in the rapid-growth temperature range, means that after the spore has germinated, fast cooling of foods is critical. Because of the potential health hazards in cooling cooked foods, the United States Department of Agriculture requires that, during cooling of certain meat and poultry products, the relative growth of *C. perfringens* should not exceed 1.0 $\log_{10}$. Researchers have developed predictive models that can be used to predict small to moderate amounts of relative growth of *C. perfringens* from spores during cooling of cooked cured and uncured meat products. The predictive equations are being used in risk assessment models for cooked meats and have been incorporated into the USDA pathogen modeling computer program available on the Eastern Regional Research Center Web site.

S14 Innovations in Retail Food Safety Management Systems and Technology

Fred Reimers, HEB Quality Assurance, 4839 Space Center Drive, San Antonio, TX 78218, USA; Tom Chestnut, Darden Restaurant Inc., 7101 Lake Ellenor Drive, P.O. Box 593330, Orlando, FL 32859-3330, USA; Richard Barnes, FDA, 15600 Fishers Lane, HFC-150, Room 1207, Rockville, MD 20857, USA; Cameron R. Hackney, West Virginia University, 1170 Agricultural Sciences Bldg., P.O. Box 6108, Morgantown, WV 26506-6108, USA; Christopher Boyles, Steritech Group Inc., 7600 Little Ave., Charlotte, NC 28226, USA

The retail food industry is no different than many other industries in the world today. It is going through a period of rapid and technology driven change. Professionals in retail food industry must not only meet the challenge of providing high quality products, exceeding consumer expectations, but they must also manage food safety concerns. Food safety and quality assurance managers and directors are driven by the desire to ensure safe food for all customers and consumers while at the same time protecting good will, reputation and brand. These individuals must harness the available science, technology tools and the best practice into the culture of the organization. Creating adult commitment and behavior change coupled with monitoring and corrective action are all essential cornerstones of a successful food safety initiative.

Recent technological advances have given retail personnel many choices to improve temperature monitoring, record keeping practices and training procedures. This symposium will discuss current innovations and proven strategies in retail food safety that include HACCP, computer food safety devices, regulations, and training. Those attending this session will be provided with recommendations, guidelines, and practices to help ensure the highest level of food safety and sanitation possible in both retail and food service operations.

S15 Alternatives in Dairy Waste Management: Create New Products or Generate Power!

Mark Fosshagee, World Water Works, 143 Old River Road, Edgewater, NJ 07020, USA; Mark D. Johnson, Gannett Fleming, 6 Pleasant St., Malden, MA 02148, USA; Clay Detlefsen, International Dairy Foods Association, 1250 H St., NW, Suite 900, Washington, D.C. 20005, USA; Michael Marsh, Western United Dairymen, 1315 K St., Modesto, CA 95354, USA; F. Michael Byers, USDA, 10200 Baltimore Ave., Room 108, Beltsville, MD 20705-0000, USA

Waste management issues continue to provide the dairy industry with some of its greatest challenges. Tighter environmental controls continue to drive the need for new technologies and strategies for process-

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ing waste streams from both farms and processing plants. Energy costs are of concern and may at the same time provide a partial solution through conversion of waste streams to energy. Separation technologies hold the key to fractionating waste streams into useable and valuable components for further appropriate processing. This symposium focuses on waste management issues in the dairy industry. Speakers will address both farm and processing plant issues. Topics covered will include; power generation, nutrient management and pathogens, separation technologies, product recovery and reuse issues.

S16 Chronic Wasting Disease and Other Transmissible Spongiform Encephalopathies

Dean O. Cliver, University of California, VM:PHR, One Shileds Ave., Davis, CA 95616-8743, USA; Katherine O'Rourke, USDA-ARS, P.O. Box 646630, 3003 ADBF, Pullman, WA 33164-6630, USA; Suzette A. Priola, Rocky Mountain Laboratories, NIH, NIAID, Laboratory of Persistent Viral Diseases, 903 South 4th St., Hamilton, MT 59840, USA; Elizabeth S. Williams, University of Wyoming, Veterinary Science, State Veterinary Laboratory, 1174 Snowy Range Road, Laramie, WY 82070, USA; Lynn H. Creekmore, USDA, 4101 Laporte Ave., Fort Collins, CO 80521, USA

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative disorders that affect both humans and animals. The opening presentation of this symposium will include an overview of the recognized animal and human TSEs.

