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ADVANCING FOOD PROTECTION WORLDWIDE

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ABSTRACTS

POSTER SESSIONS

(P1) A NEW RAPID AUTOMATED METHOD FOR THE DETECTION OF *LISTERIA* FROM ENVIRONMENTAL SWABS

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Many food and meat processors test environmental swabs to confirm the absence of *Listeria* spp. Spectral pattern changes in liquid growth medium, resulting from esculin hydrolysis by *Listeria*, were automatically monitored by the BioSys instrument in a semifluid layer (SFL). The blackening of SFL in modified MOX resulted in curves characterized by a sharp decline, which were easily detected by the instrument. All 9 *Listeria* strains tested positive. None of the gram negative organisms (*Proteus*, *E. coli*, *Pseudomonas*, *Citrobacter* and *Yersinia*) were detected in the system. While most gram positive organisms, including *Bacillus*, *Streptococcus* and *Lactobacillus* strains, were not detected by the system, some strains of *Staphylococcusaureus*, *Enterococcus faecium* and *E. faecalis* hydrolyzed esculin. As a result, they were detected in the system and produced black colonies on PALCAM and Oxford media. Good correlation was obtained between numbers of *Listeria* and detection times resulting from esculin hydrolysis. 100% correlation was obtained between the system and PALCAM plates for all swabs tested. Presence of *Listeria* resulted in fast detection: 1,000 CFU/ swab were detected in 10 to 13 h, whereas 1 to 10 CFU/swab were detected in less than 22 h.

(P2) POSTER WITHDRAWN

(P3) SUITABILITY OF SELECTIVE MEDIA FOR RECOVERY AND ENUMERATION OF SUBLETHALLY HEAT- AND ACID-INJURED *L. MONOCYTOGENES*

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The suitability of PALCAM and modified Oxford (MOX) agars for recovering sublethally heat- and lactic acid-injured *L. monocytogenes* was investigated. *L. monocytogenes* LM101M, LM103M (meat isolates), and Scott A were suspended in tryptose phosphate broth (TPB) and heated for up to 40 min. at 54°C. At selected intervals, samples were withdrawn, serially diluted, and surface plated onto tryptose phosphate agar (TPA), TPA + 4% NaCl (TPAS), PALCAM, and MOX, then incubated at 30°C for 72 h. Results showed that heat-injured LM103M was recovered in the highest numbers on all media, followed by Scott A and LM101M ($P<0.01$). TPA allowed the greatest level of recovery of all test strains, followed by PALCAM and MOX, which were not different, and finally TPAS ($P<0.01$). For acid-injury studies, uninjured and sublethally heat-injured LM103M was suspended in phosphate-buffered TPB (bTPB) and bTPB + 0.85% lactic acid (bTPBLA) and incubated at 25°C for up to 24 h. At selected intervals, test samples were plated as described above. Results showed that uninjured LM103M incubated in bTPB was recovered equally on all media, whereas sublethally heat-injured LM103M incubated in bTPB was best recovered on TPA, followed by MOX, TPAS, and finally PALCAM ($P<0.01$). Little or no difference in recovery of uninjured LM103M incubated in bTPBLA was observed with TPA, TPAS, and MOX, although recovery on PALCAM was poorest ($P<0.01$). Sublethally heat-injured LM103M incubated in bTPBLA was best recovered by TPA, followed by MOX, TPAS, and finally PALCAM ($P<0.01$). Results of this investigation reveal that recovery of *L. monocytogenes* on selective media is influenced by the type of sublethally injury (i.e., heat vs. acid injury). Provisions for resuscitation of injured cells should be made when using selective media for recovery.

(P4) IDENTIFICATION AND ENUMERATION OF *SALMONELLA* ON SAMPLE SLIDES OF POULTRY CARCASS WASH WATER USING IMAGE ANALYSIS

J. Huang,* Y. Li, M. F. Slavik and G. R. Bayyari, 203 Engineering Hall, University of Arkansas, Fayetteville, AR 72701

Rapid detection of bacteria on poultry carcasses is needed by the poultry industry. This research focused on the image analysis method for identification and enumeration of *S. typhimurium* on slide samples of poultry carcass wash water which were prepared using fluorescent antibodies and immunomagnetic beads. The criteria of morphological and optical characteristics of *S. typhimurium* cells, including area, aspect ratio, diameter, major and minor axes, maximum and minimum radii, perimeter, radius, ratio, length and width, and intensity, was developed. An algorithm that included channel extracting, median filtering, image sharpening, image dilation and erosion, image flattening, and watershed filtering was set up to analyze the acquired images. The algorithm was trained with 110 slide samples, and regression analysis was conducted for the image counting vs. plate counting results. The relationship between the image analysis and the plate counting was found to be linear with a correlation coefficient of 0.883. The sensitivity of this method was 10^4 cells/ml, and the

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time needed by the image analysis method was less than a half of that of the plate counting method.

(P5) EVALUATION OF AN AUTOMATED ENZYME-LINKED FLUORESCENCE IMMUNOASSAY (ELFA) FOR THE DETECTION OF *SALMONELLA*

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High and low concentrations of *Salmonella* in milk and whey products were compared to the BAM method using two protocols for the VIDAS system. Results indicate no significant differences in protocol #1 and the BAM method using pure cultures of *Salmonella*. The specificity and false negative rate of the VIDAS and BAM methods were 100% and 8%, respectively. Using Protocol #2, the sensitivity was 100% for the VIDAS method compared to 96% for the BAM method. The sensitivity in the presence of competing microorganisms was 99% for the VIDAS method compared to 75% for the BAM method. The false negative rate for VIDAS and BAM methods was 1% and 25%, respectively. The specificity rate for VIDAS and BAM methods was 100%.

