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POSTER SESSION

P1 MODIFICATION OF SOME SELECTIVE MEDIA FOR THE RAPID DETECTION OF *SALMONELLA* USING IMPEDANCE-SPLITTING METHOD

Pravate Tuitemwong,* T. Hongdusit, and K. Tuitemwong, King Mongkut's University of Technology Thonburi, Food Science and Technology, Bang-mod, Toongkru, Bangkok 10140, Thailand

Modifications of some selective media for the rapid detection of *Salmonella* by means of impedance splitting system were carried out. Trimethylamine oxide (0.5%) was added to selenite cystine broth (SC), while, 1 to 4% $MgCl_2$ were added to Rappaport Vassiliadis (RV) broth for the detection of changes of medium (M) and electrode (E) impedance, respectively, using BacTrac 4100 (SyLab, Austria). Yeast extract (0.3%), mannitol (0.5%), novobiocin (0.15ppm), and nitrofurantoin (10 to 30ppm) were added to both media. Modified SC stimulated growth of all strains of *Salmonella* tested (*S. paratyphi A, S. thompson, S. stanley, S. derby, S.* Enteritidis) giving high M-values of 40 to 100%. Modified RV with 3 to 4% $MgCl_2$ gave 40 to 70% E-values for all *Salmonella* strains. Both media suppressed M and E-values generated by *E. coli, E. aerogenes* and *C. freundii* to levels lower than the 15% threshold for more than 20 h. The modified SC and RV appeared to be effective for rapid *Salmonella* detection using the automated impedance-splitting system.

P2 USE OF MEMBRANE FRACTION AND SELECTIVE MOTILITY FOR THE RAPID SCREENING OF *LISTERIA MONOCYTOGENES*

Pravate Tuitemwong,* J. Wongchavalit, and K. Tuitemwong, King Mongkut's University of Technology Thonburi, Food Science and Technology, Bang-mod, Toongkru, Bangkok 10140, Thailand

The application of *Escherichia coli* E-8 membrane fraction (MF) to enhance growth of *L. monocytogenes* (LM) in selective motility method using media of the ISO (Fraser), USFDA (LEB), USDA (UVMI), and IDF (LEB) methods was carried out. Esculin and ferric ammonium citrate were added to all enrichment media tested. Selective motility utilized u-tube with both tubes filled with selective enrichment broth supplemented with MF at 0.1, 0.5, and 1.0 unit/mL. At 35°C, the bacteria grew in a tube and migrated through the 5-cm u-tube filled with either Oxford, LPM or Palcam agar supplemented with MF and then grew in the other tube changing the medium to black, which was considered presumptive positive for LM. At high level (10^8 cells), the USDA media required about 18 h while ISO/CD and IDF methods required 19 and 21 h, respectively. At low level (10^3 cells), however, all methods required about 21 to 22 h while the control required 30 to 36 h. The higher MF concentrations resulted in faster growth and shorter lag phase than those without MF (control). This method enables one to screen for LM using regular media of these standard methods to obtain presumptive results in less than 24 h.

P3 EVALUATION OF THE BAX FOR SCREENING/GENUS *LISTERIA* METHOD FOR TESTING ENVIRONMENTAL SPONGES

Joseph D. Meyer,* K. B. Mikkelson-Baldus, A. C. Borger, W. M. Barbour, and P. A. Hall, Kraft Foods, Oscar Mayer Division, 910 Mayer Avenue, Madison, WI 53704, USA

Listeria monocytogenes is an important foodborne pathogen. Often referred to as ubiquitous, complete elimination of *L. monocytogenes* from the manufacturing environment is considered impossible. The presence of *Listeria* species is often used as an indicator for the presence of *L. monocytogenes*. This study evaluated the performance of BAX® for Screening/Genus *Listeria* method for detecting *Listeria* species in environmental sponge samples. Both a 24-h and a 48-h enrichment protocol were evaluated. Sponges were also tested using a modified USDA cultural method for comparison. Of 252 sponges tested using the 24-h protocol, 56 were positive by both the BAX® and the modified USDA methods, 8 were positive by the BAX® method alone, 17 were positive by the modified USDA method alone, and 171 were negative by both methods. Of 277 sponges tested using the 48-h protocol, 128 were positive by both the BAX® and the modified USDA methods, 35 were positive by the BAX® method alone, 1 was positive by the modified USDA method alone, and 113 were negative by both

methods. The BAX® for Screening/Genus *Listeria* method offers significant time savings over cultural methods and the 48-h protocol proved to be a highly sensitive method for detecting *Listeria* species in environmental sponge samples.

P4 IMMUNOASSAY-BASED TEST FOR DETECTION OF PEANUTS IN FOOD PRODUCTS

Mohamed M. Abouzied,* S. A. Askegard, P. S. Satoh, S. L. Hefle, J. A. Nordlee, and S. L. Taylor, Neogen Corporation, 620 Lesher Place, Lansing, MI 48912, USA

A 30-min Sandwich Enzyme-Linked Immunosorbent Assay (S-ELISA) for determination of peanut concentration in foods was developed. Rabbit anti-peanut polyclonal antibodies were used as capture and detector antibodies. The antibodies are highly specific to peanut and did not cross-react with other legumes or tree nuts. The assay minimum limit of detection for peanut was 0.035 µg/mL (0.875 ppm). Samples were extracted by shaking 5 g of ground samples with 125 mL of PBS in a hot water bath. Extracts were filtered and filtrates used directly for ELISA analyses. Recovery of peanut from spiked samples varied from 75 to 92% with a mean recovery of 84%. Blue color development will indicate the presence of peanut in sample extract and absorbance readings of samples are compared with those of the standards and the concentrations in part per million (ppm) were calculated. Dark chocolate among several other food commodities were found to interfere with the assay resulting in false negative samples. A specific propriety reagent (extraction additive) was developed to eliminate this matrix effect.

P5 DETECTION OF EGG CONTAMINATION IN FOOD PRODUCTS BY IMMUNOASSAY-BASED TEST

Mohamed M. Abouzied,* C. J. Fetzner, P. S. Satoh, S. L. Hefle, E. Jeanniton, and S. L. Taylor, Neogen Corporation, 620 Lesher Place, Lansing, MI 48912, USA

An estimated 1 to 2 percent of adults, and 5 to 8 percent of children, are sensitive in some degree to food allergens. Introduction of food allergens can occur at any step of the food manufacturing process. Allergy to eggs is one of the more common food allergies especially among infants and adolescents. We developed a quantitative sandwich enzyme-linked immunosorbent assay (S-ELISA) for determination of egg contamination in food products. Rabbit anti-egg polyclonal antibodies were produced and used as capture and detector antibodies. Samples were extracted by shaking 5 g of ground samples with 125 mL of PBS in a hot water bath. Extracts were filtered and filtrates were used directly for ELISA analyses. For quantitative analyses a standard curve of egg ranging from 0 to 15 ppm was used. The minimum detection limit for egg was determined to be 0.012 µg/mL (0.3 ppm). Recovery of egg from various spiked samples varied from 76 to 89% with a mean recovery of 82%. The assay can be carried out in 30 min at room temperature.

P6 ACCURACY OF *SALMONELLA* DETECTION IN FOOD USING COMMERCIALLY AVAILABLE *SALMONELLA* ELISA TESTS

Catherine F. Smith,* K. W. Doherty, and C.-M. Chen, IDEXX Laboratories, 1 Idexx Drive, Westbrook, ME 04092, USA

The ability to detect *Salmonella* in our food supply is important to protect consumer safety. Traditional procedures require 5 to 6 days for the detection of *Salmonella* in food. Two commercially available *Salmonella* immunoassay systems, *Salmonella*-TEK™ ELISA test system (Organon Teknika Corporation) and TECRA *Salmonella* Visual Assay (VIA) (TECRA Diagnostics), were compared against standard methods (AOAC Bacteriological Analytical Methods or FSIS) for detecting *Salmonella*. Enrichment procedures recommended by the assay manufacture were used for different food matrixes. Representative samples were inoculated with *Salmonella* (1 to 3 CFU). Presumptive ELISA results were confirmed using biochemical testing and serology. Comparisons were made between the reference procedures and ELISA test results. *Salmonella* was detected in 2 raw meat samples and one sample of paprika. *Salmonella* was detected at pre-enrichment levels of <5 CFU from all methods. No method, including BAM was 100% effective. The results from *Salmonella*-Tek agreed with reference procedures in 87% of the samples tested. Presumptive positives were confirmed in 89% of the samples. TECRA VIA had 89% agreement with the reference procedures with confirmations of 84%. In conclusion, both *Salmonella*-Tek and Tecra VIA are comparable to reference methods for presumptive screening of *Salmonella* in food samples.

P7 RAPID PREPARATION OF PCR SAMPLES FROM FOOD COMBINED WITH SHORTENED PCR CYCLES FOR THE DETECTION OF *ESCHERICHIA COLI*

William K. Shaw, Jr.* and L. A. McLandsborough, University of Massachusetts, 434 Chenoweth Lab, Amherst, MA 01003, USA

Efficient and rapid removal of PCR inhibitors are needed to utilize PCR technology to detect bacteria from foods. We hypothesize that buoyant density centrifugation can be used to concentrate cells and separate PCR inhibitors to increase efficiency of PCR detection. In addition, shortening cycle times in a conventional PCR thermal cycler could decrease detection time while not

requiring additional new expensive equipment. The sample treatment method involved a 30 min centrifugation at 14,000 × g of ground beef homogenate layered over the gradient medium, Percoll®, in a flat bottom 2 ml microcentrifuge tube. Overnight *E. coli* cultures were serially diluted and added to ground beef samples, stomached, treated, and assayed using PCR for the *uidA* gene. Shortened cycle PCR reaction time was reduced from 3.5 h typical reaction time to 1.5 h with greater sensitivity. Results showed that the Percoll® treatment was able to remove enough PCR inhibitors allowing the PCR reaction, while samples without Percoll® treatment resulted in no PCR product. The method was able to detect down to 10 PCR template cells without enrichment. This sample treatment method coupled with a shortened PCR reaction has potential applications with other bacteria and food combinations.

P8 ENUMERATION OF *CAMPYLOBACTER JEJUNI* AND C. *COLI* WITHIN 36 H BY IMMUNOBLOTTING FROM MODIFIED BLOOD AGAR MEDIUM

Rama Nannapaneni,* R. Story, and M. G. Johnson, University of Arkansas, Dept. of Food Science, IFSE-Ctr. For Food Safety & Quality, 272 Young Avenue, Fayetteville, AR 72704, USA

An immunoblotting method was developed that can be used for enumeration of *C. jejuni* and *C. coli* in pre- and post-chilled chicken carcasses. *Campylobacter* was recovered from poultry carcass rinses by spread plating onto a modified blood agar (MBA) medium. MBA plates gave rise to visible microcolonies of *C. jejuni* and *C. coli* within 36 h and the colonies were twice as large as those observed in Abeyta-Hunt-Bark agar. After growth, microcolonies were transferred by overlaying MBA plate with a PVDF-membrane for 20 s. A 30-min immunoblotting assay was optimized for distinguishing the microcolonies of *Campylobacter* from the other naturally occurring microflora present in chicken carcass rinses. The endogenous peroxidases bound to the PVDF-membrane from MBA medium were inactivated with a 5-min rinse in a solution containing 0.2% BSA and 5% hydrogen peroxide. *Campylobacter* levels in rinses varied, ranging from less than 10 to 1,300 CFU/ml in samples from commercial pre-chilled and post-chilled carcasses.

P9 A SINGLE MEDIUM FOR THE QUANTITATIVE SCREENING OF THREE FOODBORNE PATHOGENS

R. Victor Lachica,* US Army Natick Labs, PENQ Team, Natick, MA 01760-5056, USA

The present study describes the successful use of a single agar medium for the rapid screening of three pathogens on slices of retail luncheon meats instead of using three selective media. Plates of the multiple target agar (MTA) medium were obtained using a rich, nonselective brucella agar base containing esculin and phenolphthalein diphosphate as diagnostic agents. Food homogenates of 1:2 dilution were prepared in stomacher bags with built-in filter. Duplicate, pre-dried plates of MTA were inoculated with 0.5 ml of the homogenates. After 24 h at 35°C, the plates were scanned for typical large colonies (>2 mm) of *Bacillus cereus* and examined with a quick 3% KOH viscosity test. Medium-sized colonies (>1 mm) that turned pink upon exposure to ammonium hydroxide were suspected as *Staphylococcus aureus*. Colonies of listeriae exhibited typical bluish hue when observed by oblique lighting; a dark halo is observed around the colonies under ultraviolet light.

Additional tests were made with suspect colonies. However, most sample were negative for the pathogens even at a detection threshold of 2 cells/g. With seeded samples, MTA provided comparable recovery of target organisms as the conventional selective media.

P10 COMPARISON OF MICROBIAL IDENTIFICATION METHODS

Marlene Celis,* J. Deabel, V. Gangar, and M. Curiale, Silliker Laboratories Research Corp. Center, 160 Armory Drive, South Holland, IL 60473, USA

Traditional techniques for identification of bacterial and yeast isolates are labor intensive and generally require numerous tests and several days to weeks to complete. Several identification kits and automated systems have been developed for rapid identification that provide quicker results. Three of these commercial systems (Biolog, Vitek Jr., and the Vitek ATB) were evaluated for their performance in the identification of *Bacillus* spp., lactic acid bacteria and yeast. A number of isolates obtained from various food products and environmental samples were used. Method agreement, defined as frequency of identical results between two methods, was determined for each group of organisms. For yeasts, there was a 14% agreement between Biolog and the traditional method and 32% agreement between Vitek Jr. and the traditional method. For *Bacillus* spp., there was a 17% agreement between Biolog and the traditional method and 67% agreement between Vitek Jr. and the traditional method. Lactic acid bacteria showed an 8% agreement between Biolog and the Vitek ATB. Overall, results indicated that determining the accuracy of any one method used for identification is very complex since each system consists of a different database. Constant taxonomic changes further complicate matters as well as the lack of a "standard" on which one can base an identification.

P11 A PCR-ELISA FOR DETECTING SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* IN FOOD

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A sensitive and specific PCR-ELISA was developed to detect *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC) in food. The assay is based on the incorporation of digoxigenin-labeled dUTP and biotin-labeled primer during PCR. The labeled PCR products are then bound to the streptavidin-coated microtiter plate and detected by an ELISA. Shiga toxin genes were the targets of the PCR. The specificity of the PCR was determined using more than 40 different serotypes of *E. coli*. The ELISA detecting system for the PCR products was able to increase the sensitivity by up to 1,000 fold and showed a much stronger signal for positive samples, compared with the conventional gel electrophoresis. With the aid of a DNA purification system, PrepMan (Perkin-Elmer), the PCR-ELISA was able to detect ca 10^5 CFU of STEC per gram of ground beef without any cultural enrichment. The assay took about 6 h. Because its microtiterplate format, the PCR-ELISA is particularly suitable for screening a large number of samples; future automation is highly possible.

P12 EVALUATION OF THE TECRA® UNIQUE™ TEST FOR RAPID DETECTION OF *SALMONELLA* IN FOOD: A COLLABORATIVE STUDY

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The TECRA Unique test for *Salmonella* uses the principle of immunoenrichment to provide a rapid and convenient alternative to cultural methods for detection of salmonellae in food. Although Unique already has AOAC Research Institute certification, a collaborative study was necessary, in order to gain AOAC Official Methods Board approval. The collaborative study is being conducted in two parts and the first part, using laboratories in Australia and New Zealand as collaborators, has been completed. Eighteen collaborators participated in the study which included 3 food types: non-fat milk powder, soy flour and ground black pepper. For each food type, 5 uninoculated control samples, 5 samples inoculated with *Salmonella* at the low level (target 1 to 5 cells/25 g) and 5 samples inoculated at the high level (target 10 to 50 cells/25 g) were analyzed by Unique and by the FDA BAM (8th edition) method. Statistical analysis using the Chi-square test at 5% level showed no significant difference between results obtained using Unique and those obtained with the FDA BAM method. The Unique test is simple to perform and gives a presumptive positive or negative result within 22 h making it very convenient for routine testing by food industry laboratories.

P13 RAPID 24-H MULTIPLEX DETECTION OF FOUR PATHOGENS IN FOOD FROM A SINGLE ENRICHMENT

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Detection of multiple specific pathogens in food using conventional microbiological techniques is a complex and time-consuming process. Individual pathogen detection is achieved by sequential growth on several selective media. This study describes a nucleic acid probe-based assay capable of simultaneously detecting less than 10^4 target cells following enrichment of four individual pathogens; *Escherichia coli, Salmonella* Enteritidis, *Listeria monocytogenes* and *Staphylococcus aureus* from a single sample within 24 h.

The food sample is enriched in a single non-selective growth medium overnight, the micro-organisms from a sub-sample are concentrated by micro centrifugation, washed and lysed to release their ribosomal RNA. The rRNA is detected in a microtitre well format via a sandwich hybridization assay using 4 pairs of specific probes, an enzyme-labelled antibody and chemiluminescence.

The probe specificity's have been checked against at least 30 of each target organism, 150 individual non-target organisms and the endogenous flora of a range of food samples including raw and cooked meats, cream, chocolates and drink powders. No false positives were generated from the food samples tested and only one significant non-target organism has been identified from the test panel. This assay provides a significant advantage in both speed to result and convenience.

P14 RAPPAPORT-VASSILIADIS ENRICHMENT PROCEDURE FOR USE WITH DNA HYBRIDIZATION ASSAYS FOR DETECTION OF *SALMONELLA* SPP. IN FOODS

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The performance of Rappaport-Vassiliadis (RV) medium for the selective enrichment of *Salmonella* species in foods was evaluated in a pre-collaborative study involving the analysis of 21 artificially and naturally contaminated foods. A total of 1050 food samples were tested in parallel

by two commercially available DNA hybridization methods (AOAC Official Method 990.13 and AOAC Research Institute Performance Tested℠ Method No. 961101) and the BAM/AOAC reference method. The steps of pre-enrichment, selective enrichment, isolation, and preliminary screening of typical *Salmonella enterica* isolates were performed as described in the *Bacteriological Analytical Manual*, 8th edition (Rev. A), 1998, except that all methods utilized RV broth medium for selective enrichment. [Selenite cystine broth is still used in the BAM method for low microbial load foods, although replacement with RV broth is under consideration.] Twenty *Salmonella enterica* serovars previously isolated from foods were used as inoculum strains. For low microbial load foods, the minculum strains were first frozen and then lyophilized to produce conditions of sublethal injury. To date, 700 samples have been tested. Results showed 99.4% agreement comparing the standard 65°C hybridization method with the BAM/AOAC culture method and 99.6% agreement comparing the DLP hybridization method with the culture method. Results of the completed study will be presented.

P15 DIFFERENTIATION BETWEEN TYPES AND STRAINS OF *CLOSTRIDIUM BOTULINUM* BY RIBOPRINTING

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The Qualicon Riboprinter™ Microbial Characterization System, currently used to provide genetic "fingerprints" or Riboprint patterns of microbiological isolates, was evaluated for its ability to differentiate between major types and individual strains of *C. botulinum*.

Pure spores of *C. botulinum* type A, proteolytic type B, nonproteolytic type B, and type E were inoculated onto modified anaerobic egg yolk agar and incubated 24 h at 35°C. Plates were rinsed with buffer (2mM Tris + 20mM EDTA) to remove cells which were heated for 10 min at 80°C, treated with lysing agent, then ribotyped using the Qualicon Riboprinter™ utilizing the enzyme *Eco*RI .

Riboprint™ patterns obtained for 25 strains of the 4 major types of *C. botulinum* most commonly involved in human foodborne botulism showed good differentiation within the genus. Pattern comparisons performed by the system's computer algorithm determined that 13 separate Riboprint™ groups exist amongst the 25 strains and that distinct and multiple banding patterns appear to exist within each major *C. botulinum* type.

This degree of differentiation between strains of *C. botulinum* will allow this technique to be used in improving the safety of our food supply, in hazard analysis and identification, HACCP monitoring and validation, environmental monitoring, outbreak epidemiology and in inoculation studies.

P16 EVALUATION OF CLEARVIEW™ AND BAX™ FOR THE DETECTION OF *LISTERIA* SPP. AND *LISTERIA MONOCYTOGENES*

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The use of new methods, rapid and efficient, to evaluate the incidence of microorganisms in foods have been evaluated everywhere. In order to compare the efficiency of *Listeria* Rapid Test Clearview™ and Bax™ *Listeria* system in the detection of *Listeria* spp. and *L. monocytogenes*, respectively, this study was developed. A total of 413 samples from different sources, collected in a chicken nuggets processing line, were analyzed by both methods and by a third ("Traditional"- pre-enrichment, enrichment and isolation on selective agars). Overall results showed an excellent correlation between the results obtained with Clearview™ and the "Traditional" method (99%). Bax™ showed a lower correlation with the "Traditional" method (71.1%). The type of sample did not affect the efficiency of Clearview that varied from 98.1% for product samples to 100% for floor, drains and food handlers while for Bax it has a marked influence. Correlation between Bax and "Traditional" method varied from as high as 100% for food handlers to 37.9% for product samples.

P17 COMPARISON OF DIFFERENT DYE INDICATORS FOR EARLY DETECTION OF MICROBIAL GROWTH *ESCHERICHIA COLI* O157:H7 USING BIOSYS 32

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The frequent occurrence of foodborne disease outbreaks and heightened public awareness about food safety has raised the need for rapid and convenient microbiological methods that are more accurate and sensitive compared to conventional techniques. This study employed a rapid automated system called BioSys 32 (MicroSys, Ann Arbor, MI) to detect *E. coli* O157:H7 grown on medium containing different dyes (Bromocresol purple, Resazurin and 2,3,5 Triphenyl-H-Tetrazolium).

E. coli O157:H7 was grown in Tryptic Soy Broth (TSB) for 12 h at 37°C, and inoculated to vials containing sterile detection media with or

without sterile milk and incubated in BioSys 32 at 34°C for 24 h. The detection time for *E. coli* O157:H7(1×10^3 CFU/ml) varied for BCP, Resazurin and TTC (5.2, 6.3 and 6.2 h respectively). Although the detection time was higher in redox dyes compared to BCP, redox dyes showed no effect with varying concentration of milk in the detection medium. These dyes could have a potential application in the development of media for early detection of food pathogens including *E. coli* O157:H7.

P18 THE INFLUENCE OF PRE-ENRICHMENT MEDIA ON THE DETECTION OF *ESCHERICHIA COLI* O157:H7 WITH A FLUOROGENIC DNA-BASED ASSAY

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E. coli O157:H7, a foodborne bacterial pathogen, has caused several large outbreaks in recent years which affected thousands of people and resulted in scores of deaths worldwide. We have developed a rapid detection method for *E. coli* O157:H7 using a fluorogenic 5' nuclease assay based on the polymerase chain reaction. This DNA-based detection system is quantitative and highly sensitive. To determine the most efficacious pre-enrichment medium for use with this assay, we monitored the growth of normal or heat-injured *E. coli* O157:H7 spiked into ground beef enrichments using either "EEB" or "mEC" media. With both media, the fluorogenic 5' nuclease assay gave positive results after 6 h of incubation with an initial inoculum of approximately 100 CFU per enrichment of normal or injured *E. coli* O157:H7 cells. However, the mEC medium allowed more rapid growth and higher final concentrations of target bacteria for both normal and injured cells. For the fluorogenic 5' nuclease assay, where interference from background microflora is not a problem, mEC medium was determined to be more effective for the rapid detection of *E. coli* O157:H7.

P19 COMPARISON OF BAX® AND ORGANON TEKNIKA® *SALMONELLA*-TEK TO STANDARD SELECTIVE ENRICHMENT METHOD FOR THE DETECTION OF *SALMONELLA* IN FOOD

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Simple, rapid, and reliable testing methods for pathogen detection are becoming essential for the food industry to ensure safety of food products. This study compared two rapid methods, BAX® for Screening/*Salmonella* PCR assay and Orgnanon Teknika® *Salmonella*-Tek to standard selective enrichment method for detecting the presence of salmonellae in various inoculated food samples. Four hundred and fifty different inoculated food samples were evaluated. The samples were inoculated with stressed *Salmonella* cells, an ATCC isolate or a food isolate at an inoculum range of 10 to 100 *Salmonella* cells per 25 g. The BAX system detected *Salmonella* in 439 of the 450 inoculated samples tested. The standard selective/enrichment and the *Salmonella*-Tek methods detected *Salmonella* in 438 of the 450 inoculated samples tested. An uninoculated set of samples was also evaluated. Of 450 uninoculated samples tested none were positive by PCR. The standard selective enrichment method yielded one presumptive positive. The *Salmonella*-Tek method yielded 36/450 false positives. Results of this study indicate that the BAX PCR assay is a rapid and definitive method for detecting *Salmonella* in foods. Comparably, *Salmonella*-Tek method yielded too many false positives to be effectively used for the routine screening of the types of food tested in this study.

P20 ISOLATION OF FOODBORNE *SALMONELLA* USING DYNABEADS® ANTI-*SALMONELLA* AND OXIOD SPRINT *SALMONELLA* MEDIUM

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IMS procedures from pre-enriched raw food samples, followed by plating, have in some instances resulted in overgrowth of target salmonellae by competitive enteric flora making identification of isolated *Salmonella* colonies difficult. This has led to the introduction of a post IMS selective enrichment in Rappaport-Vassiliadis Soya Peptone broth (RVS). The disadvantage of this cultural modification is that *Salmonella* detection takes 72 instead of 48 h. A new enrichment medium from Oxoid, SPRINT, combines the resuscitative properties of buffered peptone water and the selective properties of RVS. This medium has been evaluated in combination with Dynabeads anti-*Salmonella* for its ability to detect low levels of stressed or sublethally injured *Salmonella* from foods. The results demonstrated a significant reduction of background flora equivalent to that observed using post IMS selective enrichment, with almost pure cultures of *Salmonella* being obtained. This combination significantly reduced analysis time of raw foods by one day and is also fare more sensitive and specific than the direct plating of the SPRINT enriched samples.