There are three animal TSEs in the United States, (a) chronic wasting disease (CWD) in deer and elk, (b) scrapie in sheep and goats, and (c) transmissible mink encephalopathy (TME). Bovine Spongiform Encephalopathy (BSE), commonly known as "mad cow disease" was first diagnosed in cattle in the United Kingdom in 1986. Since then, BSE has been identified in cattle from a number of European countries and Japan, but has not been found in cattle in the United States. The beef industry and the regulatory agencies have taken steps to prevent BSE from occurring in this country in the future.

Chronic wasting disease (CWD) occurs in wild deer and elk in sections of Colorado, Nebraska, Wyoming and Wisconsin. CWD has also been identified in captive herds of elk in five states and Canada. Current post mortem detection methods used to identify CWD in deer and elk will be discussed in this symposium, as will the potential for developing a reliable live animal test. The pathogenesis, mode of transmission, and the progression of CWD in deer and elk are as yet unestablished. In vitro and in vivo models are being used as research tools to resolve these unknown questions. The status of this research will be presented. Epidemiologic studies of CWD in wildlife are being conducted to determine the extent of the problem and assess if there is a potential for CWD to cross over to cattle.

Scrapie is a fatal degenerative disease affecting sheep and goats. The first case of scrapie in the US was identified in 1947. Transmissible mink encephalophy (TME), a rare TSE of ranch-raised mink, was also first identified in 1947 in the US. A discussion on the current status of both TSEs will be presented in this symposium.

A roundtable discussion will conclude this session on animal TSEs.

S17 Applications of DNA Chip Technology in the Food Safety Area

Neil Winegarden, University Health Network, Microarray Centre, 101 College St., 5th Floor MBRC, Toronto, Ontario M5G 1L7, Canada; Bala Swaminathan, Foodborne and Diarrheal Diseases Branch, CDC, Mail Stop C07, 1600 Clifton Road, Atlanta, GA 30333, USA; Gianfranco de Feo, Affymetrix, Inc., 3380 Central Expressway, Santa Clara, CA 95051, USA; Andrew Benson, University of Nebraska, Dept. of Food Science and Technology, 330 Food Industry Complex, Lincoln, NE 68583-0919, USA; John Nash, National Research Council of Canada, Pathogen Genomics Group, Institute for Biological Sciences, Room 3135, 100 Sussex Drive, Ottawa, Ontario K1A 0R6, Canada; Jeff Farber and Franco Pagotto, Health Canada, Tunney's Pasture, Banting Research Center, Postal Locator 2203G3, Ottawa, Ontario K1A 0L2, Canada

Genetic techniques have become widely used for studying foodborne microbial pathogens. However, traditional methods in molecular biology generally work on a "one gene in one experiment" basis, so that the throughput is very low and the information one can obtain about a particular organism can be fairly limited. Over the past several years, a new technology, called DNA microarray, has attracted tremendous interest among biologists. Terminologies that have been used in the literature to describe this technology include biochip, DNA chip, genome chip, DNA microarray, and gene array. This technology promises to monitor the whole microbial genome on a single chip, and in so doing create new and exciting opportunities in fields such as bacterial taxonomy, methodology, pathogenesis, regulation and control. Thus information on whole genome sequences has the ability to change our approach to food analysis. This symposium will endeavor to provide food safety professionals with the knowledge to evaluate the impact of DNA chip technology. What exactly are "DNA chips" to begin with? How can they be used in diagnostics? What are they actually being used for today? As examples, we'll see how DNA chips are currently being applied in the food safety area, and in particular to the investigation of foodborne pathogens such as *Campylobacter* and *Listeria* spp.