(P6) ANTIBODY-DIRECT EPIFLUORESCENT FILTER TECHNIQUE (AB-DEFT) FOR RAPID, SPECIFIC ENUMERATION OF *LISTERIA* IN FOOD

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The antibody-direct epifluorescent filter technique (Ab-DEFT), which involves membrane filtration of food, fluorescent antibody staining, and epifluorescence microscopy, has been developed for direct, specific quantitation of *Listeria* in less than 1 h. The Ab-DEFT and most probable number (MPN) were compared as methods for enumeration of *Listeria* in ready-to-eat packaged salad mix. A rifampicin-resistant variant of *L. monocytogenes* was inoculated into homogenized salad mix, and the MPN and viable plate counts were performed under rifampicin selection, to compare quantitation by the two cultural procedures under conditions exclusive of indigenous microbial growth, and by the Ab-DEFT. Correlation coefficients were calculated for data comparing the MPN with viable plate counts ($r = 0.9563$); Ab-DEFT with viable plate counts ($r = 0.9590$) and MPN with Ab-DEFT counts ($r = 0.9362$). Quantitation also was performed without rifampicin selection, resulting in correlation coefficients of 0.9533 for MPN and viable plate counts; 0.9352 for Ab-DEFT with viable plate counts; and 0.9012 for MPN and Ab-DEFT. Presence of the indigenous microbial population in the salad mix at 2.1×10^5, 2.5×10^6, or 1.0×10^8 CFU/mL had no interfering effect on either the MPN or Ab-DEFT results. The general agreement between the methods shows the potential of the Ab-DEFT as a rapid alternative for quantitation of *Listeria* in food.

(P7) QUANTITATIVE SCREENING OF REACTIVITY OF *BACILLUS* AND *CLOSTRIDIUM* SPORES IN A DOT-BLOT IMMUNOASSAY

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Our laboratory has the goal of developing immunoassays to detect bacterial spores. It would be desirable to have rapid assays to detect specific organisms (species) as well as for detection of spores which occur or grow in similar environmental niches or have similar resistances and therefore may be problematic in a given food product or process. We have screened five monoclonal antibodies which were developed against *Bacillus cereus* and *Clostridium sporogenes* spores for their reactivity with a total of 33 strains of spores representing 10 species and 2 genera. A dot blot immunoassay was used and quantitative detection was afforded by application of a computerized image analysis system. Antibody 183 (type IgG) detected 8 of 10 *B. cereus*, *B. globigii*, 1 of 3 *B. coagulans*, 1 of 2 *B. subtilis*, but did not detect *B. megaterium*, *polymyxa*, *stearothermophilus*, *licheniformis*, or *C. perfringens*, or butyric anaerobes. Antibody 48 (type IgG) detected 1 of 2 *B. subtilis* and 1 of 2 *C. perfringens* but did not detect the other organisms. Type IgM antibodies have been similarly screened. These antibodies should be useful in detection methods for spores yet data suggest that cocktails of antibodies may be required for detection of the total range of spores of interest.

(P8) DETECTION OF *S. AUREUS* USING AN ENHANCED CHEMILUMINESCENT BIOSENSOR

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The need of a modern mobile military has resulted in the development of ration components that can be consumed while on-the-go. This has been accomplished by use of the hurdle preservation concept with water activity levels >0.86 to improve acceptability. The growth of *S. aureus* in these products after processing and during long term storage has been a constant concern. A sensitive and rapid biosensor was devised for detection of *S. aureus*. Commercial monoclonal antibodies specific for *S. aureus* species and non-protein A producing *S. aureus* were screened using a chemiluminescent ELISA format. Detection was accomplished with a horseradish peroxidase labeled secondary antibody that was used to catalyze the reaction of an enhanced luminol with hydrogen peroxide. A 10 fold difference in the affinity of the antibodies for *S. aureus* was found. The immunoassay performed in polystyrene tubes detected a concentration of $1.3 ¥ 10^4$ colony forming units (CFU)/ ml in a 3 to 4 hour assay. When the immunoassay was performed as a membrane biosensor, the time required to bind the microrganism was reduced to 5 min, the total assay time was reduced to 60 to 90 min with the capability of detecting 10^4 CFU/mL.

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(P9) MULTIPLEX PCR FOR THE DETECTION OF HUMAN ENTEROVIRUSES, HEPATITIS A VIRUS, AND NORWALK VIRUS

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A multiplex reverse transcription polymerase chain reaction (RT-PCR) method was developed for the simultaneous detection of the human enteroviruses, hepatitis A virus (HAV), and the Norwalk virus (NV). Using poliovirus type 1 (PV1) as a model for the human enterovirus group, three different sets of primers were used to produce three size-specific amplified DNA products of 650 bp, 192 bp, and 260 bp for PV1, HAV, and NV, respectively. Products were separated by agarose gel electrophoresis and amplicon identity was confirmed by Southern transfer followed by DNA hybridization using non-radioactive, digoxigenin-labeled internal oligoprobes. Detection limits of < 10 infectious units were achieved. Multiplex PCR offers advantages over traditional mammalian cell culture methodology and monoplex RT-PCR since it allows rapid and cost effective detection of non-culturable human enteric viruses in a single reaction tube.