P21 COLLABORATIVE TESTING OF A PROTOTYPE AUTOMATED IMS SYSTEM FOR RAPID DETECTION OF *SALMONELLA* AND *ESCHERICHIA COLI* O157 USING DYNABEADS®

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An instrument has been developed to perform immunomagnetic separation (IMS) automatically. This instrument has been evaluated at five international test laboratories. Each laboratory analyzed 150 processed or raw foodstuffs which were either naturally or artificially (spiked) contaminated samples with *Salmonella* or *E. coli* O157. All samples were processed using both manual and automated IMS. Furthermore, for *Salmonella*, samples were also examined by the ISO 6579 method. The manual IMS has previously been shown to be the most sensitive culture-based technique available for the detection of *E. coli* O157 from samples. Samples examined for *E. coli* O157 using both manual and automated IMS demonstrated a 94.6% concordance in one laboratory and an 88.5% concordance in a second laboratory. This deviation was due to 15 additional positive results obtained by the automated IMS. Twelve of these 15 samples were subsequently confirmed as true positives. Samples examined for *Salmonella* using the three methods (manual IMS, automated IMS, and ISO method), demonstrated results which were equivalent (100% concordance). Users reported that the developed instrument is suitable for routine use and therefore will make a significant contribution to sample testing based on IMS.

P22 THE USE OF RAPID METHODS TO ASSESS THE INCIDENCE AND PUBLIC HEALTH RISK OF *STAPHYLOCOCCUS AUREUS* IN FOOD AND FOOD PRODUCTION ENVIRONMENTS

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A rapid method, using the TECRA *S. aureus* Visual Immunoassay (VIA), was compared with the standard Triplicate Tube method to determine the presence or absence of *S. aureus* in 135 food and environmental samples.

Results showed that 10 of the samples tested were positive for *S. aureus* using the VIA method and all were confirmed. There were no false positives or false negatives with the TECRA method. Three samples were found to contain enterotoxigenic *S. aureus* by using the TECRA Staphylococcal Enterotoxin VIA. For the Triplicate Tube method, only 2 of the foods were positive for *S. aureus*.

This study shows that *S. aureus* is a relatively common contaminant of food and environments in which food is produced and a significant proportion of the *S. aureus* found may be enterotoxigenic. Such results indicate a need for increased surveillance for this important pathogen. This can be achieved cost-efficiently by using the TECRA method, which was more sensitive, and less labor intensive. Results are also obtained at least 24 h earlier than by traditional methods and, together with the ability to test for enterotoxins on the same enrichment sample, the TECRA method provides an extremely rapid and convenient way of assessing public health risk.

P23 EVALUATION OF THE RAPID SIMPLATE™ YEAST AND MOLD TEST FOR VARIOUS FOOD BAR PRODUCTS

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A rapid yeast and mold enumeration method for routine QA testing would be of great value in facilitating product release. The SimPlate™ Yeast & Mold (SYM) test was evaluated for a variety of meal-supplement food bar products. The objectives were to compare the performance and demonstrate equivalency of SYM test and the reference Potato Dextrose Agar with antibiotics (PDA) method. The acceptance criteria were set to be within 0.5 log between the two methods. Six varieties of meal supplement food bars were tested for yeast and mold with SYM and PDA in parallel on 10 replicate samples. Five of the 6 food bars were artificially inoculated with a mixture of yeast and mold isolates. Results showed SYM incubated at 30°C/72 h met the 0.5 log criteria for all food bars tested; counts of SYM incubated for 48 h vs. PDA were all within 1-log differences. SYM counts were consistently lower than PDA except the 72-h SYM for naturally contaminated cocoa almond samples. The SYM test yielded comparable counts to the PDA method for the products tested and it would reduce testing time by at least 2 days.

P24 COMPARISON OF TWO ELISA TESTS AGAINST STANDARD METHOD FOR THE DETECTION OF *LISTERIA* SPECIES IN FOOD SAMPLES

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The choice of methods for detecting *Listeria* spp. in food quality control is important. In the present study, Organon Teknika (OT) *Listeria*-Tek™

and Tecra® *Listeria* Visual Immunoassay (VIA) were evaluated against AOAC Bacterial Analytical Method (BAM) in 54 food samples (soft cheese, unpasteurized milk, fresh vegetables, seafood, raw meat products). All results were subjected to confirmation by selective isolation and biochemical identification. Of the 54 samples tested, the BAM confirmed the presence of *Listeria* spp. in 18 samples. OT *Listeria*-Tek detected 37 samples as positive for *Listeria* spp. with 21 samples confirmed. Tecra *Listeria* VIA detected 19 samples as presence of *Listeria* spp. with 15 samples confirmed. The ELISA positive predictive values for OT *Listeria*-Tek and Tecra *Listeria* VIA were 56.8% and 88.2%, respectively. Negative predictive values were 100% for both ELISA tests. Chi-square statistics showed significant differences between OT *Listeria*-Tek ELISA results and the BAM procedures ($P<0.01$); there were no significant differences between OT *Listeria*-Tek confirmatory results and BAM procedures ($P>0.05$). Both Tecra *Listeria* VIA ELISA and confirmatory results showed no significant differences against the BAM procedures ($P>0.05$ in both cases). Tecra *Listeria* VIA is comparable with BAM for the screening of *Listeria* spp. in food samples.

P25 *SALMONELLA* DETECTION IN FOOD: STUDY OF A TWO-STEP ENRICHMENT PROTOCOL COMBINED WITH AN ELISA

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When working with a large series of samples, the main limitations of the immunoassays concern the enrichment procedure which is usually composed of three different steps using four different broths. In this study, a two-step enrichment protocol, using first buffered peptone water then Rappaport Vassiliadis Soya broth, was combined with an ELISA for *Salmonella* detection in food. With artificially contaminated samples, the ELISA done after the two-step enrichment protocol, showed a limit of detection lower than 3 CFU/25 g of sample, demonstrating the whole method was effective for detecting low levels of *Salmonella* in food.

For the study of naturally contaminated food samples (n = 150 samples, mainly raw poultry products), the ELISA combined with the two step enrichment protocol showed 98% agreement with the standard ISO method n°6579. Fifty positive samples were found by ELISA and 52 by the ISO method, which gave a 96% sensitivity for the ELISA. The ELISA specificity reached 99%. No statistical differences were observed between both methods.

The combination of a two-step enrichment protocol and an ELISA was shown to be reliable for *Salmonella* detection in raw food matrices. This new method simplifies the enrichment procedure for *Salmonella* detection and contributed to an optimal organization of the laboratory when working with a large number of samples.

P26 CLEANING VALIDATION IN FOOD RETAIL ENVIRONMENTS BY A NEW PROTEIN ASSAY

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Implementation of the HACCP and GMP principles to ensure food safety from "farm to fork," along with increased consumer awareness of food safety issues, has made it imperative to implement cleaning validation programs in food retail establishments. To facilitate this, we have developed an easy-to-use protein assay for detection of residual protein on food preparation surfaces. The test format allows it to be used on location by food service employees with little formal training or special equipment.

This study examines the cleanliness of various locations in food service environments. The surfaces were measured for contamination with the protein test, as well as with a bioluminescence system. In a laboratory setting, both tests were able to distinguish dirty and inadequately cleaned surfaces from surfaces that had been thoroughly cleaned. This poster presents data with dairy products, cooked and raw meat, and with fruit. Similar results were observed in field studies. Many of the surfaces that were not mechanically cleaned exhibited dirty results with the protein test (4 on a scale of 0-4). These included sites such as deli slicers and coffee creamer dispensers. Testing of mechanically cleaned surfaces yielded readings lower on the scale, as did all of the dirty areas once they were thoroughly recleaned. These results suggest that this protein assay may become an important tool for monitoring surface cleanliness in the food retail environment.

P27 A COMPARATIVE MEDIA ANALYSIS OF NEWSPAPER COVERAGE OF MICROBIAL FOOD SAFETY ISSUES IN CANADA, THE US, THE UK AND AUSTRALIA, 1994 TO 1998

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It has been previously demonstrated that the formation of public perception regarding microbial foodborne risks is strongly related to on-going media coverage. Media analysis, like public opinion

surveys, is a tool to understand the formation of public opinion to look at what people are saying and what they are being told. This reliance on the media helps to define the public's sense of reality and its perceptions of risks or benefits.

A comprehensive media analysis of newspaper coverage of microbial food safety issues in Canada, the US, the UK and Australia was conducted from Jan. 1, 1994 to Dec. 31, 1998, and the results were compared with consumer responses in various public opinion surveys. Increased media coverage of microbial food safety issues was positively correlated with increased public concern as reflected in opinion surveys, and subsequent policy initiatives.

P28 STATISTICAL PROCESS MONITORING AND FAULT DIAGNOSIS IN A CONTINUOUS DAIRY PASTEURIZATION PROCESS

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Multivariate statistical techniques have gained importance in monitoring food processes because of their ability to detect abnormalities in the process with a high number of variables and observations. Methods are needed to predict deviations in the process variables from control limits continuously, and to diagnose faults in the process. A computer controlled, high-temperature, short-time pasteurization system was used to study the implementation of multivariate statistical process monitoring (SPM) and fault diagnosis (FD) techniques. Process temperatures and control valve signals (0.004-0.02 A) were acquired electronically. Computer programmed failures in process variables were imposed on the process. Hotelling's T^2 charts and squared prediction error (SPE) charts were used to statistically analyze the information from the process variables. These techniques helped detect abnormalities in process operations. The FD techniques were used along SPM charts to isolate the source of disturbance in the system. A minimum of 0.4°C (0.4%) change in temperature measurements and a minimum 0.0011 A (11.5%) change in control valve measurements could be detected. Multivariate SPM charts detected abnormalities faster and with fewer false alarms than Shewhart charts and monitored critical control points effectively on-line. The FD techniques determined successfully the source cause of deviation.

P29 CLEANING VALIDATION OF FOOD PROCESSING EQUIPMENT: A COMPARISON BETWEEN A NEW ULTRASONIC APPARATUS AND SWAB METHOD

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In this paper, we report the sucessful utilization of ultrasonic technology for the non-invasive detection of biofilm in food processing equipment. We have developed an ultrasonic apparatus which uses the mechanical effects of ultrasonic cavitation produced by generators operating at a frequency of 40 KHz. Removal of biofilm by ultrasonic technology and detection by ATP-bioluminescense allowed us to take out a patent on this new technology (November 1997). The fouling and the cleanability levels of materials (open or closed surfaces) present at four food industry were measured. The results, show a significant difference between the swab method and ultrasonic method (80 to 100% rate of residues, soil removal). The results indicate the reproducibility of biofilm's removal only by ultrasound technique. The application of ultrasound produced at 40 KHz was not found to be detrimental for low concentration of ATP standard. We have described sonication conditions that do not significantly reduce the culturability of bacterial cells. The effectiveness of four industrial cleanability were studied. The method used to dislodge bacterial cells from surfaces was sucessful in removing biofilm as seen by scanning electron microscopy. Our new ultrasonic apparatus has confirmed the ineffectiveness of existing cleaning and sanitizing procedures in eliminating to and possible growth of cells on equipment surfaces.

P30 A COMPARATIVE EVALUATION OF THE CLEANING PERFORMANCES OF A RANGE OF SEVEN FLOORS IN THE FOOD INDUSTRY

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The aim of our work is a comparative evaluation of the cleaning performances of a range of seven floors used at present time in different types of food plants (ceramic tiles, epoxy resins, methacrylate resins). Materials were chosen to represent those used in food industry and recommended by suppliers of floor materials. Their

ability for soiling and cleaning properties are studied. A cleaning procedure, close to an industrial one, was carried out on different floors soiled with industrial dirts and inoculated with spores of *Bacillus stearothermophilus* variety *calidolactis* as tracer. Six food products are directly collected in respective food processing plant and are used as soiling agents. The fouling and the cleanability levels of the different floors were measured. All the samples are submitted to sonication. This procedure will remove the overall dirt from the reference samples and the residual dirt from those that have been cleaned with a detergent. The draining suspension is cultured. The number of spores remaining after pre-rinsing gives an indication of the floor ability for soiling. Some floors retain more dirt than others do. The final level of contamination with spores gives an assessment of the level of dirt remaining after the cleaning procedure. This assessment allows a categorization of the floors into groups according to both following aptitudes: low soiling and good cleaning. No clear relationship has been observed between cleanability of a floor and its slip resistance property.

P31 INDICATIVE MICROBES ON PROCESSED SHRIMP BEFORE IMPLEMENTATION OF US FDA'S HACCP REGULATIONS

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The nature and number of microbes on raw and cooked shrimp products processed along the Gulf Coast states were determined before implementation of FDA's seafood HACCP regulations. Five unit samples of shrimp products (e.g., raw tails, raw peeled, cooked peeled and breaded) all processed on the same day (lot) were procured from eleven processors. A total of 55 samples were examined. Aerobic, psychrotrophic, coliform, fecal coliform, Enterobacteriaceae and *E. coli* counts were determined using 3M™ Petrifilm™ Aerobic, Coliform, Enterobacteriaceae and *Escherichia coli* plates. Each microbial counts did not differ ($P > 0.05$) among products, with mean aerobic (5.3 ± 0.7 Log CFU/g), psychrotrophic (5.3 ± 0.6 Log CFU/g), coliform (1.8 ± 0.4 Log CFU/g), fecal coliform (1.9 ± 0.4 Log CFU/g) and Enterobacteriaceae (2.7 ± 0.3 Log CFU/g) counts. *E. coli* ranged from 0 to 2 CFU/g was detected on 12% of products. Coliform, fecal coliform and Enterobacteriaceae were observed in all samples examined. Indicative microbiological counts on raw and cooked shrimp products had reasonable desired quality and were within ICMFS microbiological criteria before the implementation of FDA's seafood HACCP regulations.

P32 EVALUATION OF HACCP PROGRAM FOR DELI FOOD SERVICE MANAGERS

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HACCP principles are being applied to all segments of the food chain. The objective of this multi-state project was to develop a HACCP program for deli managers, small food processors, and food transportation personnel based on FDA HACCP applications and regulations. An educational HACCP workshop was designed to be modified to meet audience needs. Workshop format included time for participants to apply HACCP principles to formulations. Workshops were given for deli managers and evaluations were conducted immediately after the workshop and after 3 months. The "pre-post" evaluation tool included 11 concepts and asked participants to indicate their comfort level with each concept. "Pre" workshop, the participants rated concepts such as GMP's as familiar and concepts such as organizing HACCP teams as unfamiliar. Participants were "quite uncomfortable" with HACCP principles, whereas "post" workshop, they were "quite comfortable" with HACCP principles. From responses, participants either learned or reinforced the importance of CCP's and record-keeping. After 3 months, managers reported that they monitored CCP's and kept records on a consistent basis. For deli operations to implement HACCP, participants need to be provided assistance and opportunity to work on HACCP plans for their deli during the workshop.

P33 GROWTH OF SALMONELLAE IN PREVIOUSLY IRRADIATED GROUND BEEF

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Ground beef was irradiated to 0, 2 or 4 kGy and then inoculated with a mixed culture of five serotypes of salmonellae. The ground beef was stored at either 15°C or 25°C, and the growth of salmonellae monitored over time. Growth parameters were determined for the salmonellae using the Gompertz equation. There was no significant difference in lag phase durations or generation time, irrespective of the dose that the ground beef had previously been exposed to. Furthermore, the lag phase durations and generation times determined in this study did not differ significantly from previously published values for salmonellae. This suggests that, although irradiation eliminates a significant portion of the spoilage microflora in ground beef, the absence of this microflora

provides no competitive advantage to the growth of salmonellae in ground beef. The growth parameters of salmonellae were identical in both previously irradiated and non-irradiated beef.

P34 REDUCTION OF BACTERIAL CONTAMINATION ON HOG CARCASSES WITH HOT WATER AND ORGANIC ACID RINSES

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Hot water rinses were applied to the forelegs of hog carcasses intentionally contaminated with manure. The water temperature varied from ambient temperature (25°C) to 82°C. The hot water washes were followed by a 1.5% acetic acid rinse, and the carcasses were sampled before the hot water rinse, after the rinse and after the acid rinse. The hot water rinses reduced the total aerobic population by approximately 2 $\log_{10}$ cycles and the population of Enterobacteriaceae by approximately 2.5 $\log_{10}$ cycles. The acid rinses reduced the total aerobic population by an additional 0.3 $\log_{10}$ cycle (total of 2.3 $\log_{10}$ cycles) and the population of Enterobacteriaceae to below detectable limits (total of 4 $\log_{10}$ cycles). The experiments were repeated in a commercial slaughter establishment with scalded and skinned hog carcasses heavily inoculated with manure. The combined hot water and acid treatment reduced total aerobic bacteria and coliforms by 2 and 2.5 log cycles, respectively. After the combined treatment in the commercial slaughter establishment, 60% of the scalded and 40% of the skinned carcasses were negative for *Escherichia coli*.

P35 DISSEMINATION OF *LISTERIA MONOCYTOGENES* IN A BRAZILIAN FROZEN CHICKEN NUGGETS PROCESSING LINE

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The presence of *L. monocytogenes* in a food processing plant is cause for concern because of its capacity of surviving at environmental conditions. This study was conducted to evaluate the dissemination of *L. monocytogenes* in a chicken nuggets processing line located in Sao Paulo, Brazil. A total of 413 samples of different sources were collected and pre-enriched in Half-Fraser broth (30°C/22 h) followed by enrichment in Buffered *Listeria* Enrichment broth (30°C/22 h). They were plated onto HCLA, PALCAM and LPM agars that were incubated at 30°C/24 to 48 h. The identification of suspected strains was done by API *Listeria* and some standard biochemical tests. *Listeria* was isolated from 271 (65.6%) samples and *L. monocytogenes* from 249 (60.1%) of them. The highest *L. monocytogenes* incidence was observed in samples collected from drains and floors (100%), followed by product samples (92.9%) and equipment surfaces (43.4%). The incidence of *L. monocytogenes* in the hands of food handlers were very low (2.4%). There was no step, from raw material to final product, that favored the reduction of *L. monocytogenes* occurence with percentages varying from 83.3% to 100%. This production line ought to be a cause of concern for this meat industry.

P36 PRODUCTION OF MORTADELLA: BEHAVIOR OF *LISTERIA MONOCYTOGENES* UNDER COMMERCIAL MANUFACTURING AND STORAGE CONDITIONS

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In a previous study we found that 26.7% of the mortadella samples collected at retail stores in Sao Paulo, Brazil, were contaminated with *L. monocytogenes*. Studies were done to evaluate the cooking treatment used for the production of mortadella and the protective effect that different formulations can have on the survival of 2 different levels (1 to 10 CFU/g and 100 to 1000 CFU/g) of a *L. monocytogenes* pool spiked the product. Two formulations of mortadella were prepared, one with high quality meats and the other with filler meats, binders and and other fillers. Artificial casings, clipped at both ends were used and the product was cooked until reaching 74°C at the geometric center. Mortadellas were stored at 5°C and 25°C and were analyzed at 4 different days. The surviving *L. monocytogenes* cells were couted by a MPN procedure. No *L. monocytogenes* has been recovered from any of the samples. One can conclude that the heat treatment is effective to inactivate the microorganisms even if high levels are present. It also can be concluded that no formulation or storage temperatures affects the behavior of the pathogen.

P37 ENUMERATION OF *ESCHERICHIA COLI* IN POULTRY CARCASS RINSE USING SIMPLATE AND PETRIFILM METHODS

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The current food safety regulations specify the acceptable, marginal and unacceptable levels of *E. coli* in poultry rinse water. Consequently, there is much interest in methods for the detection and enumeration of coliforms and generic *E. coli* in meat and poultry establishments. A study was

conducted to compare a newly developed SimPlate coliform and *E. coli* (CEc) method with Petrifilm (*E. coli* count) method to assess the application of the former as an alternative method for enumeration of generic *E. coli*. Of 49 poultry carcass rinse samples tested by the two methods, 31 (63%) yielded higher *E. coli* counts with the SimPlate method than with the Petrifilm method. Sixteen (33%) samples yielded lower *E. coli* counts with the Petrifilm method and 2 (4%) samples yielded the same counts with both methods. In some instances the SimPlate method detected *E. coli* that were either missed completely or yielded "uncountable" plates (>15 CFU) on a corresponding Petrifilm plate. The SimPlate method also rated favorably in terms of the ease of use and detection and enumeration of low levels of *E. coli*. It is concluded that SimPlate CEc is a suitable alternative for enumeration of *E. coli* in poultry carcass rinse samples.

P38 SENSITIVITY OF *SALMONELLA TYPHIMURIUM* DT104 TO IRRADIATION

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Salmonella typhimurium DT104 was inoculated into sterile ground pork and then irradiated using a linear accelerator to determine the D_{10} values. The irradiation was conducted at approximately 3°C, and the composition of the ground pork was either 5%, 50% or 95% fat. There was no significant difference in D_{10} values in the pork, irrespective of fat content. The D_{10} value over all of the ground pork mixtures averaged 0.39 kGy, which is within the range of irradiation D_{10} values previously reported for *Salmonella* spp. This suggests that *S. typhimurium* DT104 possesses no unique qualities which would predispose it to survival during irradiation processing.

P39 FATE OF *SALMONELLA* ENTERITIDIS IN HARD-COOKED EGGS

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Outbreaks of salmonellosis have been associated with hard-cooked eggs. A study was done to determine D_{56}°C values of *S. senftenberg* 775W and six strains of *Salmonella* Enteritidis isolated from outbreaks associated with eggs. D_{56}°C values for *S.* Enteritidis in liquid egg yolk ranged from 5.14 to 7.39 min; the D_{56}°C value for *S. senftenberg* was 19.96 min. Two strains of *S.* Enteritidis were inoculated (10^7-10^8 CFU) into the yolk of medium and extra large eggs at 10 and 21°C, and survival was monitored using two cooking methods: (1) placing eggs in water at 22°C, heating to 100°C, removing from heat, and holding 15 min (American Egg Board method); and (2) placing eggs in water at 100°C, then holding 15 min at this temperature. Inactivation was more rapid using method 1 compared to method 2 and, within each cooking method, inactivation was most rapid in medium eggs initially at 21°C. The pathogen survived in yolk of extra large eggs initially at 10°C when eggs were cooked 9 min using method 2. The temperature of the yolk in these eggs was 62.3 ± 2°C. Of the two methods evaluated for hard-cooking eggs, the American Egg Board method is clearly most effective in killing *S.* Enteritidis in the yolk.

P40 SURVIVAL OF MULTIDRUG-RESISTANT *SALMONELLA TYPHIMURIUM* DT104 IN EGG POWDERS AS AFFECTED BY WATER ACTIVITY AND TEMPERATURE

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The ability of *Salmonella typhimurium* DT104 and non-DT104 cells to survive in whole egg powder, whole egg powder supplemented with corn syrup solids (38%) and salt (1.9%) (WECS), egg yolk powder, and egg white powder as affected by a_w (0.29-0.37 and 0.51-0.61) during storage at 13 or 37°C for 8 wk was determined. Rates of inactivation of DT104 and non-DT104 cells were similar within each set of test parameters. With the exception of WECS, death at 13°C was enhanced in powders at a_w 0.29-0.37 compared to a_w 0.51-0.61. Survival in WECS was significantly higher compared to survival in other egg powders stored at 13°C. The opposite trend occurred in powders at a_w 0.51-0.61 stored at 37°C. Rates of inactivation of DT104 and non-DT104 cells in egg white powder containing 4.9, 6.1, or 8.2% moisture at 54 or 82°C for 7 d or 8 h, respectively, did not differ. Both cell types were detected in egg white powder containing 4.9% moisture but not in powder containing 8.2% moisture when held at 54°C for 7 days. Heating at 82°C for 8 h failed to eliminate 5 $\log_{10}$ of *S. typhimurium*. Depending upon the log reduction in desired, traditional egg white powder pasteurization conditions may not be adequate to eliminate *S. typhimurium*.

P41 CONSUMER ACCEPTABILITY AND MICROBIAL INACTIVATION IN HOME-STYLE BEEF JERKY PRODUCED BY VARIOUS METHODS

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The safety of homemade jerky continues to be questioned. Producing a safe product which retains acceptable quality attributes is important. Consumer acceptability and sensory attributes of jerky prepared by four methods that have a lethal effect on *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* and the microbial lethality resulting from the most acceptable method were examined. Preparation methods were drying marinated strips at 60°C (representing a traditional method), heating strips in marinade or in an oven to 71°C prior to drying, and heating strips in an oven after drying to 71°C. A 60-member consumer panel rated overall acceptability. A 10-member descriptive panel evaluated quality attributes. Overall acceptability was greater for samples heated after drying and samples heated in marinade than for traditional. Based on the sensory data, samples heated after drying were determined to be the most similar to the traditional samples. Microbial challenge studies with the pathogens showed that oven heating after drying resulted in at least a 6-log reduction. These reductions were as great as or greater than obtained by traditional drying. In conclusion, a safer, acceptable home-dried beef jerky can be produced by oven-heating jerky strips after drying.