S18 Sanitary Design of Plants and Equipment

Donald Graham, 14318 Aitken Hill Court, Chesterfield, MO 63017, USA; Joe Stout, Kraft, NF135, 3 Lakes Drive, Northfield, IL 60093, USA; William Schwartz, NSF, 789 Dixboro Road, Ann Arbor, MI 48105, USA; Tim Freier, Cargill, 15407 McGinty Road West, Wayzata, MN 55391-2399, USA; Bruce Paulson, Evapco, 1234 Brady Blvd., Owatonna, MN 55060, USA; Jeffrey Banks, Qualicon, 3531 Silverside Road, Bedford Bldg., Wilmington, DE 19810, USA

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There are numerous intervention strategies that a food processor can use to produce safe food, including heat pasteurization, irradiation, refrigeration, formulation, and even cleaning and sanitation. Well-designed production facilities and equipment will greatly enhance the efficiency and efficacy of cleaning and sanitation activities, thus reducing the risk of producing contaminated product. This symposium will address many of the key areas of sanitary design of food plants and equipment. The session will begin with a general guide and sanitary plant and equipment design. Further discussions will relate sanitary design to product quality, discuss hygienic design of meat and poultry processing equipment (NSF Standard 14159), explore recent improvements to the sanitary design of conveyors and air handling systems, and provide a European perspective on hygienic plant and equipment design. Whether building a new plan, remodeling an existing plant, purchasing new equipment, or simply repairing existing structures or equipment, the audience will receive timely, practical information that can be implemented in their own facilities to advance food safety and quality.

S19 Risk Assessment of Food Workers' Hygiene Practices and Intervention Strategies

Barry Michaels, Georgia-Pacific Corporation, P.O. Box 919, Palatka, FL 32178-0919, USA; Chris Griffith, University of Wales Institute, Food Research and Consultancy Unit, Colchester Ave., Cardiff, Wales CF23 9XR, UK; Sabah Bidawid, Health Canada, Banting Research Center, Postal Locator #2204A2, Room 435 Ross Ave., Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada; Don Schaffner, Rutgers University, Food Safety Initiative, 65 Dudley Road, New Brunswick, NJ 08901-8520, USA; Lee-Ann Jaykus, North Carolina State University, Dept. of Food Science, Box 7624, Raleigh, NC 27695-7624, USA; Deborah Clayton, University of Wales Institute, Food Research and Consultancy Unit, Colchester Ave., Cardiff, Wales CF23 9XR, UK

Infected food workers play a significant role in the transmission of foodborne illness caused by bacterial, viral and parasitic pathogens. Although it is widely believed that hygiene practices can be devised in pre-requisite programs or HACCP plans that will prevent contamination by food workers, there is no agreement on precisely which intervention measures should be employed that would have optimum benefit. This symposium reviews outbreaks, attributed to the ill or asymptomatic food workers, to identify hazards responsible for foodborne illness outbreak morbidity and mortality. It examines the various routes of exposure and quantifies risk associated with various food-handling activities through presentation of various transmission route exposure models. The results of various personal hygiene intervention measures are explored for the development of preventive management strategies designed to improve food handling practices. Use of appropriate microbial dose response relationships, and recognition of the various data gaps and areas where there is considerable variability and uncertainty, is discussed with respect to worker, food product, preparation process, host susceptibility and agent pathogenicity. The applicability of various intervention and training approaches is reviewed by examining what is known about the psychological aspects of hygiene training and use of social marketing approaches to achieve these goals. Insights gained from this symposium can be used in developing quantitative risk assessments intended.