(P10) MODIFICATION OF THE SAMPLE PREPARATION PROTOCOL IN THE BAX™ SYSTEM FOR SCREENING *SALMONELLA* TO PERMIT DETECTION IN FOOD MATRICES WITH INHIBITORY PCR EFFECTS

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Recently, a rapid pathogen detection system based on Polymerase Chain Reaction (PCR) called BAX™ for Screening has been introduced. This PCR method utilizes a very simple sample preparation procedure and does not require the extraction of DNA. Unfortunately, some food matrices such as cocoa, tea, and certain spices can inhibit or even prevent PCR from occurring; thereby, eliminating the usefulness of a quick, genetics-based screening test for these foods. Polyphenolics are a class of chemicals which are commonly found in plant derived food products and are known to inhibit PCR reactions. A compound, polyvinyl-polypyrrolidone (PVPP), can bind polyphenolic compounds and remove them from solution. The addition of a simple PVPP treatment to the standard sample preparation protocol in the BAX™ System can allow many foods to be routinely tested. The experiments below validate the use of PVPP for removing polyphenolic compounds. Various levels of pure polyphenolic (0, 0.05, 0.5, and 5 µg/rxn) were processed with and without PVPP treatment. PCR product was quantified using picogreen fluorescence units and varied between 0.4-0.9 without PVPP treatment to a range of 19-36 with PVPP treatment. The procedure was then evaluated on food products that routinely inhibit PCR reactions with a standard preparation. These foods include cocoa, spices (thyme, basil), ice tea beverage, soy flour, wheat germ, apple cider and carrageenan (thickening agent). All foods were tested using *Salmonella* inoculated at low levels (<5 cells/25g). The standard protocol and a PVPP treatment protocol were run in parallel. The results show that PVPP treatment makes a marked improvement in the amount of PCR product produced as evidenced in Polaroid photographs. This process allows many foods, previously not testable with the BAX™ System, to be analyzed with a straightforward, easy protocol modification.

(P11) RAPID MOLECULAR METHOD FOR THE DETECTION OF *SALMONELLA* SPP. USING PCR AND LCR

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A reverse transcription polymerase chain reaction (RT-PCR) method was developed for the detection of *Salmonella* spp. Primers targeting regions 444-461 and 1001-1019 of the 16S rRNA gene sequence of *Salmonella enteritidis* were designed to produce a 557-bp amplicon fragment. Detection limits of 100 CFU were achieved. No primer cross-reactivity was noted with 13 *Enterobacteriaceae*, including the closely-related species *Citrobacter freundii* and representative strains of the *Erwinia* genus, or 8 non-*Enterobacteriaceae* species. Due to high sequence similarity between rRNA from *Salmonella enteritidis* and other members of the family *Enterobacteriaceae*, the ligase chain reaction (LCR), which can detect single base pair differences, was applied for confirmation of PCR amplicons. This RT-PCR/LCR assay provided a rapid and sensitive method for the detection of a wide range of *Salmonella* spp.

(P12) RAPID DETECTION OF *SALMONELLA* IN FECES FROM DAIRY COWS USING A FLUORESCENT PCR-BASED ASSAY

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Salmonella poses a health threat to consumers and an economic threat to the dairy industry. Early detection of *Salmonella* in dairy cows could reduce incidence of contamination in milk and in dairy herds. We analyzed fecal samples for *Salmonella* by a rapid and simple process. Inoculated and non-inoculated fecal samples were enriched overnight in selenite cystine broth. Inoculated amounts of *S. dublin* ranged from 10^0 to 10^8 CFU/mL. A silica-guanidinium isothiocyanate DNA extraction was optimized to minimize PCR inhibitors from the feces and maximize DNA output. Amplification of the IS200 region, specific to *Salmonella*, was analyzed using TaqMan™ Sequence Detection System. An internal probe bound to the IS200 region fluoresces following cleavage during PCR by the 5' nuclease activity of *Taq* polymerase. PCR products were also verified by electrophoresis. The sensitivity of the assay resulted in detection of <50 CFU of *Salmonella* per mL of feces. Results of the overall assay, including enrichment time, were obtained in less than 24 hours and correlated well with culture methods. This procedure represents

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a fast and sensitive method for the dairy industry to detect *Salmonella* and monitor herd health.

(P13) RESULTS OF TESTING A VARIETY OF FOODS FOR *SALMONELLA* USING A FLUOROGENIC PCR-BASED ASSAY

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Salmonella, a Gram-negative bacterium, is the most commonly reported cause of foodborne illness. We have developed a rapid polymerase chain reaction (PCR) based assay for the detection of *Salmonella* in food samples. Results were obtained within 24 h including preenrichment of the food sample. This closed-tube homogeneous PCR assay uses the 5' nuclease activity of AmpliTaq DNA Polymerase with a fluorogenic probe and PCR primers specific for *Salmonella*. We tested more than 20 different artificially and naturally contaminated food types including raw eggs, hot dogs, yogurt and ground turkey. The assay was sensitive and specific, detecting *Salmonella* at < 5 MPN/25 g (mL) food sample prior to enrichment. The results had an excellent correlation with results obtained using standard USDA/FDA culture methods. The combination of extraction method, assay, instrument and automated analysis provides a total system for the rapid evaluation, identification, and documentation of foods contaminated with *Salmonella*.