P42 EVALUATION OF ENVIRONMENTAL MICROFLORA IN A KOREAN MEAT PLANT FOR HACCP APPLICATION

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The environmental microflora was examined for the hygienic evaluation in meat processing plant. The sixty swabbed samples using sponge were collected from the environmental surfaces of meat plant and measured the total, coliform and psychrotrophic count by using tryptic soy agar (TSA), violet red bile agar (VRBA) and TSA, respectively. Sampling sites were the surfaces of shelves, walls, floor, cutters, cutting boards, drains, carriers, water containers, smokers, autoclaves, conveyers, packaging machines, and windows. The swabbed samples were transported to laboratory within 2 h of sampling kept in ice packed box and analyzed. Total counts of surface samples were ranged from not detectable to 10^6 CFU/200 cm^2. The level of coliform and psychrotrophic were ranged from not detectable to 10^5 CFU/200 cm^2. There were great differences among sampling sites. Especially, handling sites of raw meat showed highest level of microorganism than the rest. Despite of same stainless steel surfaces, the counts were different by the usage of processing and sampling sites. The highly contaminated sites were cutting board, drains and floor, which could be the critical control points of HACCP. Therefore, these results demonstrated clearly that special hygienic treatment is necessary those highly contaminated sites in meat processing plant.

P43 REDUCTION OF NORMAL FLORA BY IRRADIATION AND ITS EFFECT ON MULTIPLICATION OF *LISTERIA MONOCYTOGENES* ON GROUND TURKEY AT 7°C IN A MODIFIED ATMOSPHERE

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Listeria monocytogenes did not multiply faster during storage at 7°C on raw ground turkey with a gamma radiation reduced normal flora than on non-irradiated meat and there was a concentration-dependent inhibition of its multiplication by CO_2. Gamma irradiated at 5°C (1.5 and 2.5 kGy) and non-irradiated ground turkey was inoculated after irradiation with an average of 100 CFU/g *L. monocytogenes* (ATCC 7644, 15313, 49594, and 43256) and stored at 7°C for periods of up to 28 days after packaging in air-permeable pouches or under atmospheres containing 25 or 50% CO_2, 25% O_2, and 25 or 50% N_2. A dose of 2.5 kGy extended the time for the total plate count (TPC) to reach 10^7 CFU/g from 4 to 19 days compared to that for non-irradiated turkey in air-permeable pouches. Following a dose of 2.5 kGy at the end of the 28-day study, the TPCs were $10^{6.42}$ and $10^{4.98}$ under 25% and 50% CO_2 atmospheres, respectively. Under air, 25% CO_2, and 50% CO_2 atmospheres, the populations of *L. monocytogenes* after 19 days incubation were $10^{4.89}$, $10^{3.60}$, and $10^{2.67}$ CFU/g. The populations of lactic acid bacteria and anaerobic or facultative bacteria were also reduced by irradiation.

P44 MICROBIOLOGICAL CONTAMINATION BASELINES OF BEEF CARCASSES, WHOLESALE CUTS AND RETAIL CUTS

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Resulting from consumer concern about the microbiological safety of retail products, a study was conducted in six packing plants and six retail stores to determine microbiological contamination of carcasses, subprimals and retail cuts. Carcasses were sampled (by sponging or excising) after 24 to 72 h of chilling and subprimals were sampled during fabrication (by sponging) at the plants and retail stores. Samples were analyzed for aerobic

plate counts (APC), total coliform counts (TCC), *Escherichia coli* counts (ECC) and for *Salmonella* and *Listeria monocytogenes*. Steaks/roasts (from subprimals of the same lot) were also analyzed (at 0 and 48 h) for *Staphylococcus aureus* counts (SAC). Carcass sponging recovered APC, TCC, and ECC of 4.1, 1.2, and 1.0 log CFU/100 cm^2, respectively, which were lower ($P \leq 0.05$) than those recovered by excising (6.6, 3.2, and 2.8 log CFU/100 cm^2, respectively). The overall frequency of *Salmonella* isolation from sponged and excised carcasses was 0.7% and 1.7%, respectively, while overall incidence of *L. monocytogenes* was 6.9% and 15.6%, respectively. Products displayed for 48 h had mean APC, TCC, ECC, and SAC of less than 6.8, 2.7, 1.8 and 2.4 log CFU/100 cm^2, respectively. Further product handling, however, should be under sanitary conditions to control microbial growth.

P45 THERAPEUTIC ANTI-IDIOTYPIC ANTIBODIES TO *ESCHERICHIA COLI* K88 AS AN ALTERNATIVE TO ANTIBIOTIC USE IN MEAT INDUSTRY

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Enterotoxigenic *E. coli* (ETEC) causes diarrheal diseases in domestic animals and humans. Antibiotics that have been widely used to control this pathogen promote the development of resistance to ETEC and are found as contaminants in food products. Passive immunization of the animals to prevent binding of ETEC to intestinal receptors could provide a novel procedure to eliminate such diseases. Several monoclonal and polyclonal antibodies were produced against *E. coli* K88 in mice and rabbits. Purified antibodies were injected into chicken to produce anti-idiotypic antibodies (Ab2) that mimic the K88 fimbrial antigen. Competitive assays showed that antibodies bear internal images of the fimbrial K88 as they were able to compete with pure K88 fimbriae. Analysis of affinity purified receptors from porcine intestinal mucus by electrophoresis and immunoblotting using Ab2 revealed that it was bound by the 60 kDa receptor. Mice that were immunized with the Ab2 were challenged with live ETEC. The survival rate was 100% among the immunized groups while it was only 25% among the control group. Testing the efficacy of Ab2 using the pig as a model is in progress. These results demonstrated that the anti-idiotypic antibodies show promise for the control of ETEC.

P46 *ESCHERICHIA COLI* O157:H7 RISK ASSESSMENT FOR PRODUCTION AND COOKING OF BLADE TENDERIZED BEEF STEAKS

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Blade tenderization is an effective method of meat tenderization for cuts high in connective tissue. While organoleptic properties of mechanically tenderized products have been extensively researched, microbiological aspects have not been adequately characterized. Though microbial quality of blade tenderized muscle has been shown to be equivalent to non-tenderized controls on a per gram basis, bacteria may be translocated into the muscle. A study was designed to quantify bacterial relocation and define effective cooking schedules for tenderized products. Top butt beef subprimals were inoculated with 6 logs/cm^2 of *E. coli* O157:H7 on the top exterior surface, passed once through a blade tenderizer, cut into steaks of 0.5 in., 0.75 in., or 1.25 in. thickness, and cooked to one of five internal temperatures. Cross-sectional samples were evaluated for presence of the pathogen and compared to non-tenderized cooked controls. No significant differences in residual pathogen populations were found between tenderized and non-tenderized steaks (P>0.05). There was, however, significant interaction between thickness of steak and endpoint temperature. As expected, log counts decreased as temperature increased. Log counts also were lower in thicker steaks, likely due to longer cooking time. Overall, cook times tended to be shorter in tenderized versus non-tenderized samples.

P47 REDUCTION OF *SALMONELLA* CONTAMINATION ON PORK PRODUCTS USING RADIANT WALL OVEN HEATING

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Microbial contamination with foodborne pathogens continues to be a serious problem for the pork industry. Heightened media coverage of the meat industry has brought the concern of food safety to the attention of consumers. In response, the meat industry is aggressively pursuing methods to reduce the level of foodborne pathogens on its product. Developments that are practical and effective in pathogen reduction can aid the industry and enhance product safety. This study

examined the effectiveness of a short-term high heat application to reduce microbial populations using radiant wall oven heating. The radiant wall oven is relatively new and allows a product to be subjected to radiant heat to temperatures up to 81°C (150°F) for just a few seconds. Results revealed that use of the radiant wall oven has potential for addressing the issue of pathogen reduction on the food surface while resulting in minimal change in the food itself. By subjecting pork loins to 649-704°C (1200-1300°F) for 3 s, populations of *Salmonella* (including *S. typhimurium* DT 104 and the heat resistant *S. senftenburg*) were reduced by 1-1.5 logs while the product retained a fresh-like appearance.

P48 THE OCCURRENCE OF *CAMPYLOBACTER* SPP. IN SWINE CARCASS DRESSING OPERATIONS

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Campylobacteriosis is emerging as the leading foodborne illness in the US Poultry is regarded as the major vehicle, but other foods have also been implicated. A swine slaughter and dressing operation was surveyed for the incidence of *Campylobacter* spp. at several steps during carcass handling using Tran's blood-free enrichment broth and CEFEX-CAMPY agar for selective plating. Isolates were confirmed as *Campylobacter* spp. by latex agglutination and speciated by hippurate hydrolysis and API-CAMPY biochemical test strips. *Campylobacter* spp. were isolated from swine carcasses (rectal swabs and head meat) and equipment (dehairing apparatus, gambrel table, final Chad washer, and deboning tables and conveyors). The incidence of *Campylobacter* spp. on head meat was 10+/10. Isolates were identified as *C. jejuni* and *C. coli*. These results indicate that swine and pork handling equipment can carry *Campylobacter* spp. and could represent a source of these bacteria.

P49 AUTOMATED REAL TIME CCP MONITORING OF EXTERNAL COOKED SAUSAGE TEMPERATURE UTILIZING INFRARED SENSORS AND STATISTICAL PROCESS MONITORING

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Efficient monitoring of critical control points (CCPs) is a key factor in enhancing food safety. Automated monitoring of CCPs can generate significant benefits in safety. The CCP for sausage for example, is the internal temperature of the sausage after cooking. This temperature is manually measured and recorded every fifteen mins with thermocouples. A typical industrial plant may operate at a capacity of 4000 lbs an h per cooking line, which implies that at least 1000 lbs of sausage are processed between measurements.

An on-line method of monitoring the temperature of food products was developed and tested by using infrared sensor measurements of the external sausage temperature after cooking and statistical process monitoring (SPM) of the data. A digital filtering algorithm first removes the sausage temperature from the background conveyor belt temperature. SPM tools are then applied to detect process changes. Initial studies of cooked sausage indicated a linear relationship between external and internal sausage temperature. For a 1°C change in internal temperature, a 0.56°C change in external temperature was observed. An X-bar chart with a centerline of 72°C, and upper and lower control limits of 77°C and 66°C was used to detect process deviations.

P50 EXTENDING THE SHELF LIFE OF A COOKED HAM PRODUCT USING L-GLUCOSE AND D-TAGATOSE

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Negative publicity surrounding chemical preservatives has directed the food industry to search for alternatives. One approach is to use novel ingredients that are not easily recognizable by microorganisms in an effort to extend the shelf life of a food product. This study investigated the application of "unique" sugars to restrict the growth of microflora in different media. In a series of tests, L-glucose and D-tagatose were compared to D-glucose for carbohydrate utilization in growth media and a boned and rolled ham product. Results from the carbohydrate test showed that both L-glucose and D-tagatose were not easily metabolized by lactic acid (e.g., lactobacilli spp., pediococci spp.) and pathogenic bacteria (e.g., *E. coli* O157:H7, *Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus, Yersinia enterocolitica*). When L-glucose and D-tagatose were substituted for D-glucose in the cooked ham product, analyses by general linear models (SAS) revealed that D-tagatose significantly ($P<0.05$) reduced the growth rate of total aerobic and lactic acid bacteria. Enterobacteriaceae levels were not significantly ($P>0.20$) affected by any of the sugars in the meat formulation. These results indicate that D-tagatose has a negative effect on the microbial population and inhibits the rate of spoilage in a boned and rolled ham product.

P51 MICROBIAL POPULATION OF READY-TO-SERVE SALADS IN TEKIRDAG, TURKEY

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Ready-to-serve salads are naturally highly contaminated and often regarded as risky from a health standpoint. Total microbial counts as well as counts of lactic acid bacteria, total yeast and molds counts, coliform bacteria, *Escherichia coli* and *Staphylococcus aureus* were determined in 21 samples of ready-to-serve green salads and 15 samples of ready-to-serve green salads with dressing, such as mayonnaise, which had been obtained from local retail stores. The pH ranged from 4.04 to 5.90. The total aerobic count ranged from 5.0×10^5 to 2.0×10^9 CFU/g. Yeast and molds were found in all samples and ranged from 10^2 to 3.0×10^6 CFU/g. The lactic acid bacteria count ranged from 3.0×10^2 to 1.1×10^7 CFU/g. Coliforms on the samples ranged from 10^2 to 9.2×10^6 CFU/g and at the 34 samples out of 36 were found. *E. coli* was found in 32 out of 36 samples with numbers ranging from 25 to 10^4 CFU/g. Twenty-five samples out of 36 were found to contain *S. aureus* with levels ranging from 12 to 2.8×10^3 CFU/g. These results show that ready-to-serve salads, especially when they contain perishable ingredients such as mayonnaise, may constitute a health risk.

P52 A QUANTITATIVE ASSESSMENT OF THE RISK OF *ESCHERICHIA COLI* O157:H7 IN APPLE CIDER

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Unpasteurized apple cider has been shown to be a vehicle for *E. coli* O157:H7 infection. Published research has shown that this pathogen can survive in apple cider but little attempt has been made to quantify the exact risk it poses. Relevant data were collected from the scientific literature or from knowledgeable experts and translated into appropriate discrete or continuous probability distribution functions. Some of the information collected included: occurrence of *E. coli* O157:H7 in the feces of a variety of animals, antibacterial effects of chlorine and other sanitizers, adherence and detachment of bacteria to a variety of food processing surfaces and the effect of preservatives and storage temperature on growth and survival of *E. coli* O157:H7 in apple cider. In those cases where relevant data were not available, reasonable assumptions based on similar microbes were used. Monte Carlo simulation was used to integrate the data collected, and to predict which steps in cider production are most crucial to assuring food safety. Key factors which influenced the final level of *E. coli* O157:H7 in the product were the use of dropped apples, chlorine concentration in wash water and the use of sodium benzoate as a preservative.

P53 NATURE OF *ESCHERICHIA COLI* O157:H7 ATTACHMENT TO LETTUCE LEAVES AND THE EFFECT OF CHLORINE DISINFECTION

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Attachment of *E. coli* O157:H7 on lettuce leaves and the effect of chlorine disinfection were evaluated. Attachment at different inoculum levels (10^9, 10^8 and 10^7 CFU/ml) was examined by using plate counts of inoculated lettuce leaves (2 × 2 cm) at cut edges and surfaces. *E. coli* O157:H7 was found to attach preferentially to cut edges than to surfaces at all inoculum levels with greater attachment at higher inoculum levels. Longer attachment time allowed more cells to attach at both sites. Immunostaining with FITC-labeled antibody revealed cells penetrating from cut edges. Cells showed greater penetration when attached at 4°C compared to higher attachment temperatures (7, 25 and 37°C) and were found at average of 73.5 μm down from cut surfaces. Penetrating cells were mostly found at the junction of lettuce tissues. The viability of attached cells after 200 ppm chlorine treatment for 5 min was examined by plate counts and nalixidic acid elongation method. Although chlorine treatment showed significant reduction in attachments, cells remained attached at high numbers (7.9 and 8.1 log CFU/cm² for surfaces and cut edges, respectively). Elongated cells were observed in stomata and in the interior of the lettuce indicating these cells were protected from chlorine treatment.

P54 SODIUM CHLORIDE AND SODIUM BICARBONATE WASHING SOLUTION FOR REMOVAL OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157:H7 FROM THE SURFACES OF CHOPPED LETTUCE

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A washing solution containing $NaCl/NaHCO_3$ has been developed for removal of enterohemorrhagic *E. coli* (EHEC) O157:H7 from the surfaces of chopped lettuce. Chopped lettuce samples (25 g) in sterile bags were inoculated with 1 ml of decimally diluted 16 h broth cultures of EHEC

strain O157:H7 for 1 min to yield about 6.6 log CFU/g. The lettuce samples were treated with different concentrations (0.2, 0.4, 0.6, 0.8, and 1%) and pH (2.0 to 10.0) solutions of NaCl/$NaHCO_3$. The samples were washed in the treatment solutions for 1 min, rinsed with water for 1 min, and CFU/g determined. A rinse of 0.8 to 1% each of NaCl/$NaHCO_3$ (pH 8.0) reduced the log CFU/g of EHEC by about 2.5 logs and lower concentrations had about a 2.0 log CFU/g reduction. Solutions of NaCl/$NaHCO_3$ (1%) adjusted to a pH of 3.0 to 9.0 all reduced EHEC by about 2.5 log CFU/g whereas a pH of 2.0 or 10.0 had 3 log CFU/g reductions. Our results suggest that the NaCl/$NaHCO_3$ wash, over a wide pH range, can be used for removal of EHEC from chopped lettuce. The use of NaCl/$NaHCO_3$ will provide consumers and food service providers a potential tool for control of EHEC within their establishments.

P55 SURVIVAL OF *ESCHERICHIA COLI* O157:H7 IN BOVINE FECES APPLIED TO LETTUCE AND EFFECTIVENESS OF CHLORINE AS A DISINFECTANT

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A study was undertaken to determine survival characteristics of *E. coli* O157:H7 on iceberg lettuce using 0.1% peptone water and bovine feces as carriers for inocula. Four levels of inoculum, ranging from 10^0 to 10^5 CFU of *E. coli* O157:H7 per g of lettuce, were applied. Numbers of cells surviving on lettuce stored at 4°C were monitored for up to 15 days. Regardless of the type of carrier, viable cells of *E. coli* O157:H7 were detected on lettuce after 15 days, even when the initial inoculum was 10^0-10^1 CFU/g. Spray treatments of lettuce with 200 ppm chlorine solution or deionized water, then holding for 1 or 5 min, were equally effective in killing or removing *E. coli* O157:H7 from lettuce. Results of this study reveal that low numbers of *E. coli* O157:H7 cells, when applied to lettuce using bovine feces as a carrier and stored under commercial and home refrigeration conditions, can survive and are not easily removed by washing with water or treating with 200 ppm chlorine solution. Prevention of contamination of lettuce with bovine or other ruminant feces, which are potential vehicles for transmitting *E. coli* O157:H7 to humans, rather than relying on chlorine treatment, is essential for minimizing the risk of illness associated with this pathogen.

P56 SURVIVAL OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP. ON FRESH STRAWBERRIES

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For maximum shelf life, strawberries are harvested directly into retail containers and are usually washed just prior to consumption. To determine the survival of potential bacterial contaminants, cut or intact surfaces of fresh strawberries were spot inoculated with five or six strain cocktails of *E. coli* O157:H7 and *Salmonella* spp. (total CFU 10^7). Inoculated strawberries were dried for 1 h at 24°C and were stored in closed containers at 4 and 24°C. Populations of both pathogens remained constant on cut surfaces but decreased by 2 log cycles on intact surfaces when strawberries were stored at 4°C for 7 days. Populations of *E. coli* O157:H7 and *Salmonella* spp. did not decrease significantly on cut or intact strawberries stored at 24°C for 48 h. Populations of *Salmonella* spp. were reduced by 1 log cycle when strawberries were treated with a spray (5 ml) of either 200 ppm chlorine or sterile water followed by a 200 ml sterile water rinse. Soaking in either 10 ppm or 200 ppm chlorine for 5 min had no effect on pathogen levels. Bacterial pathogens survive well on strawberry surfaces and are difficult to remove with simple washing techniques.

P57 RECOVERY OF GENERIC *ESCHERICHIA COLI* FROM JUICE

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In this paper we present data that compares the relative performance of popular testing methods at recovering generic *E. coli* from various juice products.

The *E. coli* concentration was measured with undiluted and diluted juice samples using the SimPlate for Coliform & *E. coli*, Petrifilm *E. coli* count, Violet Red Bile agar (VRBA), and 3-tube MPN methods. Undiluted, 1:2, 1:5, and 1:10 dilutions of each juice sample were tested by the four methods. Numbers of recovered *E. coli* by each testing method were compared at each dilution.

The SimPlate and 3-tube MPN methods recovered *E. coli* from all dilutions and the *E. coli*

concentration as measured by SimPlate fell within the 95% confidence interval of the MPN method at least 81% of the time. The Petrifilm and VRBA methods, in general, failed to recover *E. coli* from the least dilute samples. However, both methods consistently recovered *E. coli* from the 1:10 dilution at concentrations that fell within the 95% confidence interval of the MPN method at least 61% of the time.

We conclude that recovery of generic *E. coli* from undiluted juice is best achieved by use of either the SimPlate or 3-tube MPN methods.

P58 INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP. IN UNPASTEURIZED APPLE AND ORANGE JUICE BY HIGH PRESSURE PROCESSING

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There have been reports of *E. coli* O157:H7 and *Salmonella* outbreaks associated with consumption of fresh, unpasteurized juices. The use of high pressure processing for the inactivation of *E. coli* O157:H7 and *Salmonella* spp. in fresh apple and orange juice was studied. Freshly squeezed apple and orange juices were prepared and the pH was adjusted to 3.70, 3.90, and 4.20, respectively. A mixture of 9 strains of *E. coli* O157:H7 (1.5×10^7 CFU/ml) and a mixture of 7 strains of *Salmonella* spp. (9.45×10^6 CFU/ml) were used in the study. The juice was inoculated, packaged into plastic pouches, heat sealed, and subjected to 80,000 psi pressure at room temperature for 0.5, 1.0, 2.0, and 3.0 min. The results indicate that a treatment of 80,000 psi for 0.5 min will cause at least a 5 log reduction of *E. coli* O157:H7 or *Salmonella* spp. regardless of the type of juice or pH. The study suggests that high pressure processing can be an alternative to pasteurization for inactivation of *E. coli* O157:H7 and *Salmonella* spp. potentially present in fresh, unpasteurized apple and orange juices.

P59 COLD SHOCK DECREASES THE THERMAL TOLERANCE OF BACTERIAL PATHOGENS IN APPLE AND ORANGE JUICE

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The contamination of fruit juices by bacterial pathogens is of considerable interest and concern. We investigated the potential of using prior cold shock to increase the sensitivity of pathogens to heat processing. Stationary phase cultures of *Salmonella typhimurium* (*St*), *Escherichia coli* O157:H7 (*Ec*), and *Listeria monocytogenes* Scott A (*Lm*) were grown at 37°C using a standardized protocol. An aliquot of the cells was subsequently cold shocked by incubation at 0°C for 3 h. Either cold shocked or non-cold shocked cells were added to apple or orange juice and the inoculated juice heated at 55, 58, 60, or 62°C. In apple juice, cold shocked *St*, *Ec*, and *Lm* had D-values that were lowered by 27, 26, and 24%, respectively, compared to the corresponding non-cold shocked cells. Reductions in thermotolerance were also observed when *St* or *Lm* (but not *Ec*) was inactivated in orange juice. These data suggest that cold shock prior to pasteurization of fruit juices could provide an extra measure of safety. In addition, these results would allow juice processors to reduce thermal processing requirements for pathogen control, while maintaining the fresh qualities of the juice.

P60 USE OF PGFP TO DETERMINE THE SURVIVAL OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA TYPHIMURIUM* IN MANURE APPLIED TO SOIL

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Current guidelines specify a 60-d waiting period between the application of manure to cropland and harvest. Recent studies indicate that *E. coli* O157:H7 may survive past 60 d under these conditions. It is difficult to enumerate *E. coli* O157:H7 in an environment such as soil or manure; use of selective media may not allow injured cells to be counted. This study uses a fluorescent marker to measure the survival of both *E. coli* O157:H7 and *Salmonella typhimurium* in cow manure applied to soil. pGFP was cloned into strains of *E. coli* O157:H7 and *S. typhimurium*. Cultures were inoculated into cow manure and mixed with soil. Samples were taken and enumerated by plating on non-selective agar and counting fluorescent colonies under UV light. Treatments were sampled until fluorescent colonies could no longer be detected. Although *S. typhimurium* did not survive the 60-d waiting period, *E. coli* O157:H7 was detected past 100 d. This indicates that current guidelines need to be re-examined. The use of pGFP in conjunction with non-selective media proves to be both simple and sensitive in survival studies with a mixed culture environment such as manure or soil.

P61 KEEPING QUALITY OF SPROUTS AFTER IRRADIATION AND D RADIATION VALUES FOR *SALMONELLA* AND *ESCHERICHIA COLI* O157:H7

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Recently there were a number of foodborne outbreaks due to either *Salmonella* spp. or *E. coli* O157:H7 on sprouts. As a possible means of reducing these pathogens on the sprouts, the use of ionizing radiation was investigated. The keeping quality of alfalfa, broccoli, and clover sprouts was studied after the sprouts were irradiated for 2 kGy at ambient temperature. Irradiating the sprouts increased their shelf life. The D radiation value of cocktails of *Salmonella* and *E. coli* O157:H7 used to determine the values in ground meat were obtained using the sprouts. The D radiation value in meat and on sprouts for the *Salmonella* and *E. coli* were 0.51 and 0.35 kGy, and 0.30 and 0.29 kGy, respectively. The D value for vegetable related foodborne isolates of *Salmonella* and *E. coli* were 0.28 and 0.45 kGy, respectively. There were no statistical significant difference between the previous reported D values using ground meat compared to using sprouts or between the meat vs. vegetable isolates. Ionizing irradiation is a process which can be used to reduce pathogen population and increase shelf life.

P62 BACTERIAL DECREASE OF VEGETABLE JUICE BY OZONE AND GAMMA RAY IRRADIATION

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Vegetable juice is packed after grind and squeeze fresh vegetables, not heated and washed with water. The legal regulation for vegetable juices in Korea is a storage period of 3 days. The total number of bacteria is not allowed to exceed 100 10^6 CFU/ml and *Escherichia coli* should not be detected.

The purpose of this study was to examine the efficacy of ozone water-washing and gama ray irradiation in decreasing the bacterial counts of vegetable juice. According to the classification of vegetable juice, samples were divided into four types: A (*Angelica Keisker Koidz* 100%), B (Carrot juice extract 100%), C (sample A 30%, kail 40%, apple 20%, cabbage 10%) and D (sample A 40%, kail 35%, apple 15%, beet 5%, cabbage 5%).

The total microbial load in all four vegetable juices after washing with 2.5 ppm ozone water for 60 min was in excess of the legal allowable limit.