S20 Customized Approaches to Microbiological Risk Assessment

Jorgen Schlundt, World Health Organization, Ave. Appia 1211, Geneva 27, Switzerland; Richard Whiting, FDA-CFSAN, HFS-300, 5100 Paint Branch Pkwy., College Park, MD 20740, USA; Serve Notermans, TNO Nutrition and Food Research Institute, P.O. Box 360, 3700 AJ Zeist, The Netherlands; Tom Ross, University of Tasmania, School of Agricultural Science, GPO Box 252-54, Hobart, Tasmania 7001, Australia; Leon Gorris, Unilever, SEAC – Risk Analysis Group, Colworth House, Sharnbrook, MK44 1LQ, UK; Aamir M. Fazil, Health Canada, Health Protection Branch, 110 Stone Road West, Guelph, Ontario N1G 3W4, Canada

The concept of Risk Analysis, and within that also Microbiological Risk Assessment (MRA), has received much interest in recent years. Governmental bodies (WHO/FAO; FDA/USDA; Health Canada) have set the scene for MRA studies that cover the complete chain. It has been recognized that "full blown" MRA studies are not always possible (lack of critical data) or necessary (when only the food processing/ handling links are of interest). Also, governments and industries have different needs for MRA. Therefore, different approaches to Microbiological Risk Assessment have been developed that fit their purpose, which together start to form a MRA toolbox. This symposium offers an opportunity to discuss some of such alternatives.

S21 Control of *Escherichia coli* O157:H7 in Cattle

Francisco Diez-Gonzalez, University of Minnesota, Dept. of Food Science and Nutrition, 1334 Eckles Ave., St. Paul, MN 55108, USA; Jeffrey T. LeJeune, Ohio State University, Food Animal Health Research Program, OARDC, 1680 Madison Ave., Wooster, OH 44691-4096, USA; Robin Anderson, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit, 2881 F&B RD, College Station, TX 77845, USA; Mindy Brashears, Texas Tech University, Dept. of Animal Science and Food Technology, Lubbock, TX 79409, USA; Andrew A. Potter, Veterinary Infectious Disease Organization, 120 Veterinary Road, Saskatoon, Saskatchewan S7N 5E3, Canada; Denis O. Krause, CSIRO Livestock Industries, Long Pocket Laboratories, 120 Meiers Road, Indooroopilly, QLD 4068, Australia

Gastrointestinal infections caused by *Escherichia coli* O157:H7 are a major public health problem and

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the detection of this foodborne pathogen in ground beef causes major losses to the meat industry. Ground beef is frequently associated with enterohemorrhagic diarrhea, but other foods and water have also been vehicles for transmission of *E. coli* O157:H7. Serotype O157:H7 and other shiga toxin-producing *E. coli* (STEC) are frequent inhabitants of the gastrointestinal tract of cattle, but these pathogens do not normally cause any symptoms in ruminants. Strain O157:H7 is released into the environment via fecal shedding and can be spread to carcasses, water and crops via manure contamination. A variety of solutions have been implemented throughout the food supply to reduce the incidence of disease, and strategies focused to reduce its prevalence in cattle are essential for a complete eradication of *E. coli* O157:H7 and other STEC. This symposium provides a survey of the different methods that are currently being developed to inhibit the colonization of the intestinal tract of cattle by these bacteria. The presenters will cover topics on dietary manipulations, cattle immunization, competitive exclusion, control of drinking water, feed supplements, and chlorate treatment. For each topic a review of the present knowledge and the latest findings on the method's feasibility will be presented.

S22 Current Practices in Produce Safety

Bob Gravani, Cornell University, Dept. of Food Science, 11 Stocking Hall, Ithaca, NY 14853-7201, USA; Mahipal Kunduru, Dole Fresh Vegetables, Inc., R&D Center, P.O. Box 1759, Salinas, CA 93902, USA; Trevor Suslow, University of California-Davis, Dept. of Vegetable Crops, Mann Lab, One Shields Ave., Davis, CA 95616-8631, USA; Joe Furuike, Driscoll Strawberry Associates, Inc., P.O. Box 50045, Watsonville, CA 95077-5045, USA; Frances Pabrua, Fresh Advantage, P.O. Box 80599, Salinas, CA 93912, USA

The risks of biological, physical, or chemical contamination are present at many phases of fresh fruit and vegetable production. The objective of this symposium is to discuss the impact of growing practices on the food safety of produce. Industry and university experts will present and share current knowledge regarding the application of "Good Agricultural Practices" for pre and post harvest produce.