(P14) EVALUATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY, DIRECT IMMUNOFLUORESCENT FILTER TECHNIQUE AND MULTIPLEX PCR FOR DETECTION OF *ESCHERICHIA COLI* O157:H7 IN BEEF CARCASS WASH

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In commercial beef processing, carcasses are customarily washed with water to remove physical contamination. Testing the water that runs off after washing can be a useful method to determine if the carcass is contaminated with such organisms as *E. coli* O157:H7. *E. coli* O157:H7 was seeded into carcass wash water at various levels and the bacteria were then concentrated by filtration. After collection of the bacteria in the filter units, the membranes were cut out, placed in tubes containing growth medium and mixed vigorously to remove the bacteria from the membrane into the broth. Preenrichment samples were then removed for testing by a multiplex polymerase chain reaction (PCR) and a direct immunofluorescent filter technique (DIFT). The remaining sample was subjected to 4-h enrichment culturing at 37°C after which samples were removed for testing by multiplex PCR, DIFT and an enzyme-linked immunosorbent assay (ELISA). Following 4-h enrichment culturing, detection limits using the ELISA, DIFT and multiplex PCR were 80, 0.1 and 0.8 colony forming units (CFU)/mL of wash water, respectively. On the basis of these results, testing carcass wash water by ELISA, DIFT or multiplex PCR can be useful for detection of *E. coli* O157:H7 carcass contamination and can potentially be employed as a verification tool during slaughter.

(P15) DEVELOPMENT OF PCR-BASED HOMOGENEOUS CONFIRMATIVE ASSAYS FOR *L. MONOCYTOGENES* AND *E. COLI* O157:H7

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Two highly specific, rapid assays were developed for the confirmation of suspect colonies of *L. monocytogenes* or *E. coli* O157:H7 from agar plates. The assays use PCR amplification coupled with homogeneous fluorescence detection of amplification products to screen up to 12 suspect colonies in a single test. One target colony can be detected even when mixed with 11 non-target colonies. The fluorescence signal is generated by a nucleic acid dye which is included in the reaction, thus allowing amplification and detection in a single closed tube. Appropriate assay conditions were determined by evaluation of parameters including cell concentration, lysis conditions, cycling parameters, and media compatibility. The assay steps are colony suspension, lysis, PCR and fluorescent detection. More than 1000 colonies can be processed in a single batch. Depending on batch size the assay can be completed in as little as three hours. The evaluation of single and mixed colony suspensions of over 30 *L. monocytogenes* strains and over 20 *E. coli* O157:H7 strains gave fluorescence values that were higher than corresponding control samples containing only non-target colonies. These assays represent a rapid, convenient, and highly specific means of confirming suspect colonies and, unlike other confirmative assays, can be performed on mixed isolates.

(P16) DEVELOPMENT AND EVALUATION OF A PCR-BASED ASSAY FOR THE DETECTION OF *LISTERIA MONOCYTOGENES* IN FOODS

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A simple PCR-based method was developed for the rapid detection of *L. monocytogenes* from enrichment culture. Primers specific for this pathogen were identified following screening of a panel consisting of 323 target and 30 non- *L. monocytogenes* strains. The selected primers amplified a 380-bp product in 100% of the target strains and 0% of the non-target strains. These primers were tableted along with dNTPs and *Taq* polymerase. The assay method consists of a 24-48 h selective enrichment of a food sample, followed by a simple lysate preparation, addition of 50 µl of the lysate to the PCR tablet, thermal cycling, then agarose gel detection. Forty-four samples of a wide variety of foods including deli products, soft cheeses, chilled/frozen desserts, and salads were screened unspiked or spiked (5-30 target cells/25 grams) using the assay de-

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scribed. The same foods and spike levels were run in parallel using the FDA rapid method with culture confirmation of presumptive positives. The FDA method takes a minimum of 48 hours. Following 24 and 48 hour enrichments, there was 90% and 95% overall agreement, respectively between the PCR assay and the FDA method. At 48 hours, both methods are statistically equivalent. Of the spiked samples that were confirmed culture positive, the PCR method detected 95% at 48 h and 83% at 24 h.

(P17) CONCENTRATION OF PATHOGENIC MICROORGANISMS FROM DAIRY PRODUCTS FOR DETECTION BY PCR

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Rapid, direct methods to detect pathogenic microorganisms in food products using the polymerase chain reaction (PCR) has been complicated by large sample volumes, low levels of contamination, and the presence of inhibitory compounds that interfere with enzymatic amplification. Bacterial immobilization using metal hydroxides was investigated as a method to concentrate bacteria from a complex food matrix prior to application of PCR. Using nonfat dry milk as a model system, 25 ml samples were seeded with 10^1-10^7 CFU of *L. monocytogenes* (LM) or *S. enteritidis* (SE) and clarified with 25% sodium citrate. After centrifuging as a primary concentration step, bacteria were further concentrated by immobilization with 65% solutions of zirconium hydroxide or titanous hydroxide. The efficiency of bacterial immobilization using metal hydroxides exceeded 98% in model systems. When applied to the seeded milk samples, recoveries ranged from 65-100% and 70-100% for LM and SE respectively, regardless of metal hydroxide. Recovery efficiencies were partially dependent upon the initial concentration of cells. When nucleic acids from final concentrates were extracted using a guanidinium thiocyanate method, the resulting product represented a >100-fold sample concentration factor with removal of PCR inhibitors. Future endeavors will seek to improve immobilization efficiencies and directly link concentration methods to nucleic acid amplification.