While the initial bacterial counts ($\log_{10}$ CFU/ml) of the four samples were A=6.02, B=6.03, C=6.17, D=5.80, they decreased to A=2.17, B=2.23, C=1.93, D=2.39 after gamma ray irradiation treatment with 3kGy. The numbers of initial *E. coli* in four samples were about 10 CFU/ml and they were all inactivated after gamma ray irradiation treatment with 1.25 kGy.

P63 THE STUDY ON SAFETY AND CRISP PROPERTY OF PICKLED PEELED-HOT CHILI

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Hot chili is very popular in the tropics; pickled peeled hot chili is a newly developed product in the eastern Asia area, and the preservative added and the loss in crispiness are two major problems in producing this product. Two steps of preheating are used in the improved procedure. The raw material was heated at 50°C for 60 min and then fried at 200°C for 25 s (the hot chili can be peeled completely by machine). After peeling, some seasoning was added to the hot chili and adjusted the pH to 4.3 and pasteurized at 90°C for 15 min. It can get the crisp product from the above procedure; the total count, yeast and mold, and *Lactobacillus* will be less than 25 CFU after storage for six months.

P64 MICROBIAL ANALYSIS OF LETTUCE USED IN READY-TO-EAT SALADS

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The fastest growing segment of the fresh cut-produce industry is carrots and chopped lettuce, and other leafy salad constituents. Lettuce is a low calorie bulky food that can satisfy hunger and stimulates the digestive system by providing abundant water and gentle bulk. Processing of lettuce is limited since it cannot be frozen or heated without becoming limp and losing much of its appeal. Hence, microbial safety of lettuce is of major concern to the public. In this study, lettuce samples (pre-cut/shredded) were taken from local retailers and grocery stores and analyzed for total bacterial count, coliforms and *Escherichia coli*. Standard plate count was used for total bacterial count and Reveal Bioplate was used for detecting total coliforms and general *E. coli*.

Total bacterial population on lettuce samples (pre-cut/shredded) varied from 10^4 to 10^6 CFU/ml. Reveal Bioplate population of fluoresced wells (*E. coli* Bioplate 1) ranged from 0 to 1.4×10^3. Coliform population for different lettuce samples varied from 0-4.1×10^4. The presence of *E. coli* in pre-cut/shredded lettuce poses a serious health concern to the public.

P65 DEVELOPMENT OF A STANDARD METHOD FOR ASSESSING THE SANITIZING EFFICACY OF A PROTOTYPE "GRAS" PRODUCE WASH ON TOMATOES

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The US EPA is currently seeking a standard method for evaluating the antimicrobial efficacy of produce washes for home use. An EPA Scientific Advisory Panel recommended the use of fresh produce and pathogens isolated from actual foodborne (FB) illness outbreaks for development of these methods. We developed a standard method which was consistent with these SAP recommendations and evaluated the efficacy of a prototype (PT) Fit® Produce Rinse*, comprised of GRAS ingredients against *Salmonella* strains associated with tomato FB illness. We tested the efficacy of the prototype produce wash vs. tap water and a bench-mark sanitizer, i.e. 200 ppm hypochlorite. On tomatoes inoculated with *Salmonella,* the efficacy of the GRAS produce wash after 30 s exposures resulted in an average 3-4 log reduction of *Salmonella.* The efficacy of chlorine was similar while that for water was significantly less. Statistical analysis of the data and applications of the method to other produce types is discussed. *(Until criteria are established for making produce sanitization claims and products are approved via EPA registration, no consumer produce wash can make sanitizing or germ-kill claims.)

P66 ASSESSMENT OF THE MICROBIAL EFFICACY OF A PROTOTYPE GRAS PRODUCE WASH ON ALFALFA SEEDS, SPROUTS, AND SELECTED SALAD VEGETABLES

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Several outbreaks of *E. coli* O157:H7 and *Salmonella* infections have been associated with ingestion of alfalfa sprouts. In December 1998, the US Environmental Protection Agency granted a nationwide 1-year special exemption for the use of 20,000 ppm (2%) calcium hypochlorite to sanitize alfalfa seeds destined for sprout production. The study reported here was done to evaluate the efficacy of a prototype Fit® Produce Rinse†, comprised of GRAS ingredients, against *Escherichia coli* O157:H7 and *Salmonella* on alfalfa seeds, sprouts, and selected salad vegetables. On alfalfa seeds inoculated with *E. coli* O157:H7, the efficacy of the GRAS produce wash after 15 or 30 min exposures was ≥20,000 ppm calcium hypochlorite, and resulted in >6 log reduction. On mature sprouts inoculated with *E. coli* O157:H7 or *Salmonella*, the efficacy of the GRAS produce wash after 3-, 5-, or 10-min exposure times was ≥200 ppm hypochlorite, as was its efficacy in killing these pathogens on other salad vegetables. †(Until criteria are established for making produce sanitization claims and products are approved via EPA registration, no consumer produce wash can make sanitizing or germ-kill claims.)

P67 CONTROL OF *ESCHERICHIA COLI* O157:H7 IN MILK USING A NATURAL ANTIMICROBIAL AGENT-BACTERIOPHAGE

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The emergence and reemergence of virulent forms of foodborne pathogens such as *E. coli* O157:H7 has increased the need for effective measures to maintain and enhance the safety of foods. Bacteriophage a natural antimicrobial agent that has potential to bring about lysis of growing bacteria and are stable in more diverse environments compared to bacteria. In this study, bacteriophages were tested to control *E. coli* O157:H7 inoculated in milk. Biosys 32 was used to monitor changes in the microbial growth medium. *E. coli* O157:H7 was grown overnight in Tryptic Soy Broth at 37°C and inoculated into vials containing detection medium/milk with or without bacteriophage. Growth of *E. coli* O157:H7 was monitored for 24 h in sterile milk. Bacteriophages delayed the growth of *E. coli* O157:H7 by 6 to 9 h at 34.5°C. The delay in growth was proportional to the detection time in Biosys 32. Different Dyes (Bromocresol Purple and Resazurin) did not show significant difference in detection time for *E. coli* in milk. Although the bacteriophage mediated delay in *E. coli* O157:H7, growth was significant at 34.5°C. This effect will be further enhanced at lower temperatures.

P68 EFFECT OF STARTER CULTURE AND FERMENTATION TEMPERATURE ON SURVIVAL OF *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* DURING FERMENTATION AND STORAGE OF SOY YOGURT

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Survival of *E. coli* O157:H7 and *L. monocytogenes* in soy yogurt during fermentation at 37 or 43°C and storage at 4°C was evaluated. Soymilk (water, soy flour, and sucrose) was fermented (7 h)

with starters YC-180 (*S. thermophilus* and *Lb. bulgaricus*) or YC-180 + NU-A (*Lb. acidophilus*) at 37 or 43°C, then stored at 4°C for 72 h. Soymilk, inoculated with 7 log CFU/ml of a three-strain mixture of *E. coli* O157:H7 or *L. monocytogenes,* was examined hourly during fermentation and daily during storage for lactic acid bacteria (MRS), *E. coli* O157:H7 (modified EMB), *L. monocytogenes* (MOX), pH, and acidity (% lactic acid). After fermentation, pH (3.93) was lowest and acidity was highest (1.02%) for treatment 43-3 (43°C; YC-180 + NU-A); pH was highest (4.32) and acidity was lowest (0.63%) for treatment 37-2 (37°C; YC-180). For treatments 43-3, 43-2 and 37-3, *E. coli* O157:H7 was not detected at 5 h fermentation, 7 h fermentation and 24 h storage, respectively. After 72 h of storage of 37-2, counts were 5 log CFU/g. After fermentation, *L. monocytogenes* populations in 43-3 were 2 log CFU/g; survival in the other treatments was greater. *L. monocytogenes* was not detected in treatment 43-3 by 24 h of storage, but persisted at ≥ 4 log CFU/g in other treatments after 72 h of storage. Fermentation at 43°C with YC-180 + NU-A caused greatest destruction ($P < 0.05$) of the pathogens.

P69 EFFECT OF PACKAGING ATMOSPHERE AND STORAGE TEMPERATURE ON SURVIVAL OF *LISTERIA MONOCYTOGENES* ON CULTURE MEDIA CONTAINING ELEVATED NACL AND LACTIC ACID

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The effect of package atmosphere on survival of uninjured and heat-injured (54°C, 20 min) *L. monocytogenes* on tryptose phosphate agar (TPA) containing 0.85% lactic acid and 2% NaCl (TPALAS) was investigated. Inoculated TPALAS plates were packaged in air, 100% N_2 (N_2), 30% CO_2/N_2 (CO_2/N_2), and vacuum and stored at 4 and 20°C for up to 31 days. Recovery of *L. monocytogenes* from TPALAS was influenced by injury status (i.e., injured and uninjured) of the inoculum, storage atmosphere, storage temperature, and recovery media (TPA and modified Oxford agar [MOX]) ($P<0.05$). Storage in CO_2/N_2 was more inhibitory to uninjured *L. monocytogenes* stored at 20°C than air or vacuum, but less than N_2 ($P<0.05$). When heat-injured *L. monocytogenes* was stored at 20°C, CO_2/N_2 atmosphere allowed better recovery than N_2 ($P<0.05$) but was not different than air ($P>0.05$). At 4°C, uninjured and heat-injured *L. monocytogenes* were recovered in highest numbers from samples packaged in N_2 followed by CO_2/N_2 ($P<0.05$). Overall, non-selective TPA allowed greater recovery of *L. monocytogenes* than the selective MOX ($P<0.05$). Uninjured cells stored at 4°C were recovered better than heat-injured cells on TPA ($P<0.05$). On TPA, *L. monocytogenes* stored in air was recovered better than *L. monocytogenes* stored in N_2 or CO_2/N_2 ($P<0.05$). However, recovery on MOX was best when *L. monocytogenes* was stored in N_2 ($P<0.05$).

P70 OCCURRENCE OF *LISTERIA MONOCYTOGENES* IN MEXICAN CHEESES

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The occurrence of *Listeria* spp. including *L. monocytogenes* were investigated in cheeses obtained from street vendors and retail stores in Mexico City. Three types of cheeses were evaluated, two ripened cheeses from commercial operations and fresh cheeses made at farmhouse scale.

Samples were taken from the surface and inside of the cheeses. Cold enrichment broths, PALCAM agar, *Listeria* selective Oxford medium (Oxford agar) were used to recover *Listeria* spp. from positive cheese samples. Cheeses were analyzed for NaCl, pH, moisture and fat.

Internal samples were the most contaminated. Incidence of *Listeria* spp. in fresh cheeses was 20%: *L. murray* 8%, *L. monocytogenes* 6%, and *L. inoccua* and *L. grayi* 6%. Three serotypes of *L. monocytogenes* were isolated, 1/2a, 1/2b and 4 b. Chihuahua and Manchego cheeses made with pasteurized milk were negatives.

Fresh cheese had the lowest pH and NaCl content and the highest A_w. The possible contamination may come from milk from cows with mastitis.

Fresh cheeses are health hazard for the consumers. Possible source of contamination are the use raw milk in cheese making, unsanitary conditions during processing and post-contamination.

P71 EFFECT OF SIMULATED GASTRIC FLUID AND BILE ON SURVIVAL OF *VIBRIO VULNIFICUS* AND *VIBRIO VULNIFICUS* PHAGE

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Vibrio vulnificus occasionally causes septicemia and gastroenteritis in consumers of raw oysters. Phages that lyse *V. vulnificus* have been isolated from Gulf Coast oysters. Bacteria and phages may be exposed to acid conditions in the

stomach and bile in the intestine. The objective of this study was to determine the survival of *V. vulnificus* and its phage in simulated gastric fluid or bile solutions. Survival of three strains of *V. vulnificus* and three strains of its phage was determined at 37°C after exposure to simulated gastric fluid at pH 3 to 4 or to 0, 1, and 2% bile in broth or buffer. Mean D values (min) at pH 4 and 3 were 3.3 and 1.3 for *V. vulnificus* and 97.8 and 0.7 for its phage. There were little survival differences among strains of *V. vulnificus* or its phage. Numbers of *V. vulnificus* increased 1 log in tryptic soy broth containing 1 or 2% bile after 3 h. Numbers of *V. vulnificus* and its phage remained constant in phosphate-buffered saline regardless of bile concentrations up to 3 h. Stomach acidity survivors of *V. vulnificus* and its phage may proliferate in the small intestine since they are resistant to bile.

P72 IN VITRO EVALUATION OF THE EFFECTS OF NITRITE AND NACL ON THE ANTIMICROBIAL ACTIVITY OF LYSOZYME, NISIN AND EDTA COMBINATION TREATMENTS

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A two level, five factor, full factorial experimental design was used to screen for interactions between the antimicrobials, lysozyme (0, 450 ppm), nisin (0, 450 ppm), EDTA (0, 900 ppm), NaCl (0, 2.7%) and $NaNO_2$ (0, 180 ppm), by F test (a = 0.01). Fourteen organisms of concern in the spoilage or safety of cured meat products were grown in broth media. The absorbance at 450 nm relative to inoculated controls was determined after 72 h.

Agents alone reducing growth: lysozyme (*Brochothrix thermosphacta*, *Pediococcus acidilactici*, *Enterococcus faecalis* and *Weissella viridescens*); nisin (all Gram positive organisms tested, including *Lactobacillus sake* and *Pediococcus pentosaceus*); EDTA (all); NaCl or $NaNO_2$ (*E. coli* O157:H7, *Salmonella typhimurium*, *Serratia grimesii*, and *Shewanella putrefaciens*); $NaNO_2$ (*Lactobacillus curvatus*, *Leuconostoc mesenteroides*, *L. monocytogenes*, and *Staphylococcus aureus*).

Interactions observed: lysozyme and EDTA (*E. faecalis* and *W. viridescens*); nisin and EDTA (all Gram positive organisms); EDTA and NaCl (*E. coli*, *Salmonella*, *Serratia*); EDTA and $NaNO_2$ (*E. coli*, *L. curvatus*, *Leuc. mesenteroides*, *Listeria*, *Salmonella*); nisin and $NaNO_2$ (*Leuc. mesenteroides*, *Listeria*); NaCl and $NaNO_2$ (*Salmonella*, *Shewanella*). All interactions reduced growth.

The results indicate that the use of combinations of antimicrobials will result in a wider spectrum of activity against organisms growing on cured meat products.

P73 FATE OF PGFP-BEARING *ESCHERICHIA COLI* O157:H7 IN GROUND BEEF AT 2° AND 10°C, AND EFFECTS OF LACTATE, DIACETATE, AND CITRATE

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Although beef has been implicated in the largest outbreaks of *E. coli* O157:H7, studies on the fate of the pathogen are limited because of difficulties in detecting the pathogen at levels considerably lower than those of the competing microorganisms. In the present study a green fluorescent protein-expressing strain was used, and the stable marker enabled monitoring the behavior of the pathogen in ground beef stored aerobically from freshness to spoilage at 2° and 10°C. In addition, effects of sodium salts of lactate (dry basis) (SL; 0.9 and 1.8%), diacetate (SDA; 0.1 and 0.2%), buffered citrate (SC; 1 and 2%), and combination of SL and SDA were evaluated. SL, SDA and SL+SDA, but not SC, extended the shelf life of the meat, and combination of 0.9% SL + 0.1% SDA was most inhibitory to the meat spoilage organisms. At 2°C initial numbers of *E. coli* O157:H7 (3 and 5 $\log_{10}$ CFU/g) decreased by ~1 log CFU/g when spoilage was manifest (>7 $\log_{10}$ CFU/g of spoilage microorganisms), irrespective of the treatment. There was no decline in numbers of the pathogen during storage at 10°C. Results show that the pathogen was resistant to the tested salts, and confirm that refrigerated meat contaminated with the pathogen will remain hazardous.

P74 USE OF EXTRACTS OF *NIGELLA SATIVA* (NS) TO INHIBIT SPOILAGE AND PATHOGENIC MICROORGANISMS IN RAINBOW TROUT

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The purpose of this study was to investigate the effectiveness of extracts of black cumin seeds (NS) for control of growth and survival of common spoilage and pathogenic microorganisms in vitro and in a teriyaki marinate preparation for fresh trout. In vitro, the ethanolic extract of NS was found to be highly inhibitory (zone @ 35-55 mm) against *Listeria, Staphylococcus*, *Yersinia* and *Rhodotula,* and moderately inhibitory (zone

@16-28mm) against *E. coli* O157:H7, *Salmonella, Pseudomonas, Lactobacillus,* and *Geotricum.* Freshly harvested trout fillets were stored at 0 (on ice) and at 4°C for up to 60 days using five marinate treatments (teriyaki marinate =control). Trout treated with marinate containing NS oil had significantly lower aerobic, (> 3 log), coliform, and *Listeria* counts ($P < 0.05$). Fresh trout treated with either NS ground seeds or the combination of NS oil and DMDC (dimethyl dicarbonate) exhibited moderate reduction (>1 log) in all counts ($P < 0.05$). DMDC alone was not effective. Since a marinate containing NS essential oil was highly inhibitory to pathogenic and spoilage microorganisms and significantly ($P<0.0001$) increased shelf life by over two weeks, herbal marinates may be used as a processing method for increasing shelf life and marketing of fresh fish.

P75 INHIBITION OF *ESCHERICHIA COLI* O157:H7 BY HERBAL AND SPICE ESSENTIAL OILS

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Escherichia coli O157:H7 is one of the foodborne pathogens that may cause serious complications during foodborne infections. The purpose of this research was to examine the effects of essential oils from anise, angelica, basil, cardamom, carrot, celery, coriander, dill, fennel, black cumin, parsley, oregano, and rosemary on growth and survival of *E. coli* O157:H7. The filter paper disc diffusion method was used to determine the effect of spice and herbal extracts on inhibition of growth. Antibiotic susceptibility disks were used for comparison. Minimum lethal concentrations (MLC) were determined by using the tube dilution method. Inhibition ranged from complete with oregano to no inhibition with carrot oil. Oregano oil showed the greatest inhibition (zone @ 80 mm) (MLC @ 8 ppm) of *E. coli.* Coriander, basil, and dill were also highly inhibitory (zone @ 30-60 mm) (MLC @ 25 - 50 ppm). Anise, celery, cardamom, black cumin, parsley, fennel, and rosemary were moderately inhibitory for *E. coli* O157:H7 (zone @ 12-24 mm) (MLC > 800 ppm). Angelica essential oil showed weak activity (zone @ 12 mm). Since some of the herbal and spice essential oils were highly inhibitory to *E. coli* O157:H7, they may be useful as alternatives or supplements to conventional antimicrobial food additives.

P76 MEMBRANE BIO-CATALYST AS A GROWTH STIMULATOR OF *LISTERIA MONOCYTOGENES* IN ENRICHMENT MEDIA

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The effects of an *Escherichia coli* E-8 oxygen-scavenging bio-catalyst (MF) on growth and lag phase reductions of *L. monocytogenes* (LM) in selective enrichment broth of ISO, USDA, USFDA, and IDF methods were investigated. One hundred mL each of LEB, UVM I and II, Fraser 0.5× and 1.0× supplemented with 0.1, 0.5, and 1.0 units mf/ml in a well of a microtitre plate was inoculated with 200 mL of cell suspension (10-10^5 cells) of LM. The plate was incubated at 35°C and results were read every two h using ELISA plate reader at 405 nm. Esculin and ferric ammonium citrate at concentrations used in Fraser broth were added to all media tested. MF activities against LEB, Fraser, and UVM were about 23, 31, and 24 unit/mL, respectively. The lag phase of LM with MF was shorter (4-8 h) than those without the MF (12-16 h) ($P < .05$). Fraser broth 0.5x supported the fastest growth of LM, while LEB gave the shortest lag phases. The higher the MF concentrations resulted in the faster growth and shorter lag phase of LM compared to those without MF. In conclusion, the MF successfully reduced lag phase and enhanced faster growth of LM in all media of standards methods tested.

P77 COMBINED EFFECT OF ANTIBIOTIC AND COMPETITIVE-EXCLUSION TREATMENT ON *SALMONELLA* ENTERITIDIS FECAL SHEDDING IN MOLTED LAYING HENS

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Salmonella Enteritidis (SE) is an important pathogen for the layer industry, primarily because of its ability to infect hens and ultimately contaminate egg contents. Studies have shown that stress situations such as flock recycling (induced molting) can increase SE problems in the flock. Studies were conducted which examined the use of antibiotics and competitive exclusion (CE) in reducing SE problems during a molt. The treatment comprised of oral administration of enrofloxacin (Baytril; 10 mg/kg/day in drinking water) for 10 days followed by 2 doses of oral CE treatment at 48 and 96 h. The birds were examined for SE shedding 4 and 10 days following the completion of therapy. The SE shed rate was 33%

and 25% in untreated birds 4% and 0% in the Baytril/Aviguard birds on the two test days. These results indicate that treatment of SE-positive laying hens postmolt with Baytril and CE cultures can substantially reduce SE problems due to molting and would be a good alternative to diverting eggs for pasteurization or slaughtering the infected flock.

P78 MECHANISMS OF ANTIBACTERIAL ACTIVITY OF ALLYL ISOTHIOCYANATE

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Allyl isothiocyanate (AITC), a natural compound in plants belonging to the family Cruciferae, has been shown to have strong antimicrobial activity on media as well as in its vapor form. Due to the limited understanding of its antimicrobial mechanism, AITC was tested with *Salmonella montevideo, Escherichia coli* O157:H7 and *Listeria monocytogenes* Scott A at different stages of growth for bactericidal activities. The antibiotics, streptomycin, penicillin, and polymyxin were used in a comparison study with AITC to elucidate its antibacterial activity. After exposure to AITC, extracellular filtrate was collected and concentration of magnesium ion and optical absorbance at 260 and 280 nm were determined. The two gram-negative bacteria, *S. montevideo* and *E. coli* O157:H7 were more sensitive to AITC than *L. monocytogenes*, which is gram-positive. Although optical density of bacterial suspensions did not decrease dramatically, the bacterial viability was reduced. AITC was effective at all bacterial growth stages. The physiological response of test bacteria to AITC was similar to polymyxin. High absorbance at 260 and 280 nm of cellular filtrate indicated that AITC could result in damage to the cell membrane causing leakage of cellular substances.

P79 ENHANCED INHIBITORY EFFECT OF *ESCHERICHIA COLI* O157:H7 BY CHITOOLIGOSACCHARIDE AND MONOLAURIN

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Antimicrobial activity and mode of action of combined chitooligosaccharides (COS) and monolaurin (ML) on *E. coli* O157:H7 was determined. Minimum inhibitory concentration (MIC) values of ML and COS alone were 2,000 *u*g/ml and >2,000 *u*g/ml on 932 strain, and 25 *u*g/ml and 2,000 on 933 strain, respectively. However, MIC values were lower when ML was combined with each COS in dual combination. Also, sublethal amounts of combination ML and COS gave greater inhibition than for the most active compound alone. Thus, MIC results correlated well with growth curves when studying combined effects. Mechanism of the combined effect of ML and COS showed that cells pretreated with COS became more susceptible to ML than those of ML to COS. These results suggested that the cells of O157:H7 received nonlethal damage in the pretreatment with COS and became more sensitive to ML.

P80 EFFECT OF BALSAM APPLE EXTRACT ON BACTERIA

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The Balsam apple (*Momordica* spp.) has anticarcinogenic and blood sugar-lowering effect. Due to the bitter taste, it is mixed with other fruit and vegetable juices for consumption. Little information on its anti-microbial effect is available in the literature. The objective of this study was to determine the effect of Balsam apple extract on bacteria. Paper disks wetted with the extract were placed on nutrient agar plates with bacteria inocula at level of 8 log CFU/ml. The Balsam apple extract exhibited a strong anti-microbial effect. The diameters of clear zones on the plates for tested bacteria were as follows: 0.6 cm for *Bacillus subtilis*, 0.93 cm for *Bacillus cereus,* 1.06 cm for *Escherichia coli,* 1.10 cm for *Proteus vulgaris,* 0.81 cm for *Staphylococcus aureus*, and 1.02 cm for *Salmonella* spp. The results showed that Balsam apple is a potential preservative for beverage products.

P81 WATER ACTIVITY, PH, AND POTASSIUM SORBATE CONCENTRATION EFFECTS ON THE GROWTH/NO GROWTH INTERFACE OF *SACCHAROMYCES CEREVISIAE*

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Probabilistic microbial modeling using logistic regression was used to predict the boundary between growth and no growth of *Saccharomyces cerevisiae* at selected incubation periods (50 or 350 h) in the presence of growth controlling factors such as a_w (0.97, 0.95 or 0.93), pH (6.0, 5.0, 4.0 or 3.0) and potassium sorbate (0, 50, 100, 200, 500 or 1000 ppm). The obtained model predicts the probability of growth under a set of conditions and can be used to calculate critical values of a_w, pH and potassium sorbate concentration needed to inhibit yeast growth for different probabilities.

The reduction of pH increased the number of combinations of a_w and potassium sorbate concentration with probabilities to inhibit yeast growth higher than 0.95. With a probability of growth of 0.05 and using the logistic models, the critical pH values were higher for 50 h of incubation than those required for 350 h. For lower a_w values and increasing potassium sorbate concentration, critical pH values increased. Logistic regression is a useful tool to evaluate the effects of combined factors on microbial growth.