S23 Food Safety Education Update

Christine Bruhn, University of California-Davis, Center for Consumer Research, 1 Shields Ave., Davis, CA 95616-8598, USA; O. Peter Snyder, Jr., Hospitality Institute of Technology and Management, 670 Transfer Road, Suite 21A, St. Paul, MN 55114, USA; Pete Friedman, ACH Food Companies, 7171 Goodlett Farms Pkwy., Cordova, TN 38018-4909, USA; Robert B. Gravani, Cornell University, Dept. of Food Science, 11 Stocking Hall, Ithaca, NY 14835-7201, USA; Judy Harrison, University of Georgia, Cooperative Extension Service Room 204, Hoke Smith Annex, Athens, GA 30602, USA; Laura Fox, FDA, 965 North Longfellow St., Arlington, VA 22205, USA

The starting point for safe food is education of the people who handle and prepare the food. This is a diverse group, and the educational message must be tailored to each sector. This symposium reviews the current educational practices for consumers, chefs, food handlers, elementary and preschool children, junior/senior high school students, and suppliers. It also looks at opportunities for new practices.

LECTURES

L01 ICMSF Lecture on Microbiological Sampling Plans

Susanne Dahms, Institute of Biometrics and Data Processing, Dept. of Veterinary Medicine, Free University of Berlin, Oertzenweg 19b, Berlin 14163, Germany

It is nearly 30 years ago that the International Commission on Microbiological Specifications for Foods (ICMSF) provided urgently needed guidance on the use of sampling plans and microbiological criteria for foods in international trade. Despite their wide use and adoption, microbiological criteria and sampling plans are not fully understood, especially with regard to their performance, and in relation to other risk management approaches such as HACCP.

Two general types of sampling plans, attribute plans and variable plans, are used in microbiological testing to make decisions concerning the safety or quality of foods. Attribute plans are used to evaluate qualitative data (presence/absence) or quantitative data that have been grouped (e.g., <10 CFU, 10 to 100 CFU, >100 CFU), whereas variable plans evaluate non-grouped quantitative data. Though both approaches are employed, the relationship between the two had not been rigorously evaluated. Starting with sampling plans articulated by the ICMSF, the design and characteristics of two- and three-class sampling plans are contrasted with variable plans.

"Operation characteristic" (OC) curves are used to visualize lot acceptance probabilities both in relation to the fraction of defective units and the mean concentrations of a target microorganism in food lots being sampled. In the latter case calculation of acceptance probabilities requires assumptions be made regarding the shape and spread of the frequency distribution of sample results. Thus, the effect of using an attribute sampling plan to assess mean microbial concentration in a lot is not only dependent on the microbial limits set and the number of samples required, but as well on the validity of the underlying assumptions for the frequency distribution. However, with prior experience substantiating the assumptions made, even attribute plans can be used to assess mean microbiological concentrations in lots of food.

Effective use of microbiological sampling requires a firm understanding of the statistical basis for subsequent decision-making, providing the conceptual framework for the development and implementation of microbiological testing programs. These concepts are critical if microbiological testing is to remain an important tool for evaluating the quality or safety of foods within a risk analysis context.

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L02 Risk Assessment of Microbiological Hazards in Foods: An International Approach

Sarah Cahill, Food and Agriculture'Organization, Food Quality Liaison Group, Food Quality and Standards Service, Food Quaility and Nutrition Division, Viale delle Terme de Caracalla, 00100, Rome, Italy; Peter Karim Ben Embarek, World Health Organization, Food Safety Programme, Dept. of Protection of Human Environment, 20 Ave. Appia, CH-1211, Geneva 27, Switzerland

The development of risk-based concepts in recent years has led to a fundamental change in approach to food safety. Risk assessment is increasingly being utilized as the scientific basis for decision-making and the systematic application of a framework for managing risks is increasingly being recognized at the national and international level as the most appropriate means of bringing about an on-going reduction in risks to human health. In addition risk assessment can be used at the international level to facilitate food trade.