(P18) RAPID METHODS FOR IDENTIFICATION OF LACTIC ACID BACTERIA

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Lactic acid bacteria are the primary contributors to a variety of fermentations and may be problematic spoilage organisms. However, rapid method development for these organisms has not kept pace with more clinically significant organisms. Three methods to characterize and identify lactic acid bacteria were examined. The methods were the Biolog biochemical test kit, the Microbial Identification System of fatty acid profiling, and the Qualicon Riboprinter™ method of ribotyping. One hundred and thirty-one strains of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Lactococcus* species were obtained from the American Type Culture Collection (ATCC) and throughout the food industry. When the ATCC strains were analyzed, ribotyping performed best with genus and species being identified correctly 82% and 75%, respectively. The Biolog correctly identified 72% and 53%, and the fatty acid 71% and 25%, respectively. For initial characterization of an isolate, a combination of methods was recommended to gain maximum information.

(P19) GENETIC CHARACTERIZATION OF *SHEWANELLA PUTREFACIENS* AND *PSEUDOMONAS* SPP. ISOLATED FROM FISH PROCESSING AND SPOILAGE USING AUTOMATED RIBOTYPING

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Food producers and consumers have a common interest that food is of the highest possible hygienic quality. This means that the food is free of pathogenic microorganisms and the number of other microbes is at a minimum. During fish processing, there is a substantial increase in bacterial numbers on the fish. In some cases this is no more than can be expected and is acceptable but often these bacteria can be spoilers or pathogens. When setting up a HACCP system or other quality assurance system, it is important to know which bacteria are present, at which points they contaminate the fish, and how they can be controlled. A study was conducted to examine the abundance of different bacterial types at different locations in three fish-freezing plants in Iceland. The bacterial numbers were estimated and selected isolates were identified to the group/genus level using classical microbiology methods. A subset of these isolates was chosen for genetic characterization using the RiboPrinter™ Microbial Characterization System. Thirteen strains of *Shewanella putrefaciens*, 11 strains of *L. monocytogenes*, 4 strains of *Aeromonas* spp., 7 strains of *Vibrio* spp., and 38 strains of *Pseudomonas* spp. were processed. The results showed much greater diversity (11 RiboGroups) among the *S. putrefaciens* strains than anticipated. The *Pseudomonas* spp. isolates had been segregated conventionally into groups I, II, and III-IV. As expected, these isolates demonstrated significant genetic variation (16 RiboGroups).

(P20) COMPARISON OF EXCISION VERSUS SWABBING TECHNIQUES FOR ASSESSING THE BACTERIOLOGICAL QUALITY OF PIG CARCASS SURFACES

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Two different swabbing methods (single or multiple site) were compared to the standard excision method to determine the bacteriological quality of pig carcass surfaces. Swab and excision samples were taken from 105 hog carcasses 24 h post slaughter. Excision samples were discs from the ham, belly and jowl held 24 h at 4°C prior to processing. Swab samples were taken either at one site (belly) or at 3 sites with the same swab (ham, belly and jowl). Swab samples were either processed immediately

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or after storage for 24 h at 4°C. Samples were processed for total aerobic counts as well as for total coliforms and *E. coli* counts using Petrifilm™ aerobic count plates or Petrifilm™ *E. coli* count plates, respectively. The data were analyzed by ANOVA either as counts or as incidence (counts of 5 CFU/cm^2 were treated as zero). The excision method resulted in statistically higher recovery of total aerobes, coliforms and *E. coli* when compared to either single or multiple site swabs; however, it was much more labor-intensive. Comparison of the multiple and single site swab methods indicated that the 3 site swab resulted in much higher recovery of all organisms analyzed with the increase in counts resulting from a higher incidence of bacterial contamination present on the ham as compared with the belly. The easier-to-use multiple swab method could be used for quality control surveys as well as HACCP programs.

(P21) A NOVEL TECHNIQUE FOR *E. COLI* TESTING OF BEEF AND PORK CARCASSES

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In January 1997, the USDA-FSIS mandated *E. coli* testing in all processing plants to monitor surface contamination of beef, pork, and poultry carcasses. This mandate, in conjunction with HACCP, was implemented in response to the growing number of foodborne outbreaks attributed to pathogens of animal origin. A novel technique has been developed to monitor *E. coli* contamination on carcasses through membrane filtration (MF) and m-ColiBlue24 (mCB). mCB is a MF medium that simultaneously detects Total Coliforms (TC) and *E. coli* (EC) in a 24 hour period. A study was conducted on pork and beef carcasses that compared mCB to standard methods. On pork carcasses (n = 56), the mean values for mCB and Violet Red Bile Agar (VRBA) were 6 CFU/15 cm^2 and 3 CFU/15 cm^2, respectively ($t = 3.2$, $P < 0.01$). Spiked beef carcasses (n = 57) were used to compare mCB to both TC Petrifilm™ and EC Petrifilm™. The mean TC count on mCB was 1.6×10^4 CFU/cm^2 and 9.3×10^3 CFU/cm^2 on TC Petrifilm™ ($t = 2.4$, $P = 0.02$). The mean EC count on mCB was 9.3×10^3 CFU/cm^2 and 3.2×10^3 CFU/cm^2 on EC Petrifilm™ ($t = 3.5$, $P < 0.01$). The combination of MF and mCB detected more TC and EC than VRBA and both types of Petrifilm™.

(P22) A 24-HOUR TEST FOR ENUMERATION OF TOTAL COLIFORMS AND *E. COLI* IN FOOD

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SimPlate™ for Total Coliform and *E. coli* (CEc) is a new method for the detection and quantification of total coliforms and *E. coli* in food. Quantification is achieved by incubating the CEc media in a device containing 84 wells called a SimPlate™ which serves as an autoaliquoting incubation vessel. Detection of total coliforms is based upon the enzymatic cleavage of CPRG by ß-galactosidase. Concurrent activity of ß-galactosidase and ß-glucuronidase, which cleaves MUG, indicates the presence of *E. coli*. The number of red colored wells with and without fluorescence after 24 hours of incubation is converted into the most probable number (MPN) of *E. coli* and total coliforms, respectively.