P82 SYNERGISTIC EFFECT OF VANILLIN AND POTASSIUM SORBATE COMBINATIONS TO INHIBIT MOLD GROWTH

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Individual and combined effects of potassium sorbate (SK) and vanillin (V) concentrations on the growth of *Penicillium digitatum*, *P. glabrum* and *P. italicum* in potato-dextrose agar (PDA) adjusted to a_w 0.98 and pH 3.5 were evaluated. PDA agar was prepared with sucrose and citric acid to reach a_w 0.98 and pH 3.5; and the necessary amount of V (0, 100, 200, 300 up to 1300 ppm) and/or SK (0, 50, 100, 150 up to 700 ppm) was added. Plates of each combination and for each mold were centrally inoculated, incubated at 25°C, and periodically observed. Inhibition was defined as no observable mold growth after 1 month. Minimal inhibitory concentrations (MIC) for SK and V were determined, transformed to fractional inhibitory concentration (FIC) and the FIC Index was calculated. MIC of SK varied from 150 ppm for *P. digitatum* to 700 ppm for *P. glabrum*. MIC of V varied from 1100 ppm for *P. digitatum* and *P. italicum* to 1300 ppm for *P. glabrum*. Calculated FIC Index varied from 0.60 to 0.84. FIC index as well as FIC isobolograms (curves deviated to the left of the additive line) show synergistic effects on mold inhibition when V and SK are applied in combination.

P83 MODELING AND SIMULATING GROWTH OF *CLOSTRIDIUM BOTULINUM* AT VARYING INOCULUM SIZE, TEMPERATURE, PH, AND SALT CONCENTRATION

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This project studied the growth and lag time kinetics of *Clostridium botulinum* 56A at inoculum sizes ranging from 1 to 10,000 spores per sample. Our experimental design also included three other variables: temperature, pH and sodium chloride concentration for a total of 81 experimental conditions. *C. botulinum* spores were heat shocked then inoculated into Brain Heart Infusion (BHI) broth. Two hundred mL of inoculated BHI broth was dispensed into each well of a 96 well microplate. The distribution of spores expected in the 200mL samples was also estimated by plating multiple samples on BHI agar. Change in turbidity at 620nm was measured hly for at least 14 days. Turbidity changes were fitted to a Gompertz equation and cell growth rate was estimated. Temperature and salt concentration had the greatest effect on growth rate while inoculum size and pH had more influence on lag time. SAS statistical software was used to predict cell growth rate as a function of temperature, pH, salt concentration and inoculum size. These data, together with spore germination data previously collected in our lab, were used to create a computer simulation which can be used to analyze the risk of *C. botulinum* growth under different conditions.

P84 MODELING THE BACTERIAL SPOILAGE OF READY-TO-DRINK BEVERAGES

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This project studied the ability of spoilage bacteria to grow in cold-filled ready-to-drink beverages. A Box-Behnken experimental design included five variables at three levels. The variables and levels for these experiments were: pH (2.8, 3.3, and 3.8), titratable acidity (0.20%, 0.40%, and 0.60%), sugar content (12.0, 14.0, and 16.0°Brix), sodium benzoate (150, 225, and 350 PPM), and potassium sorbate (150, 225, and 350 PPM) for a total of forty-two beverage samples. Samples were filtered through sterile 0.20 µm disposable filter units and cold-filled into sterile, 50 ml centrifuge tubes. A cocktail consisting of equal proportions of *Acinetobacter calcoaceticus* and *Gluconobacter oxydans* (5×10^5 CFU/ml) was prepared. Duplicate samples were inoculated with the bacterial cocktail (100µl/50 ml) and stored at 25°C. The inoculated samples were plated on Malt Extract agar at time zero and after one, two, four, six, and eight weeks. Regression analysis was conducted on the data using JMP® software. A predictive model was created that quantifies and predicts the growth of spoilage bacteria in response to the various formula factors. Product developers can use the predictive model to estimate microbial stability as they consider formulation options and compare product costs, quality, and flavor impact implications.

P85 USE OF *BACILLUS MEGATERIUM* SPORE GERMINATION AND CELL PARAMETER DISTRIBUTIONS TO PREDICT SPOILAGE TIMES AT LOW INOCULUM SIZE AND DIFFERING ENVIRONMENTAL CONDITIONS

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Low numbers of bacterial spores are typically present in many processed foods. Samples inoculated with low spore numbers exhibit broadly variable spoilage times. We hypothesize that small populations of spores behave randomly, with the variability observed due to inherent properties of population size. The objectives of this research were to study spore germination and cell growth as functions of pH, sodium chloride and inoculum size and, predict time-to-spoilage in a model food system. *Bacillus megaterium* ATCC 14581 spore germination and outgrowth kinetics were observed by microscopy. The environmental conditions tested were pH (4.5-8), sodium chloride (0.5-4% w/v) and inoculum size (1, 10^2, 10^4). Growth curves were produced by automated turbidity instrumentation. Computer simulation was performed using risk analysis software. Spore germination and outgrowth data resulted in negative exponential and logistic distributions. A Poisson distribution described spore inoculum variability. Growth kinetics showed normally distributed growth rates. The distribution values were input to the simulation to validate spore behavior in small populations under varying pH and sodium chloride combinations. The understanding of this behavior and its contribution to the variability of spoilage times can lead to improved mathematical models for microbial food safety and quality.

P86 SURVIVAL OF *ESCHERICHIA COLI* O157:H7 IN DRIED BEEF AS AFFECTED BY WATER ACTIVITY, SODIUM CHLORIDE, AND TEMPERATURE

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The general effect of lowering a_w of high-moisture foods such as raw beef is to retard or prevent microbial growth. Sodium chloride (salt) and other solutes can be used to reduce the a_w of dried beef products. The objective of this study was to determine the rate of inactivation of *E. coli* O157:H7 in a commercially processed beef powder as affected by a_w (0.34 ± 0.06 - 0.68 ± 0.01), salt content (0.05, 3.0, and 20%), and temperature (5 and 25°C) over an 8-wk storage period. Retention of viability of acid-adapted, acid-shocked, and control cells was determined. Overall, there were no significant ($P \leq 0.05$) differences in survival among the three types of *E coli* O157:H7 cells subjected to the same test parameters. At each a_w and within cell type, an increase in salt concentration resulted in significant reductions in the number of viable cells after a given storage time. The rate of inactivation was enhanced at a_w 0.34 ± 0.06 compared to a_w 0.68 ± 0.01 and at 25°C compared to 5°C. Results indicate that factors other than temperature and osmotic stress imposed by salt influence viability of *E. coli* O157:H7 cells in dried beef.

P87 CRITICAL TEMPERATURES TO INHIBIT *ZYGOSACCHAROMYCES BAILII* GROWTH IN MANGO PUREE PRESERVED BY COMBINED FACTORS

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Probabilistic microbial modeling using logistic regression was used to predict the critical temperatures to inhibit, at least during 30 days, *Zygosaccharomyces bailii* growth in mango puree (pH 3.5) formulated with selected a_w (0.99, 0.98 or 0.97) and 1000 ppm of preservative (potassium sorbate or sodium benzoate). Mango purees were inoculated with 10^5 CFU/g *Z. bailii* and incubated at 5, 15 or 25°C. Periodically during incubation the survival count was determined. The probability of growth under a set of conditions and critical temperatures needed to inhibit yeast growth for different incubation times were calculated with the obtained model. With a probability of growth of 0.05 and using the logistic model, the critical temperatures of incubation were higher when potassium sorbate was added than those required using sodium benzoate. Increasing a_w values and with longer incubation times, critical temperatures to inhibit *Z. bailii* growth increased. *Z. bailii* growth in mango puree formulated with a_w 0.97, 1000 ppm potassium sorbate and stored at 5°C was delayed for 30 days and only for 20 days when sodium benzoate was used. Preservative resistant yeast growth control based on the hurdle concept can find in probabilistic microbial modeling a practical approach to evaluate the effects of select factors.

P88 GROWTH AND RECOVERY OF SELECTED GRAM NEGATIVE BACTERIA IN RECONDITIONED WASTEWATER

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We previously reported that *E. coli* O157:H7, *Salmonella* spp., and *V. cholerae* could grow in nutrient-limited, reconditioned wastewater over the temperature range of 4 to 46°C. The BOD

of this water was <2 while its AOC was >2. In this current study, we investigated the growth response of *V. parahaemolyticus*, *Shigella* spp., *V. vulnificus*, and *P. aeruginosa*; both selective and non-selective media were used for their recovery. *V. parahaemolyticus* did not grow or survive at any temperature; *Shigella* spp. did not grow but survived for > 28 days at 4-25°C; *V. vulnificus* did not grow but survived for > 21 days over the entire temperature range; *P. aeruginosa* survived and grew during 14 days at 13-35°C. Non-selective media gave statistically higher recovery than the respective selective media commonly used for these pathogens. These results indicate that caution should be used in attempting direct recoveries of these four Gram negative bacteria from water using selective media.

P89 CONTAMINATION OF COLD-SMOKED FISH WITH *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes is a foodborne pathogenic bacterium that can be found in seafood products. The aim of this work was to determine methods of contamination of cold-smoked fish with *L. monocytogenes*. The study was carried on four factories producing cold-smoked fish. The presence of *L. monocytogenes* was investigated on processing products and environment using an ISO 11 290-1 based method. *L. monocytogenes* strains differentiation was based on serotyping and on resistance to tetracyclin, cadmium and arsenic. *L. monocytogenes* was isolated from 20.7% of fresh eviscerated fish, from 33.6% of finished product and from 14.8% of environmental samples. Incidence of *L. monocytogenes* in finished products varied from less than 9% to 52% depending on factory. Typed strains belong to serogroups 1/2 (92.7%) and 4 (7.3%). Serogroup 1/2 was divided in four different biotypes called A to D. Most of the strains isolated from fresh eviscerated fish samples belong to biotypes A and C. Most of the strains isolated from environment samples belong to biotype B, and finished product samples were contaminated with strains belonging, mainly, to biotypes A, B and C. We conclude that contamination of finished products with *L. monocytogenes* may be related both to raw material and environmental contamination.

P90 THE EFFECT OF TEMPERATURE ON THE SURVIVAL OF *SHIGELLA FLEXNERI* AT LOW PH

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Shigella is a major foodborne pathogen, although rarely isolated from incriminated foods. Its reported ability to survive for long periods at low pH suggests that the bacterium may be acid-tolerant. However, few systematic studies are available. We determined the survival characteristics of *Shigella flexneri* in Brain Heart Infusion broth as a function of pH (2-5) and temperature (4-37°C). Stationary phase cells were inoculated into media to give initial populations of 6-7 $\log_{10}$ CFU/ml. Survival increased as temperature decreased and pH increased. Populations reached undetectable levels (<1.3 log) at 28, 19, 12, and 4°C after 7, 22, 65 and 69 days, respectively, in media of pH 4, and after 3, 9, 16 and 29 days, respectively, in media of pH 3. At pH 2, populations were stable for 4-9 h at 19°C or lower and reached undetectable levels after 30 h, while at 28 and 37°C bacteria were not detected after 8 h. In media of pH 5, bacterial levels decreased only 0.5-1.5 logs after 75 days at 4°C, decreased to undetectable levels after 135 days at 12°C, while growth occurred at the higher temperatures. These results show that *S. flexneri* is acid resistant, and suggest that even acidic foods may serve as vehicles for this bacterium.

P91 MODELS FOR GROWTH OF *ZYGOSACCHAROMYCES BAILII* IN HIGH-ACID FOODS

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Models to predict growth of *Zygosaccharomyces bailii* in high acid foods were developed to provide tools to facilitate spoilage risk assessment and reduction. Measurements of growth at various pH, NaCl, fructose, and acetic acid levels were made over a 29-day period using an automated turbimetric method. Statistical analysis and modeling was done using SAS LIFEREG procedures. Challenge studies in acid products from US retail stores validated the models' predictions. One model predicts days to growth versus combined molar concentration of fructose + salt and undissociated acetic acid and indicates a growth/no-growth boundary similar to that established by Tuynenburg Muys in 1971 that still forms the basis of industry assumptions about the stability of acidic foods. Another model predicts days to growth versus fructose, salt, acetic acid and pH levels varying independently. Predictions from this model agree closely with observations made in laboratory media and in product challenge studies, giving confidence that the model is applicable to acidic foods. The models represent a significant improvement in the tools available. They provide quantitative indications of spoilage potential rather than a simple growth/no-growth indication and the ability to isolate the effects of the independent factors fructose, salt, acetic acid and pH.

P92 SURVIVAL OF *ESCHERICHIA COLI* O157:H7 IN MARGARINE, REDUCED FAT SPREADS AND LIQUID WATER-IN-OIL TOPPINGS

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The ability of *E. coli* O157:H7 to survive, for extended periods, in acid foods such as apple cider, fermented sausage, and selected "oil-in-water" based mayonnaise and salad dressing food emulsions has been well documented. The unusual tolerance of this pathogen to low pH conditions continues to underscore the concern that various acid foods could serve as vectors for EHEC mediated foodborne infection. This study was undertaken to determine the fate of *E. coli* O157:H7 in 5 commercial "water-in-oil" based food emulsions representing the reduced fat "margarine," spread, and liquid topping categories, the principal risk concern being incidental contamination and/or food handling abuse. The aqueous phase pH of the products ranged from 3.6 to 4.5, the salt from 2.4 to 6.2% and the oil content from 37% to 70%. To assess pathogen-clearing ability, the products were challenged with approximately 1×10^5 CFU/g of a 3-strain mixture of *E. coli* O157:H7 and incubated at 5, 10, 15 or 20°C. Sample populations were assayed using Sorbitol MacConkey and Standard Methods Agars. *E. coli* O157:H7 did not grow in any of the products. In all instances, at the least, a 2 to 3 log drop in *E. coli* O157:H7 numbers was observed by day 3 and a 3 to 5 log drop by day 6. Although only minor differences in EHEC neutralization ability were noted, aqueous phase pH and acidulant type (lactic acid > citric acid) appeared to exert the greatest impact. In only one instance, a 70% oil/fine water droplet emulsion system, did lower storage temperatures noticeably decrease the EHEC neutralization rate. Increasing potassium sorbate level, present at concentrations ranging from 0.12 to 0.20%, also appeared to enhance lethality.

P93 GROWTH RESPONSE OF *LISTERIA MONOCYTOGENES, SALMONELLA* ENTERITIDIS AND *SALMONELLA TYPHIMURIUM* DT104 IN PASTEURIZED AND RAW LIQUID WHOLE EGG HELD AT CHILL ABUSE

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The purpose of this study was to determine the effect of temperature abuse on the outgrowth of *L. monocytogenes* (LM), *Salmonella* Enteriditis (SE), and *Salmonella typhimurium* DT104 (ST-DT104) in both commercially pasteurized and raw whole egg. Same day pasteurized whole egg was obtained from a local egg breaking facility. Grade A shell eggs were sanitized with 70% ethanol, aseptically broken, pooled and "homogenized" using a hand-held mixer. A portion of the latter was also used to prepare a scrambled egg mix. Washed cells from 2 culture strains of SE, LM and ST-DT104 were individually inoculated into bulk whole egg samples, to deliver 1 to 10 CFU/ml of egg, and 100 ml volumes of each incubated at 2, 5, and 10°C for 14 days. Samples were taken daily and surface plated on XLD (*Salmonella*), MOX (*Listeria*), and Standard Methods Agar (all). In the pasteurized whole egg samples both SE and ST-DT104 strains grew well at 5 and 10°C but not at all at 2°C. A 2 log increase was recorded in 4 to 5 days, and a 3 to 5 log increase in 7 to 9 days, at 5°C, with a 4 to 5 log increase observed in 7 days at 10°C. The 2 LM strains grew at all three temperatures showing a 2-3 log increase in 13 days at 2°C, 7 to 9 days at 5°C and 5 to 6 days at 10°C. In the fresh whole egg sample sets, both SE and LM strains failed to grow at any of the temperatures trialed, while ST-DT104 numbers steadily decreased, during the 14 day study period. In the scrambled egg mix, the addition of milk to raw whole egg had little effect on LM inhibition but did obviate *Salmonella* inhibition. A significant impact on the outgrowth of the SE, ST-DT104, and LM strains in pasteurized whole egg was made by adding back lysozyme, conalbumin, avidin and ovoflavoprotein, all heat sensitive components of egg albumen.

P94 MODULATION EFFECTS OF ANTIOXIDANT VITAMINS ON OCHRATOXIN A-INDUCED OXIDANTIC TOXICITY IN MICE

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Ochratoxin A(OA), a naturally occurring mycotoxin, has been known to cause renal and hepatic lesions in humans and animals. This study was carried out to investigate the modulation effects of antioxidant vitamins on OA-induced lipid peroxidation associated with oxidative damage. Vitamins C (10 mg/kg/day) and E (63.8 mg/kg/day) were administered by intraperitoneal (i.p.) injection to male ICR mice, and 1 h later, OA (4 mg/kg/day) was injected into mice by i.p. During 4 successive days, superoxide dismutase (SOD) activity, catalase activity and malondialdehyde (MDA) formation in microsomes of liver and kidney were measured. Additionally, the relationship between cell damage and modulation effects of antioxidant vitamins was evaluated by a comet assay. Results were as follows; (i) 0 SOD, catalase activity and MDA level were significantly increased by OA treatment, (ii) SOD, catalase activity and

MDA formation were significantly decreased by antioxidant vitamins combine treated, (iii) blood cell damage associated with lipid peroxidation, induced by OA, also modulated by antioxidant vitamins. These results indicated that antioxidant vitamins might be used for prevention of renal and hepatic damage due to ochratoxicosis.

P95 DRY REHYDRATABLE FILM METHOD FOR THE RAPID ENUMERATION OF *STAPHYLOCOCCUS AUREUS* IN FOODS: 3M PETRIFILM RAPID *S. AUREUS* COUNT PLATES

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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. The 3M™ Petrifilm™ Rapid *S. aureus* Count (RSA) plate has been developed in order to provide quantitative, confirmed *S. aureus* results from food within 26 to 30 h using Petrifilm plate and thermostable nuclease detection technology. The performance of the method was demonstrated by comparing the counts from inoculated and naturally contaminated foods using both the Petrifilm RSA plate and the FDA *Bacteriological Analytical Manual* (BAM) methods. For comparison to the Petrifilm plate method, the BAM method enumerated *S. aureus* on Baird-Parker agar followed by tube coagulase confirmation of positive presumptive colonies. The mean log difference (log RSA – log BAM) for a group of 22 inoculated food types was -0.002, -0.051, and 0.017 at the low, medium, and high inoculum levels, respectively. Regression analysis for 22 naturally contaminated food types showed a correlation coefficient of 0.95, a y-intercept of 0.05, and a slope of 0.95. The mean log difference was 0.05. These results suggest that the Petrifilm RSA plate method gave similar, quantitative, confirmed results in approximately one-third the time of the BAM method.

P96 MICRORESTRICTION FINGERPRINTING: A NEW TOOL FOR STUDYING THE MOLECULAR EPIDEMIOLOGY OF *ESCHERICHIA COLI* O157:H7

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Microbial subtyping methods have become an invaluable tool in the epidemiology of infectious diseases. Here, we describe a method for interstrain differentiation of *E. coli* O157:H7 that, unlike others, is based on cutting the genome of interest into very small fragments. This is achieved by the use of restriction endonucleases with very frequent recognition sites (four base cutters), or restriction digestion using two enzymes, which results in digestion of a major portion of the genome in very small fragments (less than 2 kb). However, depending on the enzyme, there will be a few larger fragments in the approximate size range of 1-18 kb. These larger fragments form a very differentiative RFLP pattern.

Using a set of *E. coli* O157: H7 cultures of known epidemiological history, several endonucleases were tested to determine their utility in interstrain differentiation of *E. coli* O157:H7 isolates. Among the restriction enzymes tested, *Sau*3A and *Hae*III were found to have the highest discriminatory power.

A collection of 30 isolates from two multistate outbreaks were tested with the method (20 isolates from the 1992 restaurant associated multistate outbreak and 10 isolates from the 1994 salami related outbreak). The 10 isolates from the salami related outbreak had identical MRF patterns with each of the two enzymes. Twenty *E. coli* O157:H7 isolates from the 1992 outbreak which had an identical PFGE pattern (Using *Xba*I, data not shown) were tested by the MRF method. The 20 isolates had identical RFLP patterns with the two enzymes. Twenty-five isolates with no apparent epidemiological, geographic or spatial linkage were tested with the two enzymes. The results of the two enzymes (*Sau*3A, and *Hae*III) when combined divided the 25 isolates into 25 groups. Six *E. coli* O157:H7 strains from three outbreaks that were untypeable using PFGE (Timothy Barrett, CDC, personal communication) were typed by the PFGE and MRF method. Several attempts at typing the strains using the PFGE method failed to produce a RFLP pattern. All six isolates were typeable using the MRF method. Each of the three pairs of isolates showed a unique MRF pattern. To investigate the stability of the RFLP pattern an *E. coli* O157:H7 strain was consecutively passed in culture fifty times. The MRF pattern of the frozen stock culture was identical to the culture after 50 passages.

P97 MODEL FOR THE IMPLEMENTATION OF HACCP IN THE FOOD INDUSTRY OF DEVELOPING COUNTRIES

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Many food processors from our country have still not implemented HACCP, and they probably will not implement it unless it becomes mandatory. However, even if HACCP is not included in the food law, regulatory agencies need to encourage the use of HACCP in the food industry. This study presents a model of cooperation between public

health officials, food industry technologists, and HACCP and academic experts for facilitating the use of HACCP in small and medium size food processors. The process starts with workshops where preliminary HACCP models for high risk or high consumption foods are given. Models are subsequently tested in selected industries, reviewed, and adjusted. Then generic HACCP models are prepared for teaching similar processors.

This collaborative approach is being applied in two areas of Columbia, and is beginning to show benefits. First, experts from industry, regulatory and academia are developing a new relationship; second, food safety and HACCP concepts are now being widely discussed and incorporated; third, foods are becoming safer. The models being prepared are basic. Further improvement of these models will require more technical expertise to be provided by international food safety experts, HACCP authorities, and HACCP organizations.

TECHNICAL SESSION

T1 REDUCTION OF *ESCHERICHIA COLI* O157:H7 CONCENTRATIONS IN RUMINAL CONTENTS IN VITRO; BACTERIOCIDAL EFFECT OF SODIUM CHLORATE

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Preharvest control of *E. coli* O157:H7 is a priority to the food industry. Since chlorate, an analog of nitrate, is reduced by respiratory nitrate reductases (enzymes possessed by *E. coli* and other enterobacteria) to cytotoxic chlorite, we conducted an experiment to see if chlorate could rid *E. coli* O157:H7 from ruminal contents, a reservoir of the pathogen. Bovine ruminal contents, inoculated with a novobiocin and nalidixic acid resistant *E. coli* O157:H7, were mixed (1:1) with a phosphate buffer (pH 6.2 or 6.8) supplemented with cellobiose, glucose, soluble starch, and xylose (1% wt/vol each). These were incubated (39°C) anaerobically with sodium chlorate as indicated. *E. coli* O157:H7 concentrations ($\log_{10}$ CFU/ml), determined via colony counts (MacConkey's plus novobiocin and nalidixic acid; 25 and 20 μg/ml, respectively), declined from initial levels (5.9) to 5.7 and 5.1 following 24 h incubation of the cultures (pH 6.2 and 6.8, respectively) without added chlorate. Chlorate addition (1.25 or 5 mM) effected little the most probable number of total culturable anaerobes (ranging from 9.9 to 10.7 $\log_{10}$ CFU/ml) but markedly reduced the 24 h concentrations of *E. coli* O157:H7 to ≤10 CFU/ml. Thus chlorate was bacteriocidal to *E. coli* O157:H7 but not to potentially beneficial bacteria.

T2 INCIDENCE OF *ESCHERICHIA COLI* O157:H7 IN FROZEN BEEF PATTIES PRODUCED OVER AN EIGHT H SHIFT

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The microbiology laboratory of a ground beef patty processor found *E. coli* O157:H7 in five production lots using a commercial PCR assay. Detection of *E. coli* O157:H7 in several lots during a six-week period stimulated a research project to determine the incidence of the pathogen and its potential entry points during processing. During one shift, 119,000 lbs (71 pallets) of patties were produced. Three laboratories analyzed 710 patty samples using two different methods. Two outside laboratories employed immunoassay screening tests; the other used PCR (BAX® for Screening *E. coli* O157:H7). Indicator organisms were estimated in one sample portion from each pallet to determine the relation of indicators to *E. coli* O157:H7 contamination. Of the 710 samples tested, a total of 53 cultural-confirmed results were observed. The PCR assay detected 29 cultural-confirmed positives, while the two immunoassay methods detected 19 and 5, respectively. The highest incidence of contamination was observed in a 1.5-h period, where 77% (34) of the cultural-confirmed isolates were detected. Indicator microbial populations were extremely low for these ground beef products. This study indicated that testing of larger analytical units, the use of genetic screening technologies such as PCR and improved confirmation methods may be necessary to detect low levels of *E. coli* O157:H7 in ground beef products consistently.

T3 COMMERCIAL EVALUATION OF MULTIPLE-SEQUENTIAL INTERVENTIONS FOR DECONTAMINATION OF BEEF CARCASSES

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The objective of this study was to evaluate the efficacy of multiple interventions in reducing beef carcass microbiological contamination during commercial application. Evaluation involved sponge swabbing of the flank, brisket and rump regions combined (300 cm^2) of 40 carcass sides at various in-plant locations in each of 8 packing plants. Samples were analyzed for total plate counts (TPC), total coliform counts (TCC) and

Escherichia coli counts (ECC), as well as for the presence of *Salmonella* spp. Mean (log_{10} CFU/cm^2) TPC, TCC and ECC on the exterior hide were in the ranges of 6.2 to 10.5, 4.0 to 5.9 and 3.5 to 5.5, respectively, while corresponding levels of contamination on carcass surfaces following hide removal but prior to the application of any decontamination intervention were in the ranges of 4.1 to 7.1, 1.0 to 4.0 and 0.6 to 3.3, respectively. After decontamination and a 24 to 36 h chilling period, mean TPC, TCC and ECC on carcass surfaces were 0.3 to 3.3, -1.1 to -0.7 and -1.1 to -1.0, respectively. Presumptive *Salmonella* isolation rates decreased from 14.7% before to 1.9% after carcass decontamination. Results support the concept of multiple intervention decontamination processes as a means of improving the microbiological status of beef carcasses.