Foodborne illness is among the most widespread of public health problems and creates social and economic burdens as well as human suffering while restrictions on a countries food trade lead to additional economic burdens. Although risk assessment is a tool that countries can use to address these issues, it is not within the capacity of many countries to carry out a quantitative microbiological risk assessment.

FAO and WHO are playing an ever-increasing role in the development of microbiological risk assessment. The objectives of both organizations are to provide risk assessment advice for use in the elaboration of food hygiene standards at the international (Codex) level as well as making risk assessment a viable tool for use at the national level. In order to achieve these goals activities have been focused in five main areas: (1) generation of scientific information – risk assessments; (2) elaboration of guideline documents (3) data collection and generation (4) information and technology transfer; (5) use of risk assessment within a risk management framework. Such an approach is required to truly demonstrate the utility of the risk assessment as a tool in the management of microbiological hazards in foods.

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Abstract Book Addendum

as of June 24, 2002

MONDAY MORNING — JULY 1, 2002

(S04) **Speaker Change — Tracking Sources of Environmental Contamination (*Listeria monocytogenes*) in Latin-Style Cheese —** Martin Wiedmann, Cornell University, Ithaca, NY, USA will replace Kathryn J. Boor

(T01) **Speaker Change — Effects of Dried Plum Purées on Suppression of Growth of Foodborne Pathogens in Uncooked Pork Sausage —** LESLIE K. THOMPSON and Daniel Y. C. Fung, Kansas State University, Manhattan, KS, USA will be presented on Monday morning instead of Wednesday afternoon (T62)

(T10) **Speaker Change — Comparison of Shelf Life and Microbial Profile of Immersion-chilled and Air-chilled Broilers —** A representative from the University of Nebraska, Lincoln, NE, USA will replace Ngah Wan (Jennifer) Phoon

(P003) **Withdrawn — Evaluation of COMPASS L. mono, a New Chromogenic Medium for Highly Specific Isolation of *Listeria monocytogenes***

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MONDAY AFTERNOON — JULY 1, 2002

(S05) **Title/Speaker Change — Food Security-Protecting the Source —** Gordon Meriwether, Core Processes, Inc., Alexandria, VA, USA will replace Jeff Goldburg

(T16) **Withdrawn — Characterization of Multiple Fluoroquinolone Resistance among Avian *Escherichia coli* Isolates from North Georgia**

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TUESDAY MORNING — JULY 2, 2002

(S11) **Speaker Change — Identification of Potentially Unique Genetic Markers and Virulence Attributes of Epidemic-associated Strains of *Listeria monocytogenes* —** Bala Swaminathan, CDC, Atlanta, GA, USA will replace Sophia Kathariou

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(T32) **Speaker Change — Evaluation of the Use of Lactic Acid Bacteria to Control Pathogens on Alfalfa Sprouts —** Mindy M. Brashears, University of Nebraska-Lincoln, Lincoln, NE, USA will replace Marsha R. Harris

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TUESDAY AFTERNOON — JULY 2, 2002

(S15) **Speaker Change — Product Recovery — Keeping Dairy from Going Down the Drain** will be presented by Mark D. Johnson, Gannett Fleming, Malden, MA, USA will replace F. Michael Byers

(S15) **Title/Speaker Change — A Survey of Food and Dairy Technologies for Creating Products from Waste —** CLAY DETELEFSEN, International Dairy Foods Association, Washington, D.C., USA

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WEDNESDAY MORNING — JULY 3, 2002

(S18) **Speaker Change — NSF Standard 14159 Hygenic Requirements for the Design of Meat and Poultry Processing Equipment —** Charlie Cook, NSF, Ann Arbor, MI, USA will replace William Schwartz

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WEDNESDAY AFTERNOON - JULY 3, 2002

(T62) **Speaker Change — Effects of Dried Plum Purées on Suppression of Growth of Foodborne Pathogens in Uncooked Pork Sausage —** LESLIE K. THOMPSON and Daniel Y. C. Fung, Kansas State University, Manhattan, KS, USA will be presented on Monday morning at 8:30 a.m. as (T01)

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