Regression analysis of data from SimPlate™ for CEc versus Petrifilm™ testing a variety of food matrices generated r=0.95 for total coliforms and r=0.97 for *E. coli*. SimPlate™ for CEc demonstrated better recovery of *E. coli* than Petrifilm when high concentrations of total coliforms were present. SimPlate™ for CEc versus VRB+MUG generated r=0.98 for total coliforms and r= 0.97 for *E. coli*. Data from two commercial reference laboratories demonstrated 97% agreement of concentrations of *E. coli* from SimPlate™ for CEc with multiple tube fermentation. It is concluded that SimPlate™ for CEc is a suitable alternative for total coliform and *E. coli* testing in food.

(P23) THE OCCURRENCE OF NON-COLIFORM BACTERIA ON VRBA

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The coliform bacterial count in food is one of the most convenient methods to check the sanitary condition during food manufacturing. Recently, government authorities strengthened the regulations on the inspection of coliform bacteria because of frequent occurrence of food poisoning due to the *E. coli* O157. But on Violet Red Bile Agar (VRBA), non-coliform bacterial colonies are often formed and counted as coliforms. The occurrence of these colonies may cause confusion to the food manufacturers. Therefore, this study was carried out to measure the proportion of non-coliforms to coliforms by identifying the colonies appeared on VRBA. LTLT fresh market milks collected in Korea were inoculated and incubated at 32°C for 24 h, after preincubation at 35°C for 24 h. A total of one hundred and twenty-nine colonies were isolated from 41 plates positive on VRBA. The number of coliforms isolated was 95 (73.6%) and non-coliforms 34 (26.4%). Among them, 36 colonies (27.9%) were *Enterobacter* spp. which was the highest in number and then, *E. coli* 24 (18.6%), *Citrobacter* 18 (14.0%), *Klebsiella* 12 (9.3%), *Alcaligenes* 8 (6.2%), *Pseudomonas* 6 (4.7%), and other gram negative red 15 (11.6%), respectively. The results of this study indicate that the simple measurement of the number of colonies on VRBA is not sufficient to judge the sanitary condition of milk and dairy products.

(P24) EVALUATION OF A NOVEL METHOD FOR THE DETECTION OF *S. AUREUS* IN DAIRY SAMPLES

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A study was undertaken to compare the performance of a new immunoassay for detecting the presence of *S. aureus* in foods to a reference cultural method. In the first part of the study, the sensitivity of the TECRA *S. aureus* Visual Immunoassay (VIA) was found to be as low as 10^5 cells/mL for some strains and by testing over 50 non-*S. aureus* cultures undiluted, the kit was found to be highly specific for *S. aureus*. In the second part of the study, over 100 dairy foods were surveyed for *S. aureus* by the two

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methods. The products included raw and pasteurized milk, cream, milk powder, cheese, yogurt, dip, fermented drink, custard, dairy dessert, sour cream, butter and ice cream. In addition, 25 environmental samples obtained from dairy factories were tested. The kit showed excellent correlation with the standard plating technique and was able to detect *S. aureus* from samples with as little as 1 cell per gram of food. A further analysis of the samples which contained *S. aureus* found approximately half were enterotoxigenic and could potentially pose a health risk if time and temperature abuse occurred prior to consumption of the food. Most of the enterotoxigenic isolates produced enterotoxins A or D. The TECRA *S. aureus* VIA was found to be a highly sensitive and specific method for screening foods for *S. aureus*. The method was extremely easy to use and results were available at least one day sooner than for the reference method.

(P25) THE EVALUATION OF AN AUTOMATED RAPID MICROBIAL DETECTION SYSTEM FOR STERILITY TESTING OF AN ASEPTICALLY PROCESSED TOMATO-BASED VEGETABLE BEVERAGE

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The rapid detection of microbial contamination of aseptic products is desirable for routine QA monitoring. The conventional commercial sterility test usually involves product incubation followed by selective plating on recovery media, requiring 2 weeks to obtain results. The ESP Microbial Testing System (Difco Labs, Detroit, MI) has been shown to save labor and time. ESP is a fully automated instrument, which measures changes in head space pressure in a closed system based on production and/or consumption of gases by microbial growth. The objective of this study was to evaluate the instrument's capability to detect various spoilage organisms in an aseptic tomato-based vegetable beverage (pH 3.9 - 4.2). Bottles containing 150 ml of a specially formulated low pH medium (pH 4.2) were inoculated with various levels of microbial cultures with and without product (10-ml sample). Inoculated media bottles were monitored continuously in the ESP System for 5 days at 35°C. Results showed <10 CFU/bottle of lactics (e.g. *Lactobacillus plantarum*), yeast, and mold were detected in the product in <72 h, and <100 CFU/bottle of a *Bacillus* spp. isolate were detected in <48 h. One strain of *B. coagulans* tested, which was inhibited at pH 4.2, was recovered at <10 CFU/bottle in <48 h in a medium adjusted to pH 5.3. The ESP System is sensitive and provides shorter detection times for the organisms tested compared to conventional commercial sterility testing. This could shorten or eliminate preincubation of product.