T4 VERIFICATION OF THE EFFECTIVENESS OF A SECOND GENERATION STEAM PASTEURIZATION™ SYSTEM FOR DECONTAMINATING PRE-RIGOR BEEF CARCASS SIDES IN A COMMERCIAL SLAUGHTER FACILITY

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Previous studies demonstrated the antimicrobial effectiveness of the Steam Pasteurization™ System on pre-rigor beef carcass sides in commercial beef slaughter facilities. The first generation units (SPS 400) were modified by eliminating the internal moving car, forming a static steam chamber. This second generation system (SPS 400-SC) was tested in a commercial beef slaughter facility at seven temperatures (71.1°, 73.9°, 76.7°, 79.4°, 82.2°, 85.0°, and 87.8°C). For each temperature, samples were excised from the midline of 20 carcass sides immediately before and after steam pasteurization. Total aerobic bacteria were reduced by at least 1.4 logs at 85.0°C and 87.8°C from an initial mean level of 1.8 log CFU/cm^2. At 82.2°, 85.0°, and 87.8°C, total coliforms, generic *Escherichia coli,* and Enterobacteriaceae were reduced to detectable levels (detection limit of 0.42 CFU/cm^2). Bacterial reductions in the second generation unit were comparable to the first generation unit. The Steam Pasteurization™ Systems offer processors a reliable and effective method for reducing bacterial populations on beef carcasses, and can be used as a critical control point in HACCP programs.

T5 EFFECTIVENESS OF POTASSIUM LACTATE AND LACTIC ACID AGAINST *CAMPYLOBACTER* ON CHICKEN BREASTS

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Campylobacter is the most frequently associated foodborne pathogen on poultry. This study examined the efficacy of potassium lactate (KL) and lactic acid (LA) to control *Campylobacter*. Boneless, skinless chicken breasts were injected with three levels of KL (0, 1.5, 2%), in conjunction with four levels of LA. The LA was injected (0, 0.1, 0.2, 0.3%) as well as applied directly to the surface (0.1%). The chicken fillets were surface inoculated with a mixture of *Campylobacter* species to obtain 3.0 log CFU/ml. The greatest inhibition was found using 2% KL in conjunction with either 0.3% LA (injected) or 0.1% LA (surface application), with greater than 1.0 log difference than the positive controls which received no treatment. The 2% KL and 0.1% LA surface inoculation treatment was significantly different than all treatments containing 0% KL and 1.5% KL with less than 0.3% LA ($P<0.05$). The 2% KL and 0.3% LA treatment was significantly different than all treatments containing 1.5% KL with less than 0.2% LA, except for the 0.1% surface application of LA ($P<0.05$). Results of this study indicate that KL and LA can be used to control the growth of *Campylobacter* on boneless chicken breasts.

T6 CHLORINATION OF CHILL TANKS REDUCES SALMONELLAE ON PROCESSED BROILER CARCASSES

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To reduce salmonellae and other bacterial contamination on processed broiler chicken carcasses, the majority of the US Poultry Industry have instituted similiar processing techniques over the last 5 years. One of these changes is to increase the initial level of chlorine in the chill tank from 20 ppm or below to a current usage of from 35 to 50 ppm. In 1983, FSIS scientists looked at 9 federally inspected processing plants and found 5.5% of carcasses entering the chill tank and 11.6% of carcasses exiting the chill tank to be contaminated with salmonellae. In the present study, samples were collected from 4 different processing plants. Sixty randomly selected carcasses were sampled for salmonellae from each of 12 flocks.

Overall, 239 of 720 (33%) pre-chill carcasses were salmonellae positive compared to 86 of 720 (12%) post-chill carcasses, and lower levels of salmonellae were seen from all 12 groups after chilling. These data demonstrate that when 35 ppm or greater chlorine is used in the water entering the chiller that the previously observed salmonellae cross-contamination between carcasses is basically eliminated and that overall levels of salmonellae on the final processed carcass are reduced.

T7 CROSS-CONTAMINATION MODEL FOR *SALMONELLA* IN POULTRY CHILLING PROCESS

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Microbial cross-contamination during chilling is a major concern to the poultry processors. The objectives of this research were to determine the effects of time, temperature and initial *Salmonella* level on cross-contamination and develop a predictive microbial model. Chicken skins from pre-chill chicken carcass were mounted on skin holders and then placed in chiller water. Chicken skins or chiller water was inoculated with *Salmonella typhimurium* to study bacterial transfer between water and skin. *Salmonella* in skin and water samples was enumerated with Most Probable Number Method. The results showed that bacterial transfer occurred at the beginning of chilling, and the *Salmonella* number reached equilibrium between water and skin in 5 s. The model was determined as: $\log N_t = (A + B \log N_0 + C (\log N_0)^2 + D (\log N_0)^3)/(1 + e^{k(q-t)})$, where N_t is transferred cell number (CFU/skin or CFU/L); N_0 is the inoculated cell number on skin (CFU/skin) or in water (CFU/L); A, B, C and D are parameters depending on initial cell number and transfer route; k and q are constants. Temperature in the range of 4 to 20°C did not affect bacterial transfer significantly.

T8 A COMPUTER SOFTWARE APPLICATION OF ASSESSING THE RISK AND SEVERITY OF *SALMONELLA* AND *CAMPYLOBACTER* INFECTIONS FROM POULTRY PRODUCTS

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The Poultry Food Assess Risk Model for Human Pathogens or Poultry FARM is a simulation model that assesses the risk and severity of *Salmonella* and *Campylobacter* infections from poultry products produced by specified hatch to table scenarios. Poultry FARM is constructed in an Excel spreadsheet and is simulated using @Risk. A sequence of pathogen events (contamination, growth, and reduction) is used to model the change in pathogen load of poultry servings as they move from hatch to table. Users define the incidence and extent of pathogen events and variability of infection dose. During simulation of Poultry FARM, model settings entered by users and random numbers generated by @Risk are used to calculate the change in pathogen load of poultry servings as they move from hatch to table. In addition, model settings and random numbers are used to calculate the risk and severity (infected, sick, doctor, hospital, chronic sequelae, death) of *Salmonella* and *Campylobacter* infections from consumption of the poultry servings simulated. By simulating and comparing different hatch to table scenarios, users can develop control strategies to optimize management of the risk associated with human pathogens that contaminate poultry products.

T9 CHANGES IN THE NATIVE MICROFLORA, WEIGHT, AND PH OF THE CECA OF TURKEYS SUBJECTED TO FEED WITHDRAWAL

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The effects of feed withdrawal (FW) and crating on the weight, pH, and bacterial flora of the ceca of turkeys were examined. Ceca from turkeys subjected to FW for up to 24 h (on litter or in crates) were weighed then blended in distilled water. The pH of cecal suspensions were measured; and aerobes, Enterobacteriaceae, and lactic acid bacteria were enumerated. No significant changes in cecal weight occurred during FW. The pH of cecal samples from turkeys not subjected to FW was 6.48, whereas the pH of samples from turkeys subjected to FW for 24 h on litter or in crates was 6.51 or 7.03, respectively. Cecal aerobes in turkeys subjected to 24 h FW on litter increased log 1.69 colony-forming-units (CFU)/g, while there was a log 0.99 CFU/g increase in turkeys in crates. Enterobacteriaceae increased log 0.69 CFU/g in turkeys subjected to FW on litter, but there was no change in this population in turkeys in crates. In contrast, cecal lactic acid bacteria in turkeys subjected to 24 h FW decreased log 0.95 CFU/g in turkeys on litter and log 1.36 CFU/g in turkeys in crates. Findings indicate that FW effects the cecal microflora of turkeys and effects differ on litter and in crates.

T10 USE OF WHEY-BASED EDIBLE FILM CONTAINING ANTIMICROBIAL AGENTS TO INHIBIT *LISTERIA MONOCYTOGENES* IN FRANKFURTERS

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The ability of edible film containing organic acids to inhibit *L. monocytogenes* was examined in all-beef frankfurters. Frankfurters were treated with edible film containing 0.3% sorbic or 0.3% proprionic acid and inoculated with *L. monocytogenes* at 10^5 to 10^7 CFU/ml. Immediately following treatment, frankfurters were vacuum sealed and stored at 4° and 13°C. Frankfurters were analyzed at 1, 4, 7, 14, 28, and 56 days for the presence of *Listeria*, Lactic Acid Bacteria, Aerobic Plate Count, yeasts, and molds. After 7 days of storage, *Listeria* counts were reduced from 7.8×10^6 CFU/ml to 9.6×10^3 CFU/ml in frankfurters containing edible film with sorbic or proprionic acid in comparison to control frankfurters. Likewise, after 28 days, the counts were reduced from 9.2×10^{10} to 1.2×10^6. Aerobic Plate Counts were (reduced from 9.1×10^{10} CFU/ml to 7.8×10^9) CFU/ml in frankfurters containing edible film. In the presence of edible film, an increase in Lactic Acid Bacteria was observed after 4 days of storage. However, yeasts and molds exhibited no signs of growth until 28 days of storage. Moreover, sorbic acid was shown to be more effective at inactivating *L. monocytogenes* than proprionic acid.

T11 LEVELS OF MICROBIOLOGICAL CONTAMINATION OF PORK CARCASSES DURING SLAUGHTER

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The objective of this study was to develop microbiological profiles for pork carcasses under a variety of swine processing systems currently used in United States slaughtering plants, as they initiated operation under the new inspection regulations. In addition, the 3-site (ham, belly and jowl) surface sampling protocol was compared with a 2-site (belly and jowl) surface sampling protocol. Data were collected in twelve geographically dispersed pork packing facilities on two separate visits (May-June and November-January). Samples were analyzed for aerobic plate counts (APC), total coliform counts (TCC) and *Escherichia coli* counts (ECC), and for incidence of *Salmonella* spp., *Campylobacter jejuni/coli, Listeria monocytogenes* and *Yersinia enterocolitica*. *Campylobacter jejuni/coli,* was the most commonly found (7.9%) pathogen in samples taken from chilled carcasses, followed by *L. monocytogenes* (5.0%), *Salmonella* spp. (4.6%) and *Y. enterocolitica* (0.9%). The 3-site sponge sampling protocol was more effective than the 2-site sponge sampling protocol in detecting *Salmonella* spp., with an incidence of 4.6% and 2.1% on chilled carcasses, respectively. Mean (log CFU/cm^2) APC, TCC and ECC were generally lower during the winter than the summer season, and lower for market hogs than sows. The application of a 2% lactic acid spray on carcasses following the final carcass washing cabinet was effective in reducing mean APC (log CFU/cm^2).

T12 EXTENT OF MICROBIOLOGICAL CONTAMINATION ON PORK VARIETY MEATS

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The objective of this study was to develop a microbiological profile for United States pork variety meats, by determining aerobic plate counts (APC), total coliform counts (TCC) and *Escherichia coli* counts (ECC) and the incidence of *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni/coli* and *Yersinia enterocolitica*. Samples of cheek meat, head meat, salivary gland, tongue, liver, heart, kidney, stomach, chitterlings, bungs and front feet were collected for analysis from 10 geographically dispersed pork packing facilities. Contamination was relatively high for most variety meats sampled, with mean APC ranging between 4 and 7 log CFU/g, but with hearts being the exception and having a mean APC of 3.4 log CFU/g. Means of TCC and ECC ranged between 1.7 to 4.6 and 1.1 to 4.3 log CFU/g, respectively. *Yersinia enterocolitica* was not detected on pork variety meats, and *C. jejuni/coli* was detected in fewer than 1% of variety meat samples. *Salmonella* spp. and *L. monocytogenes* were the most frequently detected pathogens, with isolation rates of 15% and 16%, respectively. The results indicated that there may be a need for proper sanitation procedures, good manufacturing practices and application of decontamination interventions to improve the microbiological status of pork variety meats.

T13 MODELING THE GROWTH BOUNDARY OF *STAPHYLOCOCCUS AUREUS* FOR RISK ASSESSMENT PURPOSES

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Knowing the growth boundary of *Staphylococcus aureus* is critical for food-safety risk assessment. Previous studies have led to the presumption that *S. aureus* is osmotolerant. However, most studies and resulting models have focused on growth-kinetics using NaCl as the humectant. In this study, glycerol was used to investigate the effects of a glass-forming nonionic humectant to avoid specific metabolic aspects of membrane ion transport. The experiments were designed to produce a growth boundary model as a tool for risk assessment. The effects and interactions of RH (84-95% adjusted by glycerol), initial pH (4.5-7.0 adjusted by HCl), potassium sorbate (0-1000 ppm) and calcium propionate (0-1000 ppm) on the aerobic growth of a five strain *S. aureus* cocktail in Brain Heart Infusion broth were explored. Inoculated broths were distributed into microtiter plates and incubated at 37°C over appropriate saturated salt slurries to control RH. Growth was monitored by turbidity, measured over a six-month period, and confirmed by plate counts. The 1536 generated data points were analyzed by SAS LIFEREG procedures which showed all studied parameters significantly affected the growth responses of *S. aureus* with interactions between RH and pH. The resulting growth/no growth boundary will be presented.

T14 RESPONSE SURFACE MODELS FOR EFFECTS OF PREVIOUS SODIUM CHLORIDE AND TEMPERATURE ON GROWTH KINETICS OF *SALMONELLA TYPHIMURIUM* ON COOKED CHICKEN BREAST

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Response surface models were developed for effects of previous NaCl (0.5 to 4.5%) and temperature (10 to 40°C) on lag time (l) and specific growth rate (μ) of *Salmonella typhimurium* on cooked chicken breast (CCB). Fifty-five growth curves for model development and 16 growth curves for model validation were fit to a two-phase linear growth model to obtain direct estimates of l and m of *S. typhimurium* on CCB. Response surface models for natural logarithm transformations of l and m as a function of previous NaCl and temperature were obtained by regression analysis. Previous NaCl did not alter ($P > 0.05$) or interact with temperature to alter subsequent growth kinetics of *S. typhimurium*. However, l and m of *S. typhimurium* were affected ($P < 0.0001$) by linear and quadratic effects of temperature. Models were validated against data not used in their development. Mean absolute relative error of predictions (model accuracy) was 26.6% for l and 15.4% for m. Median relative error of predictions (model bias) was 0.9% for l and 5.2% for m. Results indicated that the models developed accurately predicted growth kinetics of *S. typhimurium* on CCB within the matrix of factors modeled.

T15 BACTERIOPHAGE ACTIVITY AGAINST *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP.

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Control of foodborne pathogens is of utmost importance to ensure the safety of our food supply. In recent years the emergence and reemergence of virulent forms of foodborne pathogens including *Salmonella* spp. and *E. coli* O157:H7 has increased the need for development of effective hurdle systems for foods; to include novel biological and physico-chemical methods. Some natural antimicrobial agents have a unique advantage over many other preservatives because of the low level of their toxicity to humans. Among natural antimicrobial agents bacteriophage has the potential to bring about rapid lysis of bacteria. Phages also survive harsher environments compared to bacteria. Hence, they are ideal for inclusion in hurdle systems. In this study sewage samples were passed through 0.22m filter, adsorbed on *E. coli* O157:H7 and plated using an agar overlay method. Electron Microscopy of isolated bacteriophages revealed that STU3, ETU1, and ETU2 were structurally different. Also, ETU1 and ETU2 are virulent against *E. coli* O157:H7 and STU3 is active against *E. coli* O157:H7 and *Salmonella* spp. These phages were also checked for virulence against many other strains of *E. coli* and *Salmonella*. Phages are currently being investigated for applications in food systems.

T16 EFFECT OF CHLORINE TREATMENT ON HEAT INACTIVATION OF *ESCHERICHIA COLI* O157:H7

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The effect of sublethal chlorine treatment on thermal inactivation of *Escherichia coli* O157:H7 was determined. D_{58} values were calculated for stationary phase cells treated with 0.6 mg/l total available chlorine and untreated cells in commer-

cial shelf stable apple juice (pH=3.6). D_{58} values (mins) for untreated and chlorine treated cells in buffer were 5.45 and 1.65 respectively (P<0.01). Death curves of chlorine treated and untreated cells in apple juice were non-linear. Untreated cells heated in apple juice exhibit a three min delay before onset of linear inactivation, whereas chlorine exposed cells exhibit a sharp decline between 0 and 0.5 mins followed by a linear inactivation. D_{58} values (mins) taken from the linear portions of the death curves are 0.77 for untreated cells and 1.19 for chlorine treated cells (P=0.05). The linear portions represent 1-5% and 19% of the population for treated and untreated respectively. Time (mins) required to kill the initial 90% of cells is 3.14 for unexposed versus 0.3 for chlorine exposed cells (P<.001). Thus approximately one to five percent of the total population exhibit marginal induced thermotolerance, but chlorine treatment also promotes the heat inactivation of *E. coli* O157:H7 by eliminating the delayed onset of linear kill.

T17 APPLICATION OF TREATMENTS TO REDUCE CONTAMINATION OF PORK VARIETY MEATS

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The objective of this study was to determine the effectiveness of decontamination treatments in reducing aerobic plate, total coliform, *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes,* and *Yersinia enterocolitica* counts on pork variety meats (cheek meat, heart, liver, tongue, stomach, chitterlings and salivary gland). Decontamination treatments evaluated included immersion in solutions of chlorine, hydrogen peroxide, trisodium phosphate, hot water (75-80°C), acetic acid, lactic acid or acidified sodium chlorite immersions, or spraying with hot water (75-80°C), steam, acetic acid, or lactic acid. Immersion in chlorine did not reduce bacteria counts, while hot water, steam and hydrogen peroxide treatments resulted in discoloration of the red variety meats. Immersion in hydrogen peroxide generated a foam that interfered with application and product packaging. The highest reductions in contamination were obtained with trisodium phosphate, acetic acid and lactic acid. Lactic acid immersion was the most effective and consistent (> 2 log CFU/g reductions) for reducing *Salmonella* spp., *L. monocytogenes* and *Y. enterocolitica. L. monocytogenes* was more resistant to decontamination treatments than *Salmonella* spp., while *Y. enterocolitica* was the least resistant. Use of decontamination treatments that are efficacious for reducing microbiological contamination on carcasses will reduce the microbiological contamination on pork variety meats.

T18 INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* ON APPLES USING OZONE, CHLORINE DIOXIDE, SODIUM HYPOCHLORITE AND PERACETIC ACID

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The ability of ozone (3 ppm), chlorine dioxide (4 ppm), sodium hypochlorite (200 ppm) and peracetic acid (80 ppm) to reduce populations of *L. monocytogenes* and *E. coli* O157:H7 in an aqueous model system and on inoculated apples was determined. Samples of each sanitizer solution were inoculated to contain ca. 10^7 CFU/ml of either pathogen after which aliquots were removed at 30 s intervals over a period of 5 min and appropriately plated to determine D-values. Alternatively, whole apples were inoculated by dipping to contain ca. 10^5 *L. monocytogenes* or *E. coli* O157:H7 CFU/g, dried overnight, submerged in each sanitizer solution for 5 min and then examined for survivors. In the model system, populations of both pathogens decreased > 5 logs following 2 to 5 min of sanitizer exposure. Based on D-values, ozone was most effective (15 s) followed by chlorine dioxide (19-21 s), sodium hypochlorite (25-27 s) and peracetic acid (70-75 s). All sanitizers except peracetic acid completely inactivated both pathogens on inoculated apples after 2 to 5 min of exposure.

T19 MICROBIAL REDUCTION OF LABORATORY INOCULATED PRODUCE SURFACES BY RINSING AND WIPING WITH PAPER TOWELS AND COMPARISON TO 200 PPM CHLORINE DIP

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In previous studies of produce, it was shown that if the surfaces are contaminated, then cutting may result in contamination during slicing. Several investigators have stated that lettuce leaves should be washed individually to reduce the risks. Many consumers rinse and air dry produce. In the experiments described here, 18 different types of produce, many of which are commonly eaten raw, were contaminated with inoculum consisting of either tryptone soya broth or hamburger meat containing marker bacteria and assayed for the effectiveness of rinsing, rinsing and wiping with paper towels or dipping in 200 ppm sodium hypochlorite solution. The different inoculum types contained either *Serratia marcescens, E. coli* O157:H7, or a cocktail of *Salmonella*

typhimurium, Listeria monocytogenes, Escherichia coli O157:H7 and *S. aureus* at inoculum levels of approximately 10^8 CFU. Independent of contamination type, there was a significant difference between water rinsing of contaminated produce and rinsing and drying with paper towels ($P< 0.05$). Approximately 2.5 log reduction was seen with rinsing and wiping of smooth produce with around 1.8 log reduction found on leafy vegetables with diminishing effectiveness shown on produce having complex surfaces. With the difficult to remove hamburger inoculum, rinsing and wiping was superior to the chlorine dip.

T20 EFFICACY OF ULTRAVIOLET LIGHT TO ELIMINATE *ESCHERICHIA COLI* O157:H7 IN UNPASTEURIZED APPLE CIDER

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Due to recent outbreaks of illness from *E. coli* O157:H7 associated with consumption of unpasteurized apple cider, alternative means of assuring the safety of unpasteurized cider have been sought. This study examined the efficacy of ultraviolet light for the elimination of *E. coli* O157:H7 in cider. Cider inoculated with a five-strain mixture of acid resistant *E. coli* O157:H7 (6.3 log CFU/ml) was pumped through a thin-film ultraviolet disinfection unit. Variations in UV dosage were accomplished by adjusting the flow rate of cider. Reduction was dependent on flow rate as well as the level of background microflora in cider. Populations of *E. coli* O157:H7 in cider with <1.30 log CFU/ml of background flora were reduced to undetectable levels at flow rates of 0.264 GPM. At flow rates of 1.713 GPM and with background flora of 3.68 log CFU/ml, *E. coli* O157:H7 was reduced from 6.32 log CFU/ml to 2.76 log CFU/ml. Results of this study indicate that ultraviolet light can be an effective method for the elimination of *E. coli* O157:H7 from apple cider. However, results will vary with the level of background microflora as well as the specific equipment used.

T21 INHIBITION OF GROWTH AND AFLATOXIN PRODUCTION OF *ASPERGILLUS PARASITICUS* BY KOREAN SOYBEAN PASTE (DOEN-JANG) AND IDENTIFICATION OF THE ACTIVE COMPONENT

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The inhibitory effect of methanol extract of Korean soybean paste on the mold growth and aflatoxin production of a toxigenic strain of *Aspergillus parasiticus* ATCC 15517 was studied using different concentrations of the extract in yeast-extract sucrose (YES) broth. Reduction of mycelial weight as result of addition of the extract was observed to range between 1.5 to 12.9% while reduction of aflatoxin production quantified by HPLC ranged from 14.3 to 78.1%. This study indicates that soybean paste could also be an effective inhibitor of aflatoxin production even though mycelial growth may be permitted. The main active component identified by GC-MS was linoleic acid.

T22 CRITICAL ROLE OF *PEDIOCOCCUS* SP. CYTOPLASMIC MEMBRANE IN THERMAL RESISTANCE

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Pediococcus sp. is a nonpathogenic heat-resistant microorganism that has been used as a test organism in milk pasteurization studies. Data from this laboratory suggested that thermal inactivation of this microorganism was dependent on the growth conditions. The objectives of this study were to determine: (1) the effect of growth conditions on membrane fatty acid composition; (2) the critical role of cytoplasmic membrane in thermal resistance of *Pediococcus* sp. Membrane lipid composition and thermal resistance (*D* value) of *Pediococcus* sp. were determined for mid-exponential and stationary phase cells grown in tryptic soy broth and tryptone glucose yeast extract at 28°C and 37°C. Growth conditions markedly affected *Pediococcus* sp. membrane fluidity by changing the membrane lipid composition. An increase in membrane fluidity due to changes in growth conditions corresponded with a decrease in the *D* values of this bacterium. Heating caused destabilization of the cytoplasmic membrane, allowing penetration of red fluorescent nucleic acid stain. Data from this study suggest that the cytoplasmic membrane of *Pediococcus* sp. plays a critical role in thermal resistance. Work is currently underway to study the effect of thermal inactivation on ribosomal RNA in *Pediococcus* sp.

T23 ANTIBIOTIC RESISTANCE OF GRAM-NEGATIVE ENTERIC PATHOGENS ISOLATED FROM RETAIL MEATS

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The purpose of this experiment was to assess the trend of antibiotic resistance in *Salmonella* spp. and *Escherichia coli* isolated from retail meats. A total of 200 meat samples were obtained

from retail stores including ground beef, ground chicken, ground pork, and ground turkey. The bacteria were isolated using the methods described in the FDA bacteriological analytical manual. Antibiotic resistance was determined using the Dispens-O-Disk susceptibility test system of Difco. Among the 330 *Escherichia coli* isolates 169 (51%) were resistant to at least one antibiotic. Tetracycline was the most common antibiotic (35%) that *E. coli* were resistant to. The most frequent multiple resistance pattern was streptomycin-sulfoxazole- tetracycline which was observed in (7%) of the isolates. Among the 364 *Salmonella* isolates 217 (60%) were resistant to at least one antibiotic. Tetracycline was the most common antibiotic (58%) that *Salmonella* was resistant to. The most frequent multiple resistance pattern was ampicillin-streptomycin-tetracycline-sulfoxazole which was observed in (19%) of the isolates. This study suggests that antibiotic resistant bacteria are widespread in animal meat products. Research is needed to determine if the limited use of antibiotics in animal feeds will lead to reductions in the number of antibiotic resistant bacteria in our food supply.

T24 DISTRIBUTION AND ROLE OF INTEGRONS IN MULTI-RESISTANT *SALMONELLA*

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Integrons are genetic elements containing the determinants of a site-specific recombination system which allows for capture and expression of genes. Integrons have been associated with multiple antibiotic resistance among *Salmonella*. This study seeks to elucidate the distribution of integrons among *Salmonella* and the significance of their role in multiple antibiotic resistance. A genetic probe produced by PCR to detect the presence of the most common integron type, known as class I, was used to screen 1000 clinical and nonclinical *Salmonella* animal isolates. The primary factor associated with the presence of integrons is serotype with *Salmonella typhimurium* accounting for 80% of the integron positive isolates. Chromosomal resistance to more than 1 of 5 major antibiotics including ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline is most often associated with multi-resistant *Salmonella*. Ninety-six percent of the integron positive *Salmonella* isolates are resistant to 2 or more of these 5 antibiotics versus only 7% of the integron negative isolates. This study suggests that integrons play a fundamental role in the development of multiple antibiotic resistant *Salmonella*.

T25 DEVELOPMENT, IMPLEMENTATION AND ANALYSIS OF AN ON-FARM FOOD SAFETY PROGRAM FOR THE ONTARIO GREENHOUSE VEGETABLE GROWER'S MARKETING BOARD

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Fresh fruits and vegetables are increasingly recognized as a vector for foodborne illness. Consequently, methods of growing, handling, processing, packaging and distribution of fresh produce are receiving attention in terms of identifying, and controlling microbiological hazards. Consistent with principles outlined by the US Food and Drug Administration, the US Dept. of Agriculture and others, an on-farm food safety program for the Ontario Greenhouse Vegetable Grower's Marketing Board was developed, and implemented. This paper reviews on-farm obstacles to successful implementation and highlights the challenges of implementing an on-farm food safety program across a producer association with varying farm sizes and incomes, through producer surveys conducted before and after implementation of the on-farm food safety program.

T26 MICROBIAL COLONIZATION WITH BIOFILM FORMATION ON PACKAGING FILM AND VEGETABLE TISSUE OF READY-TO-USE PACKAGED SPINACH

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Scanning electron microscopy (SEM) was used to demonstrate microbial attachment to polypropylene film used to package ready-to-use spinach, microbial colonization of the spinach itself and tissue textural changes after prolonged storage at refrigeration temperatures. Fresh spinach, packaged in polypropylene films with and without microperforations, was stored at 4°C and 10°C for up to 30 days. Packaging film and vegetable samples from fresh (control) and stored products were fixed in gluteraldehyde, post-fixed in OsO_4, dehydrated through an ethanol series and critical-point-dried under liquid CO_2. The packaging film was freeze-fractured in liquid nitrogen prior to drying. All samples were sputter-coated with gold and examined under SEM. The packaging film for products towards the end of their shelf life, (total counts $>10^7$ CFU/g), showed distinct microbial attachment and biofilm formation within and on the film. Microbial colonization was also observed on the spinach samples but not on control samples. Aged vegetable samples lost cellular

turgidity, integrity and definition. SEM has demonstrated microbial attachment on packaging film-material, vegetable tissues and textural changes after prolonged storage. Knowledge of microbial attachment and biofilm formation on packaging film for minimally processed products has important implications for design and application of packaging materials in the food industry.

T27 EFFECT OF MICROWAVE COOKING ON THE RECOVERY OF *CRYPTOSPORIDIUM OOCYSTS* FROM SPINACH

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Raw spinach was inoculated with 10^4 *Cryptosporidium parvum oocysts* using a nebulization apparatus designed for this project. The apparatus was an enclosed chamber with a small volume nebulizer that dispersed *oocysts* evenly over the product. Experimental samples were cooked for 45 s in a 1000-watt microwave oven. The cooking time was established by timing consumers who followed basic cookbook instructions to cook until spinach appeared "bright green and wilted." Cooked and raw samples were processed in a stomacher with surfactant solution, filtered to remove spinach debris, and centrifuged. A five-milliliter aliquot of the supernatant was vacuum filtered through a membrane filter. Intact *oocysts* trapped on the membrane were quantitated by direct fluorescent antibody technique. Mean *oocyst* recoveries in uncooked positive controls were 309+/-15 per sample. This represents a consistent 4% *oocyst* recovery rate. Compared to the control samples, the samples microwaved for 45 s exhibited a 2-log reduction in contamination load ($P<0.05$). Since natural contamination levels for *C. parvum* on produce is not documented, this study cannot predict whether the 2-log reduction is sufficient to eliminate risk of infection, especially for immune-compromised individuals.

T28 SURVEY OF PRODUCTION PRACTICES USED BY VIRGINIA APPLE CIDER PROCESSORS

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During the summer of 1998, Virginia cider producers were surveyed to gain a better understanding of current production practices. The survey included questions covering production levels and sales location, orchard management, facilities, processing, preservation measures, and additional safety measures. Most operations are seasonal, produce less than 5,000 gal annually, and confine sales to Virginia. Although few producers fertilize orchards with manure, animals often graze nearby. Also, drop and damaged apples are sometimes used. Most process in separate, enclosed areas and test water for bacteria. All indicate that equipment and facilities are cleaned and sanitized daily. The majority of producers sort apples before washing, use refrigerated storage, prevent contamination during storage, and wash and brush apples before pressing. However, most use only water for washing and only 37% use a chemical sanitizer following the wash step. Few pasteurize cider or add preservatives; however, almost all store cider refrigerated or frozen. Although only 25% have an operating HACCP program, most have considered implementing HACCP. Few producers conduct microbiological tests on cider or include code numbers or expiration dates on labels. Finally, most producers expressed an interest in the use of alternative processing technologies to help assure the safety of their cider.

T29 SCIENCE, SOCIETY, AND CIDER: A COMPARATIVE ANALYSIS OF INTEGRATIVE FOOD SAFETY RISK MANAGEMENT IN CANADA AND THE US

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The 1983 NAS-NRC risk analysis model explicitly distinguished between three stages of risk analysis: risk assessment, risk management and risk communication. The principles of risk assessment have been endorsed and incorporated by the US National Advisory Committee on Microbiological Criteria for Foods. Yet despite these theoretical separations, in practice, food safety risk assessment, management and communication are often integrated, consistent with a risk analysis Framework endorsed by the 1997 US Presidential/Congressional Commission on Risk Assessment and Risk Management.

Of particular importance is that the Framework is conducted in collaboration with stakeholders and using iterations if new information is developed that changes the need for, or nature of, risk management. Using the Framework, this paper will review the key interactions of science, society and public policy in developing risk management strategies to reduce microbial illness related to unpasteurized cider in Canada and the US and suggest areas for improvement.

T30 A QUANTITATIVE RISK ASSESSMENT FOR DETERMINING THE EFFICACY OF VARIOUS HAND WASHING PRACTICES

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Some foodborne illness outbreaks have been attributed to contamination by food service workers' hands prompting some states to enacting "No Bare Hand Contact" regulations. This study was undertaken in an attempt to increase the level of scientific understanding of the factors that influence the concentration of bacteria on food service worker hands. Relevant data were collected from the scientific literature and translated into appropriate discrete or continuous probability distribution functions. Some of the information collected included: the initial level of bacterial contamination on hands, and the effect of rings, soap type (regular soap, E2 soap, and soap containing antibacterial compounds), sanitizing solution, and drying method (cotton or paper towel, air drier) on bacterial contamination of hands. Graphical data obtained from the literature were converted to numerical form using Ungraph software. The appropriate statistical distribution for each set of numerical data was determined using BestFit software. Distributions were entered into Excel worksheets, and simulations were conducted using @risk software. Tornado plot analysis revealed that the primary factors influencing the final concentration of bacteria on human hands were sanitizer type, drying method and the initial concentration of bacteria present. This research represents an initial framework from which sound policy can be promulgated.

T31 THE DYNAMICS OF SURFACE CLEANING AND SANITIZATION

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Food contact surfaces may play an important role in transmission of foodborne illness in the household kitchen. Counters and surfaces have previously been found to harbor numbers of *S. aureus*, coliforms, and fecal coliforms such as *Escherichia coli,* potentially compromising food safety. Test methods used to show efficacy of disinfectant and cleaning agents in many cases fail to replicate actual in-use conditions. As virtually all kitchen disinfectants are neutralized to some extent by organic build-up, test inoculum and cleaning regimen is a significant part of overall efficacy. Using 7 commonly employed test media (water, tryptone soya broth (TSB) with and without 5% horse serum, chicken juice, whole milk, egg (white and yolk mixed) and ground beef inoculated with *Serratia marcescens* and *E. coli* O157:H7, several different cleaning/sanitization experiments were performed on mineral resin surfaces. The distinction between bacterial inactivation due to kitchen disinfectant and that provided by physical surface cleaning, as by water rinse and/or paper towel wiping was shown. Inoculum drying times of 5 min and 1 h were used. It was found that as drying time increased, contaminants became more refractory to removal or inactivation. In many instances, disinfectant and paper towel wiping results in synergistic effects.

T32 OCCURRENCE OF *LISTERIA MONOCYTOGENES, SALMONELLA, ESCHERICHIA COLI* O157:H7 AND OTHER SHIGA-LIKE TOXIN-PRODUCING *ESCHERICHIA COLI* IN RETAIL FRESH VEGETABLES AND GROUND BEEF

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Seventeen hundred and five fresh ground beef samples along with 175 samples of fresh vegetables were purchased from Seattle, Washington area retail outlets. Ten gram samples were homogenized in 90 mL of mTSB and enrichment cultured overnight with agitation. The cultures were then screened using BAX® system PCR-based assays for *Salmonella*, and *E. coli* O157:H7 and for Shiga-like toxin-producing *E. coli* (SLTEC) using a non-commercial multiplex PCR assay (for Shiga-like toxin genes and *eaeA* gene). A subset of 467 beef samples and all of the vegetables were screened for *L. monocytogenes.* We attempted to confirm all positive results using cultural methods. Three of the 175 vegetable samples and 20 of the 1705 beef samples were PCR-positive for O157:H7. Fifteen vegetable samples and 268 beef samples were positive for SLT by PCR and 50 beef samples and 11 vegetable samples were positive for the *eae*A gene. Eleven vegetable samples and 262 beef samples were positive by PCR for *Salmonella* and 1 vegetable sample and 14 beef samples were positive by PCR for *L. monocytogenes*. These results, in conjunction with our previously reported ground beef study, support the necessity of monitoring fresh produce as well as ground beef for pathogens.

T33 BEHAVIOR OF *ESCHERICHIA COLI* O157:H7 ON ALFALFA SPROUTS DURING THE SPROUTING PROCESS AS INFLUENCED BY TREATMENTS WITH VARIOUS CHEMICALS

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The behavior of *E. coli* O157:H7 on alfalfa seeds and sprouts subjected to conditions similar to those used commercially to grow and market sprouts, as affected by daily application sanitizers, was determined. Spray application of 2,000 ppm NaOCl, 200 and 2,000 ppm $Ca(OCl)_2$, 500 ppm acidified ClO_2, 1% Vegi-Clean™, 80 ppm Tsunami™, or 40 and 80 ppm Vortexx™ to germinated seeds significantly reduced the population of *E. coli* O157:H7, but application of 1% Na_3PO_4 or H_2O_2 was ineffective. With the exception of acidified $NaOCl_2$ at 1,200 ppm, these chemicals did not significantly reduce populations of *E. coli* O157:H7 or control its growth on sprouts during the sprouting process. Populations on alfalfa sprouts peaked at 6-7 $\log_{10}$ CFU/g 48 h after initiation of the sprouting process and remained stable, despite spraying at 24-h intervals with chemicals and subsequent storage at 9 ± 2°C for up to 6 days. Recommended procedures for sanitizing alfalfa seeds failed to eliminate the pathogen. None of the chemical treatments evaluated eliminated or satisfactorily reduced *E. coli* O157:H7 on alfalfa seeds and sprouts.

T34 OUTBREAKS OF VIRAL GASTROENTERITIS ASSOCIATED WITH IMPORTED RASPBERRIES

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Several episodes of food poisoning affected more than 300 people in the Quebec City region during July and August 1997. An epidemiological investigation isolated the foodborne origin of the outbreak and identified the viral agent responsible. Two retrospective cohort studies were undertaken with two groups that participated in a communal meal (cohorts A and B). An alert and case recruitment process was set-up, and food investigation was initiated. Bacteriological analyses were done on food and stools form sick individuals. The epidemiological investigation of cohorts A and B revealed that the consumption of raspberries increased the likelihood of contracting gastroenteritis (A, RR=2,6 p=0,001, B RR=4,7 p=0,015). Other families also complained of being sick after having eaten raspberry desserts. Food analyses identified a common ingredient to all these desserts, namely raspberries originating from Bosnia. A PCR protocol revealed the presence of calicivirus in five stool samples and two raspberry samples. Further analyses demonstrated that the nucleotides found in stools and in the raspberries were identical. No simple foodborne virus detection tests are presently available. It is thus important to set-up efficient surveillance systems that permit the early detection of viral outbreaks in order to quickly undertake epidemiological investigation and design the necessary interventions.

T35 AN EPIDEMIOLOGICAL STUDY OF *PSEUDOMONAS AERUGINOSA* STRAINS ASSOCIATED WITH MASTITIS AMONG DAIRY ANIMALS AND HUMAN INFECTIONS BASED ON AUTOMATED RIBOTYPING WITH THE RESTRICTION ENZYME *PVU*II

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Pseudomonas aeruginosa has been linked as a causative agent of bovine mastitis, and an infectious agent to humans having open wounds, such as burns, and also respiratory infections. This study was designed to gain a better understanding of the genetic diversity among these pathogenic *P. aeruginosa* strains based on automated ribotyping. *Pvu*II was selected as the restriction enzyme for this study, because previous studies suggest that this restriction enzyme is optimal for characterizing these organisms by ribotyping. Ninety-eight (98) strains representing clinical isolates of both human and dairy origin were processed; among these strains, 52 distinct ribotypes (RiboGroups) were observed. The most populated RiboGroup contained 8 strains; 2 RiboGroups contained 5 and 4 strains, respectively; the remaining RiboGroups contained 3 or fewer. In this study, we confirmed the previous report on *Pvu*II as the preferred restriction enzyme for this organism. The flexibility of the automated RiboPrinter® Microbial Characterization System allowed the selection of *Pvu*II as a replacement for the standard *Eco*RI process used with the system. Based on the diversity that was observed, ribotyping was found to be an excellent tool for epidemiology studies.

T36 FATE OF *LISTERIA MONOCYTOGENES* AND *ESCHERICHIA COLI* O157:H7 IN DAIRY FOODS

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In recent years, outbreaks of several diseases have been traced to contaminated milk or milk products. These outbreaks have directed our attention to milkborne pathogens especially the emerged or emerging ones such as *L. moncytogenes* and *E. coli* O157:H7. *L. monocytogenes*

survived for more than one year in Cheddar, 140 d in Colby, and 90 d in Feta cheeses. Also, the pathogen survived in sweetened condensed milk during storage at 7 or 21°C for 56 or 28 d, respectively. The organism grew in evaporated milk.

E. coli O157:H7 had the ability to grow during fermentation of yogurt and during manufacture of Domiati cheese. The pathogen survived in yogurt for 7 d held at 7°C. During storage of Domiati cheese, the pathogen could not be recovered on the plates after 4 weeks (use of appropriate hygienic procedures during milk production products), and delivery of safe food to the consumer will save much money for industry and ultimately the public.

T37 BIOCHEMICAL COMPARISON OF *LACTIS LACTIS* SPP. *LACTIS BIOVAR. DIACETYLACTIS* WRP297 AND WRP298, PHAGE RESISTANT VARIANTS, WITH ORIGINAL SENSITIVE CULTURE USED FOR CHEESE MANUFACTURE

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Lactic phage is considered to be a major threat to any dairy plant. Presently, starter failure due to bacteriophage may not be a serious problem for Indian dairy industry, but the increasing demand for cheese, which is expected to exceed 30000 tonnes per annum by 2000 AD, indicates that phage related problems could soon become very real in India. Our study focused on the possibility of selecting phage resistant strains which may be of industrial importance. The phage insensitive mutants WRP297 and WRP298 were isolated from a zone of lysis on M17 agar and a phage-infected M17 broth culture, respectively. Identification studies revealed that both the strains belong to the same species as that of the parent culture. The sensitive host strain and the two resistant strains were studied for their cell surface hydrophobicity using two-phase hydrocarbon assay. Three hydrocarbons (xylene, hexane, n-hexadecane) were employed. None of the test samples exhibited a detectable difference in the hydrophobicity pattern when compared with the parent strain. Our results contradict some earlier studies which reported a correlation between cell surface hydrophobicity of lactic cultures and their phage adsorption properties. Compositional analysis of these cultures and cell fractions is underway.

T38 A COMPARATIVE STUDY OF A COLORIMETRIC ATP MEASUREMENT TEST, ATP BIOLUMINESCENCE AND PROTEIN DETECTION FOR HYGIENE MONITORING

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Any contamination in food production leads to the loss of high value product and production time and contaminated products release. These factors dictate using monitoring and control systems to ensure the quality standards are maintained. Hygiene monitoring (HM), commonly just a visual inspection, assesses the status of critical control points.

A new colorimetric ATP test uses enzyme reagents, which amplify trace amounts of ATP or ADP in food and generates a visual color change. The performance of this test against ATP bioluminescence and protein detection systems was studied. 30% (w/v) suspensions of food samples were subjected to limited stomaching (60 s at medium speed). The samples were diluted to 1/1000 and three detection systems were evaluated. For meat samples, the colorimetric ATP test and bioluminescence systems out-performed the protein tests. The protein tests did not detect any vegetable residues while the colorimetric ATP test and bioluminescence systems detected vegetable residues in 1/100 to 1/1000 dilution range. For processed food, the protein tests detected 1/10 dilutions compared to 1/100 for the colorimetric ATP test and bioluminescence systems. The colorimetric ATP approached that of ATP bioluminescence and outperformed protein tests in a real food-processing environment and on a variety of sanitizers.

T39 AN ISOLATION AND DETECTION SYSTEM FOR *LISTERIA MONOCYTOGENES* USING FLUOROGENIC AND CHROMOGENIC SUBSTRATES FOR PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C

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The efficacy of the BCM® *L. monocytogenes* detection system (LMDS) was determined using pure cultures and naturally and artifically contaminated sponges. BCM® *L. monocytogenes* pre-enrichment broth killed most gram negative and some gram positive bacteria, but allowed the growth of *Listeria* and the resuscitation of heat-injured *L. monocytogenes*. BCM® *L. monocytogenes* selective enrichment broth containing a fluorogenic substrate (4-meth-ylumbelliferyl-myo-inositol-1-phosphate), which detects phosphatidylinositol phospholipase C (PI-PLC), inhibited the growth or killed additional Gram positive bacteria such as the enterococci, staphylococci, micrococci, and *Bacillus cereus,* and provided a potential presumptive positive test for the presence of pathogenic *Listeria* (*L. monocytogenes* and *L. ivanovii*). On BCM® *L. monocytogenes* plating medium, these pathogens were the only *Listeria* producing a turquoise convex colony due to the enzymatic activity of PI-PLC on the chromogenic substrate, 5-bromo-4-chloro-3-indoxyl-

myo-inositol-1-phosphate. Other potential false positive microorganisms, *B. cereus*, *Staphylococcus aureus*, *Bacillus thuringiensis* and yeasts, were eliminated by one or more of BCM® LMDS media. In an analysis of 162 environmental sponges from USDA inspected facilities, the respective values for identification of *L. monocytogenes* by BCM® LMDS and USDA methods were 30 and 14 sites, with respective sensitivity and specificity values of 85.7 and 100% vs. 40.0 and 77.5%. BCM® LMDS isolated no false positive samples, whereas, USDA method had 26.5% false positive samples.

T40 DETECTION AND TRACKING OF *LISTERIA MONOCYTOGENES* IN SMOKED FISH PLANTS

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The zero-tolerance ruling for *L. monocytogenes* in ready-to-eat foods presents a serious challenge for the food industry. We used molecular techniques to detect and characterize *L. monocytogenes* collected from smoked fish processing plants. Five-hundred fifty-five fish and environmental samples collected during 5 visits to each of 3 plants were examined with the BAX® *L. monocytogenes* PCR kit. Fifty *L. monocytogenes* isolates yielded 10 different ribotypes by automated analysis with the RiboPrinter®. The ribotype occurrence frequencies from these samples were compared with their frequencies among 105 human clinical *L. monocytogenes* isolates. Ribotype DUP-1039 was most frequent among fish plant isolates (32%), but represented only 5% of human clinical isolates. Among 5 isolates from finished ready-to-eat smoked fish products, four were ribotype DUP-1039 and one was DUP-1046 (which represents 0/105 human isolates), suggesting that these particular strains are not commonly associated with human illness. In combination with the fact that only 3 of 13 known *L. monocytogenes* serotypes have been responsible for > 95% of human listeriosis cases, our findings confirm that not all *L. monocytogenes* strains present the same human health risks. Our work further demonstrates that molecular techniques provide efficient strategies for specific detection and tracking of *L. monocytogenes*.

T41 EFFECTS OF CRYOGENIC COOLING AND TRADITIONAL COOLING ON *SALMONELLA* ENTERITIDIS POPULATION IN TABLE EGGS

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The response of *Salmonella* Enteritidis (SE) in table eggs cooled in a manner similar to that of the present commercial industry (traditional cooling) versus cryogenic cooling was determined. SE was injected into freshly-laid eggs at one of two locations (yolk or albumen) to provide an initial population of ca. 10^7 CFU/ml of egg content. Eggs were then subjected to the two cooling treatments. Traditional cooling was achieved by placing eggs into an incubator in which the temperature was 25.5°C on day 1, 18.3°C on day 2, 12.8°C on day 3, 11.1°C on day 4 and 7.0°C on days 5 to 15. Cryogenically cooled eggs were cooled to ca. 7°C in ca. 6 min with gaseous CO_2, then held at 7°C for 15 days. SE was enumerated from each treatment (2 injection sites × 2 cooling methods) on day of inoculation (day 1), and on 3, 6, 9, 12, and 15 days of storage. Site of inoculation did not affect the response of SE; however, cooling method and storage time interacted to affect ($P<0.001$) SE populations. SE populations in traditionally cooled eggs increased by 1.2 log/ml at 3 d of storage, after which they decreased by 0.2 log/ml each sampling day. In contrast, cryogenic cooling led to a decrease in SE population of ca. 1.1 log/ml from day of cooling until the end of the storage period. At the high inoculum levels used in this study, SE populations significantly increased in traditionally cooled eggs, but decreased in cryogenically cooled eggs, which suggests that cryogenic cooling may afford protection against SE growth in table eggs.

T42 THE IMPACT OF TRAINING ON GROCERY STORE SEAFOOD EMPLOYEES' FOOD SAFETY AND SANITATION KNOWLEDGE, PRACTICES, AND DEPARTMENT PROFITABILITY

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An important consumer issue is seafood quality and safety at the retail level. Retail seafood handlers desperately need training. However, previous training efforts in Atlanta had limited success because grocery store chain administrators believe that training is not cost effective.

A 44 question, six-part seafood quality and safety survey was completed by 274 employees representing 113 southeastern US grocery stores. Mean seafood safety and sanitation scores improved with increasing on-the-job training, when training was presented at the employee's request, with the completion of two to four food-related courses, and with years of seafood experience. The following knowledge and action correlations were significant: (1) hand-washing knowledge and practices to prevent cross-contamination of RTE foods and (2) understanding temperature effects on bacterial growth and seafood room temperature holding times at receiving and display case setup.

The survey found 78% of the workers knew that raw molluscan shellfish posed the greatest health risk to consumers and 88% knew the purpose of shellfish tags on unshucked oysters, but only 45% kept the tags for the required 90 days.

Seafood dept. profitability increased when (1) surveyed employees responded that special seminars were most helpful to their job, (2) specified clerks determined spoilage, and (3) reduced quality seafood was repackaged, frozen, and displayed in the freezer. The use of reduced quality seafood for customer samples decreased profitability.

T43 MICROBIOLOGICAL MONITORING OF "BOBBY" CALF SLAUGHTER AND DRESSING: THE NEED FOR A STAND-ALONE PROGRAM DESIGN

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The implementation of HACCP requires premises to identify interventions along the slaughter chain that contribute to microbiological contamination of carcasses, apply control measures at identified interventions to minimize contamination, and verify the microbiological performance of the HACCP plan as a whole. The design of this "microbiological verification" program is paramount in determining the effectiveness of the HACCP plan. Under the USDA PR/HACCP Final Rule, sampling for *Escherichia coli* on bovine calf carcasses is performed as for adult carcasses. In New Zealand, however, bovine "bobby" calves are generally processed using interventions more representative of "inverted" ovine and cervine chains. An intervention-by-intervention visual assessment of bobby calf processing indicated that interventions different to those for adult cattle result in contamination of bobby calf carcasses, and hence "alternative" sampling sites (forward rump, standard flank, and outside foreleg) are required for verification programs. The small size of bobby calves, usually less than two weeks of age and smaller than lamb carcasses, prevents sampling of an area greater than 25 cm^2. A comparative microbiological survey against the PR/HACCP sites confirmed the appropriateness of the "alternative" sites, and reinforced the view that it is not always appropriate to use "standard" microbiological verification programs even within species.