(P26) SIMPLATE™ FOR YEAST & MOLD: A NEW METHOD FOR RAPID FUNGI ENUMERATION IN FOOD

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SimPlate™ for Yeast and Mold (SYM) uses IDEXX's Multiple Enzyme Technology for the rapid detection and enumeration of foodborne fungi in food. The presence of yeast and mold in the food sample is revealed by blue fluorescence under a long wavelength ultraviolet lamp (365nm). The SimPlate™ Most Probable Number (MPN) device, was used in conjunction with the SYM medium to enumerate fungal concentration in food in the present study.

SYM was evaluated in parallel with the standard 5-day Potato Dextrose Agar (PDA) supplemented with chlortetracycline (100 µg/ml) and chloramphenicol (100 µg/ml). Food samples used in this study included beverages (e.g. soda, fruit juice, juice drinks, fruit juice concentrates, etc.), ingredients (e.g. wheat products, corn meal, flour, seasoning, pie filler etc.), dairy products (e.g. raw milk, cheese, ice cream, yogurt, etc.), and other prepared food products (e.g. ketchup, pickles, salad dressing, etc.). The SYM test, when incubated at 30°C for 2 days, showed a strong agreement with standard PDA yeast and mold counts (5 days @ 25°C) with a correlation coefficient of 0.96. Furthermore, a strong linear correlation (r = 0.97) was also obtained between SYM (3 days @ 25°C) and the 5-day PDA counts (25°C incubation). The SYM medium was also shown not to cross react with bacteria at a level of ~10^8 CFU/test after 5 days of incubation.

(P27) BIOLOGICAL PROPERTIES OF A BACTERIOCIN-LIKE INHIBITORY SUBSTANCE PRODUCED BY A NEWLY ISOLATED *BACILLUS SUBTILIS*

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To determine the biological properties of a bacteriocin-like inhibitory substance (BLIS) of a newly isolated *Bacillus subtilis*, the BLIS in a culture supernatant was precipitated via 55% saturated ammonia sulfate at 4°C, and then desalted and partially purified by gel filtration. The partial purified BLIS exhibited inhibitory activity against Gram-positive bacteria, including foodborne pathogens *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus*. On SDS-PAGE gel, the BLIS demonstrated a molecular weight of approximately 3,500 daltons. The activity of BLIS in this study could be inactivated by two pancreatic peptidases, beta-chymotrypsin and delta-chymotrypsin. Results of this study suggest that the BLIS produced by this strain of *B. subtilis* is a bacteriocin with activity against Gram-positive bacteria and has a potential use as a food preservative.

(P28) USE OF HPLC TO DEMONSTRATE AFLATOXIN B_1 DEGRADATION BY *FLAVOBACTERIUM AURANTIACUM* IN CORN

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Biodegradation of aflatoxins in culture media by *Flavobacterium aurantiacum* has been well documented. In this study, this bacterium was used to degrade aflatoxin B_1 (AB_1) in a corn system, where AB_1 contamination is a serious concern. A USDA recommended immunoaffinity column clean-up procedure was used with modifications

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to extract AB_1 (spiked at 4 ppm) combined with a reversed-phase HPLC system to detect residual AB_1 at 0 h and 72 h of incubation. We further looked at differences in the cytosolic protein profile of the bacterium before and after exposure (72 h) to AB_1, using a 14% PAGE technique. The extraction efficiency of the immunoaffinity column clean-up and HPLC detection system was 89%. It was observed that the bacterium degrades 78% of AB_1 in 72 h. Results from PAGE of the cytosol fraction of the bacterium suggest that there is an increased expression of 14.5 and 31 Kda molecular weight range proteins when the bacterium is exposed to AB_1 for 72 h. Further protein characterization studies are currently underway in our laboratory.

(P29) OCCURRENCE OF MOLDS AND LEVELS OF AFLATOXINS AND FUMONISINS IN VENEZUELAN CORN

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Corn samples (20) of the 1995 harvest season were purchased from supermarkets in Caracas. These samples were analyzed for mold occurrence and incidence of aflatoxin and fumonisin using TLC and HPLC, respectively. Incidence of mold on DRBC, DCPA and AFP ranged from 10^2 to 10^6 CFU/g. Four samples (20%) were positive for aflatoxin with levels between 4 to 10 ppb. Fumonisin was detected in 15% of the samples with levels ranging from 58 to 117 ppm. *Aspergillus flavus* isolated from corn samples was positive for aflatoxin and only one isolate produced aflatoxin using rice as substrate. Moisture levels of corn samples ranged between 14.2% to 21.7%. These values are higher than the value of 12% established by official standards (COVENIN). A positive relationship between moisture levels and levels of *Aspergillus* or aflatoxin was obtained but not with fumonisin occurrence and incidence of *Fusarium*. Main species of mold identified in this study were *A. flavus*, *F. moniliforme*, *P. citrinum*, *P. aurantiogriseum*, *A. versicolor*, *F. oxysporum*, *A. oryzae*, *A. niger*, *A. terreus*, *R. stolonifer*, *Syncephalastrum* and *A. ochraceus*. *A. flavus* and *F. moniliforme* represent 45% and 9.4% from the isolates, respectively.