T44 SPECIES AND STRAIN DIFFERENTIATION OF *PSEUDOMONAS* SPP. BY RIBOTYPING

Kathryn J. Boor,* M. Wiedmann, J. W. Czakja, D. Weilmeier, S. Dineen, and R. Ralyea, Cornell University, Dept. of Food Science, Ithaca, NY 14853, USA

Pseudomonas spp. in rRNA homology group I are major contributors to the spoilage of food products and also include potential human pathogens such as *P. aeruginosa*. The taxonomy of *Pseudomonas* represents a major challenge to sanitarians and microbiologists. The objective of this research was to evaluate the use of the Qualicon RiboPrinter™, an automated ribotyping system for species identification and strain differentiation of *Pseudomonas* isolates. Approximately 140 rRNA homology group I *Pseudomonas* isolates, including *P. aeruginosa*, *P. fluorescens*, *P. putida* and *P. stutzeri*, were characterized by automated ribotyping and API20NE. For most isolates, production of proteases, lipases and lecithinases and biochemical reactions using the Biolog system were also evaluated. 16S rRNA sequencing was used to speciate selected isolates. More than 70 different *EcoR*I ribotypes were identified. Isolates grouped into distinct clusters that were consistent between the APINE20 and ribotyping. Each cluster appears to represent a different species, with *P. putida* and *P. fluorescens* biovars separating into distinct clusters. Our study shows that ribotyping provides a rapid, sensitive tool for differentiation of *Pseudomonas* isolates and therefore can be used for tracking pseudomonads in food production systems. Ribotyping also shows strong potential for rapid species identification of *Pseudomonas* spp.

T45 A SINGLE-STEP POLYMERASE CHAIN REACTION FOR COMBINED GENE DETECTION AND EPIDEMIOLOGICAL TYPING (COGEDET) OF *LISTERIA MONOCYTOGENES* STRAINS

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The development of a rapid method for the simultaneous detection and typing of *L. monocytogenes* would be extremely useful in the monitoring of listeriosis outbreaks. Thus, we set out to develop a single-step PCR for combined gene detection and epidemiological typing (COGEDET) of *L. monocytogenes*. Initially, work was done with five gene primer sets, including

HLYA, ISP, FL2A, PRFA and RG1/RL1, and five RAPD primers, including M13, HLWL 74, HLWL 85, UBC 155, and UBC 156. Amplification conditions were optimized to generate discriminatory and reproducible banding patterns by testing primer concentrations, annealing temperatures and different DNA extraction methods. Three PCR primer/RAPD primer combinations led to the discrimination of a subset of five *L. monocytogenes* strains of different serotypes. These combinations were as follows: HLYA/M13, FL2A/UBC 156 and ISP/UBC 155. The COGEDET method appears to have great promise for the rapid identification and typing of *L. monocytogenes* strains in a single PCR reaction tube. Further work is being done on a larger variety of strains, as well as on detecting and typing *L. monocytogenes* isolates directly from food enrichment broths.

T46 DEVELOPMENT OF A HYBRIDOMA CELL LINE FOR THE PRODUCTION OF A MONOCLONAL ANTIBODY TO THE PESTICIDE BROMACIL

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A monoclonal antibody (Mab) was developed for a rapid and efficient immunoassay of the pesticide, bromacil. Spleen cells of BALB/C mice previously immunized with bromacil- hapten-protein conjugate, 3-[5-bromo-3- (1-methylpropyl)-2, 4(1H, 3H)-pyrimidine dione-6-y1] propanoic acid coupled to keyhole limpet hemocyanin (KLH), were fused with mouse myeloma cell line (P3 × 63 Ag8. V653). The hybridoma cell line producing a monoclonal antibody specific for bromacil was grown in tissue culture and as an ascites tumor. The ascitic fluid had a suitably high dilution titer (1:100,00) by indirect competitive enzyme-linked immunosorbent assay. The sensitivity was 0.01ng/ml with a working range up to 100ng/ml for bromacil. With this assay format, the developed Mab showed a high affinity for bromacil and minimum cross-reactivities for several fungicides. This Mab appeared to be a very promising immunoreagent for the future development of a specific and sensitive quantitative ELISA for the pesticide, bromacil in water and environmental samples.

SYMPOSIA

S1 SCIENCE-BASED CRITERIA FOR HARMONIZING FOOD SAFETY REGULATIONS

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As we approach the new millennium, international trade in food commodities is becoming an increasingly important factor in our global economy. As the past decade illustrates, food in international commerce can play a major role in foodborne disease outbreaks. Various approaches to this issue have been undertaken by different countries and regions around the world. This has resulted in a number of microbiological guidelines, standards, and regulations that often do not agree. To facilitate global trade, organizations such as Codex Alimentarius and the International Committee on Microbiological Specifications for Foods (ICMSF) continue to work toward harmonization of microbiological criteria for ensuring the safety of foods. The intent of this symposium is to explore the need for harmonizing food safety regulations based on objective, scientifically justified criteria wherever possible. One of the issues to be addressed is what constitutes "scientifically justified" criteria. The session will open with an overview of the scientific basis for setting performance standards and then examine issues of harmonization of tolerance limits for *Listeria monocytogenes* from the European perspective. Harmonization of acceptance criteria for microbiological methods will be evaluated as well as the issue of equivalency of food inspection in international trade. The issue of verotoxigenic versus other *Escherichia coli* standards will also be discussed as a potential area for harmonization. The session will conclude with a roundtable discussion of this critical topic.

S2 FRUITS AND VEGETABLES: ARE THEY SAFE ENOUGH?

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Fruits and vegetables are highly popular food items among the health conscious consumer of the '90s. During the past several years, consumption of fresh produce has greatly increased. Today, due to a greater availability of product, consumers can purchase a wide variety of fresh fruits and vegetables year round.

Food operators and consumers have generally considered fresh fruits and vegetables as safe to eat. Historically, people associated foodborne illness with meats, poultry, seafood, and dairy products. But recent well-publicized outbreaks and a review of foodborne disease data are causing us to change the way we view these products. In fact, the concern over fresh produce is now increased since there is no final cooking or commonly accepted control step to eliminate pathogens.

The potential for fresh produce to become contaminated with pathogens exists because of their exposure to a wide variety of conditions during growth, harvesting, distribution, and processing.

This symposium will consider outbreaks involving fresh produce, risks associated with their production, and risk management strategies. Products discussed will include alfalfa sprouts, unpasteurized apple juice, and lettuce.

S3 MINI WORKSHOP FOR DAIRY PLANT EMPLOYEES AND REGULATORS

Charles Price, Sr., FDA, Central Reg-Chicago, 20 N. Michigan Avenue, Room 510, Chicago, IL 60602, USA; Gaylord Smith, Mohawk Associates, Inc., 1385 Regent Street, Schenectady, NY 12304, USA; Ruth Fuqua, Quality Chekd Dairies, Inc., 7236 Lebanon Road, Mt. Juliet, TN 37122, USA; Don Graham, Graham Sanitary Design Consulting, 14318 Aitken Hill Court, Chesterfield, MO 63017, USA

The dairy program for the 1999 IAMFES Meeting is made up of three symposia. Each of these symposia are directed at dairy plants whether it be fluid, manufacturing or anything related to plants using dairy products. The program covers topics from basic plant functions to more detailed HACCP and regulatory programs. It is designed for anyone working in or interested in what makes a dairy plant function from an in-house perspective or from a regulatory viewpoint. It is designed not only for management and supervisory people, but anyone involved in production, maintenance, or quality assurance of dairy plants.

The three symposia encompass presentations needed for knowledge of a total quality control type approach for plant organization and management. This will be accomplished by presenting perspectives from regulatory, engineering, quality objectives and standards, and product safety viewpoints.

The symposia will also include informative presentations regarding HACCP prerequisite programs and preventive maintenance issues. Also included will be an in-depth look at the responsibilities of federal and state regulatory, USDA, OSHA, EPA, Bureau of Weights and Measures and Codex from an international standpoint.

S4 GLOBALIZATION OF FOODBORNE DISEASE

Ewen Todd, Health Canada, Health Protection Branch, Sir Frederick G. Banting Research Centre, Postal Locator 2204A2, Ottawa, Ontario, K1A 0L2; Yasmine Motarjemi, WHO, Food Safety Unit, Division of Food and Nutrition, CH-1211, Geneva 27 Switzerland; Ellen Morrison, FDA, Division of Emergency Investigation and Operations, Parklawn Building, 5600 Fishers Lane, Rockville, MD 20857, USA; Hiroshi Takahashi, National Institute of Infectious Diseases, Shinjuku, Toyko, Japan; Trish Desmarchelier, CSIRO, Food Science Australia, P.O. Box 3312, Tingalpa, Queensland 4173, Australia

The importance of foodborne disease around the world is being recognized. This applies to developing as well as industrialized countries, and can affect migrant workers and refugees, as well as long-time residents. In addition, such disease isn't confined to local outbreaks. Contaminated products made in one part of a country can affect people in another part or in different countries if they are exported. Examples of these are *Clostridium botulinum* in cheese, *Cyclospora* in raspberries, *Escherichia coli* O157 in radish sprouts, *Salmonella* in alfalfa sprouts, *Salmonella agona* in a snack product, and *Staphylococcus aureus* in lasagne. Action has to be taken where shellfish, which have ingested toxic marine

phytoplankton or been contaminated with enteric pathogens, need to be quarantined rapidly and sales stopped. Bovine spongiform encephalopathy in the UK and other countries has affected consumption of beef in Europe and trade in beef products worldwide. The purpose of this symposium is to see how food, particularly imported products, have caused major or international outbreaks around the world, what surveillance systems are set up to detect them, and what means are in place to control these.

S5 MANURE AND WATER: PRODUCE SAFETY IMPLICATIONS

Dean O. Cliver, School of Veterinary Medicine, Department of Population Health and Reproductive Medicine, University of California, Davis, CA 95616, USA; Joan Rose, Department of Marine Sciences, University of South Florida, St. Petersburg, FL 33701, USA; Trevor Suslow, Department of Vegetable Crops, University of California, One Shields Drive, Davis, CA 95616, USA; Michelle Smith, FDA/CFSAN, OPDFB (HFS-306), 200 C Street SW, Washington, D.C., USA; Frances Pabrua, California Strawberry Commission, 41 Hangar Way, P.O. Box 269, Watsonville, CA 95077-0269, USA

Manure and water are both recognized as potential sources of enteric pathogens that may result in the contamination of fruits and vegetables. Manure is an important source of enteric pathogens; contamination of produce can occur through direct inadvertent contact with human or animal waste, through application of improperly treated manure or indirectly via contaminated water sources. A continued adequate supply of high quality water, used extensively in the produce industry for irrigation, cooling, washing, and movement of produce in processing facilities, also presents an enormous challenge. As the demands on limited water resources increase, so do pressures to use various methods for recycling and reclamation of water waste from our ever-expanding urban centers. Also, the increased focus on the environmental impact of dairy farms and feed lots will continue to present agricultural challenges well into the next century. The symposium will provide a broad overview of the produce safety issues associated with manure and water and will specifically address the challenges of irrigation water, manure management for conventional and organic farming, and issues of field sanitation as they relate to farm workers as sources of contamination. A panel discussion held at the end of the presentations will attempt to summarize current opinions and discuss issues of mutual concern.

S6 DAIRY PLANT QUALITY AND SAFETY PROGRAMS

Chris Newcomer, New-Tech Consulting, Inc., 9 Burnham Street, Cincinnati, OH 45218, USA; Dean Sommer, Alto Cheese, N3545 County Road EE, Box 550, Waupun, WI 53963-0550, USA; Randy Dougherty, NSF International, 2100 Commonwealth Boulevard, Suite 100, Ann Arbor, MI 48105, USA; Clandia Coles, Washington State DPA, P.O. Box 42560, Olympia, WA 98504-2560, USA; Steve Sims, FDA, Milk Safety Branch 200 "C" Street SW, Washington, D.C. 20204, USA

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S7 PROBLEMS AND POSSIBLE SOLUTIONS FOR THE DEVELOPMENT OF PATHOGEN RESISTANCE TO TRADITIONAL PROCESSING

Thomas J. Montville, Cook College, Rutgers University, Dept. of Food Science, 65 Dudley Road, New Brunswick, NJ 08901-8520, USA; P. Michael Davidson, University of Tennessee, Dept. of Food Science and Technology, P.O. Box 1071, Knoxville, TN 37901-1071, USA; Alejandro S. Mazzotta, National Food Processors Association, 1350 I Street, NW, Suite 300, Washington, D.C. 20005, USA; Robert E. Marquis, University of Rochester, Rochester, NY 14642-8672, USA; Lynne M. Sehulster, National Center for Infectious Diseases, CDC, Mailstop C-16, 1600 Clifton Road, Atlanta, GA 30333, USA; Elsa A. Murano, Texas A&M University, College of Agriculture and Life Science, 310 Kleberg Center, College Station, TX 77843-2471, USA

Our food supply is safeguarded by the application of well-established preservation and processing techniques. Concerns over the possible development of microbial resistance to traditional processes and antimicrobials, such as the recent finding of acid-tolerant *Escherichia coli* O157:H7, have led scientists to question the potential for development of pathogen resistance to other treatments. This symposium will explore the potential for the emergence of pathogen resistance or adaptation to current preservation techniques and mechanisms of microbial resistance. Strategies to address this issue and assure the continued integrity of food processing systems, such as irradiation, will also be discussed.

S8 OVERVIEW OF DAIRY PLANT REGULATIONS

Cary Frye, IDFA, 1250 H Street NW, Suite 900, Washington, D.C., 20005 USA; Philip Wolff, USDA, P.O. Box 96456, Room 2750-S, Washington, D.C. 20090-6456, USA; John Wolgemuth, J. W. Safety Management and Training, 30 E. Second Street, P.O. Box 368, Hummelstown, PA 17036, USA; Paul Hoge, PDA, Division of Milk Sanitation, 2301 N. Cameron Street, Harrisburg, PA 17110-9408, USA; Michael Pinagel, Michigan Dept. of Agriculture, 940 Ventine Lane, Williamston, MI, 48985, USA; Rob Byrne, NMPF, 2101 Wilson Boulevard, Arlington, VA 22201, USA

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S9 GENERAL SESSION — ANATOMY OF A MULTI-STATE USA LISTERIOSIS OUTBREAK: ISSUES, INSIGHTS AND TAKE-HOME MESSAGES

Martin Wiedmann, Cornell University, Food Science Dept., Ithaca, NY 14853, USA; Paul Mead, CDC, 1600 Clifton Road, Atlanta, GA 30333, USA; Dane Bernard, National Food Processors Association, 1350 I Street NW, Suite 300, Washington, D.C. 20005-3305, USA

Listeria monocytogenes is a foodborne pathogen which in the mid 1980s through to the early 1990s caused much concern in the food industry and to regulatory agencies throughout the world. Attempts to control the organism through the advent of HACCP, as well as increased knowledge of the pathogen through research efforts, led to a reduction in the number of cases observed in the USA, and, as a result, much less attention has been paid to this organism in the last 5-6 years. Recently, however, a couple of outbreaks, one linked to soft cheese in France and the other to deli meats and hot dogs in the USA, has caused renewed concern about this organism and to a push for enhanced activities in the risk assessment and policy areas.

This symposium will examine the latest USA outbreak which began in late summer of 1998 and continued on into 1999. In this latter outbreak, 101 cases occurred, with 15 adult deaths and 6 miscarriages/stillbirths. The events surrounding this outbreak, including the potential sources of contamination, as well as microbiology, molecular typing and epidemiological issues, will be discussed by the various speakers, with a view to providing novel insights. An industry view of the

situation will close off the symposium. Roundtable discussions will include the issue of improving communications between industry and regulatory agencies.

S10 USDA RISK ASSESSMENT OF *ESCHERICHIA COLI* O157:H7 IN GROUND BEEF

Mark Powell, USDA/FSIS/OPHS, 1400 Independence Avenue, SW, Room 3718, Franklin Court, Washington, D.C. 20250-3700, USA; Eric Ebel, USDA/FSIS, 555 S. Howes, Fort Collins, CO 80521, USA; Tanya Roberts, USDA/ERS, 1800 M Street, NW, Room 3077, Washington, D.C. 20036-5831, USA; Peter Cowen, USDA/FSIS/OPHS/ERAD, 1400 Independence Avenue, SW, Room 3718, Franklin Court, Washington, D.C. 20250-3700, USA; Clare Narrod, USDA/FSIS/OPHS, 1400 Independence Avenue, SW, Room 3718, Franklin Court, Washington, D.C. 20250-3700, USA; Wayne Schlosser, USDA/FSIS, 555 S. Howes, Fort Collins, CO 80521, USA; Peg Coleman, USDA/FSIS/OPHS, 1400 Independence Avenue, SW, Room 3718, Franklin Court, Washington, D.C. 20250-3700, USA

This report describes a farm-to-table quantitative risk assessment of the exposure of the human population to *E. coli* O157:H7 in ground beef and products containing ground beef. The first stage of the model is the production module, which estimates the annual number of *E. coli* O157:H7-positive cattle presented for slaughter in the US. It models the movement of cattle from the farm through livestock markets to slaughter plants. Both intestinal carriers and hide contaminated cattle are simulated.

The second stage of the farm-to-table assessment is the slaughter module, which simulates the prevalence and level of *E. coli* O157:H7 in beef prior to grinding. This is a multi-path model that simulates the slaughter process of receiving through dehiding, evisceration, carcass splitting, carcass chilling, pre-grinding fabrication, and decontamination measures for each iteration of the model. Slaughter plant classes are distinguished by size and treatment technologies. This multi-path approach requires two types of variables: product fraction variables and concentration variables. Slaughter concentration variables determine the likelihood of a level of contamination and microbial survival, growth and inactivation. Slaughter product fraction variables determine the amount of product that goes into each pathway. The output for the slaughter module consists of the proportion of contaminated pieces of beef and the concentration of bacteria on contaminated pieces of beef used for grinding. The third stage of the assessment is the preparation module, which simulates grinding, distribution, and preparation of all ground beef products on each iteration. Product fraction variables reflect uncertainty in the amount of product that goes into each pathway (e.g., the proportion of hamburger that is prepared in the home). Concentration variables reflect both uncertainty and variability in the amount of bacteria present in the product. The output of the preparation module consists of the number of contaminated servings and the concentration of bacteria in contaminated servings.

The final stage of the farm-to-table assessment is the public health module, which combines the output of the preparation module with data on the frequency and size of servings containing contaminated ground beef consumed annually by age class. A surrogate pathogen dose-response model that incorporates model uncertainty is used to predict annual cases of illness associated with *E. coli* O157:H7. Uncertainty about the progression of illness to more severe health outcomes (i.e., hospitalization, HUS/TTP, or death) is modeled using epidemiological data. The symposium concludes with a description of the analysis, process, and methods used to characterize, present, and disseminate the model and its results to policymakers, risk managers, stakeholders, and the general public.

S11 ANIMAL WASTE MANAGEMENT AND ITS RELATIONSHIP TO FOOD SAFETY

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Animal waste is not necessarily an end-stage byproduct, but can be a valuable resource for farmers as fertilizer for food crop production or processed into animal feed. Intensive food animal production has steadily increased in the past decade, and the scale of such operations is much larger than existed previously. Animal manure is now produced at a daily rate nearly 200 times that of human waste in the United States. Animal waste management, particularly from large facilities, has posed new considerations for examining the impact of various practices on the microbiological quality of environmental sources (such as water), animal waste itself (manure), and derivative products (waste-derived animal feeds). This symposium will present a review of microbial

pathogens that are known to cause human disease and that persist in manure. Waste management practices currently used in many dairy, poultry, and swine operations will be described. The long-term survival of enteric pathogens in manure will be discussed as will the environmental persistence of viruses that are pathogenic for humans. Processed animal waste is sometimes used as animal feed, and microbiologic aspects of this practice, particularly the persistence of pathogens in such feed, will be delineated. The jurisdictions of the federal regulatory agencies concerned with animal and wastewater management will be outlined along with their complex relationship to state and local authorities. The need for communication and coordination among the stakeholders will be underscored as necessary prerequisites to improving food safety along the farm-to-fork continuum.

S12 NEW EMERGING PATHOGENS—*MYCOBACTERIUM* SPP.

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Mycobacteria are probably most well known as the causative agents of tuberculosis, but the incidence of clinical disease in humans caused by non-tuberculous mycobacteria (NTM) has increased dramatically over the last 10 years. These organisms can cause soft tissue skin infections, lymphadenitis, as well as pulmonary and disseminated disease. The main routes of infection of NTMs are considered to be via inhalation of aerosols, through skin lesions or cuts, or through ingestion of NTMs from water and foods. Evidence suggests that disease is caused by primary infection of, for example, the gastrointestinal tract, rather than reactivation of existing latent infection or person-to-person contact.

NTMs are ubiquitous in the environment, and are found in soil and most water sources, both fresh and salt. They have been isolated from domestic sources of treated, potable water, and bottled water and have been found in milk, fruit and vegetables, oysters, beef, pork and eggs, making food and water both potential sources of human infection.

Mycobacterium paratuberculosis is thought to be the etiologic agent responsible for Johne's disease, an inflammatory enteric infection in cattle, goats and sheep, and has been linked to Crohn's disease in humans. *M. paratuberculosis* has been detected in milk from infected cows and several studies have shown that these mycobacteria may be capable of surviving HTST pasteurization.

Fruit and vegetables in stores are often sprayed with water to keep them fresh, and can be contaminated with mycobacteria either directly from the water supply or from biofilms formed within the spray equipment. With the relative resistance of these organisms to disinfection, procedures for cleaning of equipment or washing of foodstuffs may need re-examining.

In order to properly assess the risk to individuals it is necessary to understand more about the occurrence of mycobacteria, in both water and food, so preventative measures to limit the spread of infection can be implemented.

S13 HACCP IN RETAIL OPERATIONS

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HACCP is not seven principles documented in a manual on a shelf. It is time to realize that regulatory inspection does not assure safe food. Safe food is achieved by a knowledgeable manager in a retail operation who knows the likely hazards in the food and the operating environment and who teaches and coaches the employees to control the hazards in that operation. This symposium focuses on practical solutions from actual retail operating environments. The presenters will give practical solutions to effectively deal with the hazards in the food and environment and how they have found answers to effective employee control.

S14 USDA HACCP IMPLEMENTATION – WHERE HAVE WE BEEN; WHERE ARE WE GOING?

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The most recent HACCP document (1997) from the National Advisory Committee on Microbiological Criteria for Foods defines HACCP as "a systematic approach to the identification, evaluation and control of food safety hazards." Industry, academia, and the government agree that protecting the health of the consumer is the number one objective of any food safety management system. HACCP has been embraced as a means to achieve that end. There is much speculation over how successfully a science-based concept, such as HACCP, can fit into a historically prescriptive, regulatory-based process, such as the USDA FSIS Meat and Poultry Inspection Program.

USDA began implementation of the HACCP requirements of the 1996 Pathogen Reduction; HACCP Final Rule in 1998. Implementation will be completed based on plant size (number of employees) through the year 2000. During this past year, changes have been made in the approach that USDA has taken in addressing the needs and in implementing HACCP for large (>500 employees) and small (10 to 499 employees) plants. USDA has also designed the HACCP-based Inspection Models Project to test whether new government slaughter inspection procedures, applied in conjunction with extended plant HACCP controls can improve food safety and increase consumer protection.

This symposium will highlight the experiences of HACCP plan implementation in large and small USDA inspected meat and poultry plants and address regulatory challenges and perspectives for the future.

S15 *CAMPYLOBACTER* AND FOOD SAFETY: THE STATE OF THE SCIENCE

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The last several years have brought an increasing amount of attention on *Campylobacter* as a foodborne pathogen. Surveillance data have shown that *Campylobacter* spp. are among the most important causes of human diarrheal diseases in most developed countries. This symposium will bring together researchers from industry and government to update attendees on the most recent developments in the field. Topics will include up-to-date data on the prevalence of the organism and long-term effects of human exposure. Recent advances in risk assessment applied to this organism will be shared. Additional speakers will address modern methodology for the isolation and typing of *Campylobacter* spp. Attendees will obtain a broad-based overview of the key issues surrounding this increasingly important organism, as well as a deeper understanding of the state-of-the-art in methodology for the organism.

S16 METHODS FOR THE DETECTION OF INFECTIOUS VIRUSES IN FOODS

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Outbreaks of Hepatitis A, Norwalk and related viral illnesses have been associated with a variety of foods. Methods for the detection of these viruses are under development. Presentations will focus on enteric virus extraction and assay procedures for shellfish and for produce. Conventional, cell culture-based assays to detect and enumerate enteric viruses will be discussed along with emerging molecular biological techniques for the detection of potentially infectious viruses. Information will be presented on limitations in both cell culture and molecular biological-based methods that restrict their use in detecting infectious viruses. Novel methods coupling cell culture and molecular biological assays, methods known collectively as integrated cell culture-

polymerase chain reaction techniques, will be discussed. The role of molecular epidemiology in outbreak investigations will be addressed. This symposium will provide timely information on the role of emerging technologies for the isolation, identification and control of foodborne viral pathogens.

S17 THE SEAFOOD SAFETY INITIATIVE

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This symposium focuses on the impact of the Presidential Food Safety Initiative implemented during Fiscal Year 1977. In response to concerns that the incidence of foodborne disease is increasing, the President directed the implementation of the Presidential Food Safety Initiative and Produce and Imported Food Safety Initiative involving multiple science-based agencies. One of the key components of the Food Safety Initiatives (FSI), is the focusing in food safety research efforts and resources on gaps in knowledge that limit our ability to effectively assess and assure the safety of the United States food supply. Integral to this effort is an increased level of interagency research coordination, communication, and cooperation to eliminate redundancies of effort. Food safety practices and programs must be based on sound scientific research to effectively prevent or control microbial pathogens and their toxic metabolites and respond efficiently to foodborne disease outbreaks. Interagency cooperation between the Food and Drug Administration, United States Department of Agricultural and Center Disease Control Center are working together to develop strategies to assess and manage microbial pathogens and their toxins. Ultimately, critical elements are identified that focus research and lead to developing cost-effective means for lowering the risk of illness.