(P30) ENUMERATION AND CHARACTERIZATION OF *AEROMONAS* SPP. IN VEGETABLE PRODUCTS FROM VENEZUELA

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Quantitative recovery of *Aeromonas* spp. from 104 samples of fresh fruits and vegetables and minimally processed vegetable salads, 32 samples of ready-to-use salads, with and without dressing and 24 samples of commercial salads, packed in heat-sealed plastic bags was determined using Starch Ampicilin Agar. The presumptive *Aeromonas* were characterized biochemically using the API 20E and API 20NE systems and the APILAB Plus software. Biological tests (CAMP-like factor and suicide phenomenon) and pathogenicity tests (-hemolysis, hemolysin activity, enterotoxin cholera-like and invasivity). The populations of presumptive *Aeromonas* spp. of the products ranged from $<1 \times 10^2$ to 3×10^6 CFU/g. There was significant positive correlation between populations of *Aeromonas* spp. and pH in ready-to-use salads. In fresh fruits and vegetables and commercial salads *A. caviae* was the main species found, followed by *A. hydrophila* and *A. sobria* while in ready-to-use salads *A. hydrophila* was in higher proportion than *A. caviae* and *A. sobria*. Identification using CAMP-like factor and suicide phenomenon was not able to separate completely between species of *Aeromonas*. The majority of isolates of *A. hydrophila* and *A. sobria* showed hemolysis. Hemolytic activity was mainly detected in isolates of *A. hydrophila*. All the *A. hydrophila* studied were negative for enterotoxin cholera-like and invasivity test. Results indicate that the presence of significant populations of *Aeromonas* spp. in almost all products establishes the ubiquity of these microorganism in vegetable products suggesting that *A. hydrophila* may be a potential vector of transmission of gastroenteritis.

(P31) INHIBITION OF MICROBIAL GROWTH AND TOXIN PRODUCTION IN HONEY

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As food products, such as honey, are used in new applications, new questions about microbial safety emerge. The objectives of this study were to investigate whether *Staphylococcus aureus* and selected mold species grow and produce toxins in honey. *S. aureus* did not grow in any honey samples and in fact declined from 10^4 CFU/g to below the limits of detectability within six to twelve days. Pre-exposure of staphylococci cells to higher concentrations of carbohydrate did appear to influence survival. Increasing the water activity of the honey (to mimic the microenvironment that may develop on the top of a barrel of honey) did not lead to increased survival of *S. aureus*. No enterotoxin production was detected. Likewise, germination and growth of molds in honey was inhibited. There was about a one log reduction in 21 days for *Aspergillus flavus* spores on the surface of honey and when spores were mixed into the honey. The number of viable spores of *Penicillium citrinum* and xerophilic molds was reduced in a way similar to that of *A. flavus* spores. Neither aflatoxins nor citrinin were detected in the honey. Microscopic examinations of *A. flavus* and *P. citrinum* spores showed that there was no formation of germ tubes, while spores of xerophilic molds showed emergence of short germ tubes but no subsequent growth. Inoculation of honey by staphylococci or mold species would be unusual and would result only from unacceptable practices by the processor. However, if contamination occurs, outgrowth and/or toxin production is unlikely. These data are important for those setting public health standards or purchasing specifications.

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(P32) EFFECT OF DIET ON THE INDICATIVE AND PATHOGENIC MICROBIOLOGICAL QUALITY OF AQUACULTURED PACU (*PIARACTUS MESOPOTAMICUS*)

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The qualitative and quantitative numbers of bacteria were determined on water used for culturing pacu as well as on pacu (*Piaractus mesopotamicus*) following processing. Pacu fingerlings weighing approximately 72 g were fed three different diets (a) Zucchini (0.5% protein); (b) Commercial aquaculture feed P32 (32% protein); and (c) Commercial aquaculture feed P36 (36% protein) and raised for 24 weeks. Microbial analyses on growing waters included aerobic counts, psychrotrophic counts, total and fecal coliform counts as well as pathogens assayed at 6 week intervals. At 24 weeks, twenty pacu were randomly selected, gutted and analyzed for pathogens using AOAC procedures. Five fish from the pathogen analyses were used for analyzing the indicative microbial quality using 3M™ Petrifilm™. The mean counts (range) for aerobes, psychrotrophs, total coliforms, fecal coliforms, and *Escherichia coli* ranged among 5.05 (3.72-6.81 log CFU/g), 5.05 (2.49-6.81 log CFU/g), 2.66 (0.85-4.21 log CFU/g), 2.92 (0.85-4.00 log CFU/g), and 0.13 (0.00-0.20 CFU/g), respectively. The indicative microbial quality differed significantly (P <0.05) among the treatments, except for *E. coli*. *L. monocytogenes*, *Yersinia enterocolitica*, *E. coli* O157:H7 and *Salmonella* spp. were not isolated from the sampled fish. Pacu grown on P32 and P36 diets exceeded the ICMSF limits for fecal coliform counts for freshwater fish (M = 400/g) and hence were concluded to be of unacceptable bacterial quality.

(P33) ANTIBIOTIC RESISTANT BACTERIA IN AQUACULTURED CATFISH FILLETS

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Fresh aquacultured channel catfish (*Ictalarus punctatus*) fillets were surveyed for the presence of antibiotic resistant bacteria. Five fresh catfish fillets were obtained during different processing seasons (e.g., summer, fall, winter and spring) from processors in the southern United States. Five fish fillets were randomly selected for determination of antibiotic resistant bacteria. The standard plate counts were enumerated using 3M™ Petrifilm™ Aerobic Count plates. The antibiotic resistant bacteria were determined for the following antibiotics viz. ampicillin, novobiocin, oxytetracycline and Romet™. Filter sterilized solutions of the antibiotics were added to thermally sterilized and tempered (@48°C) Standard Methods Agar. Ampicillin, novobiocin, oxytetracycline and Romet™ were added at 30.0, 20.0, 25.0 and 25.0 µg/mL, respectively, and all plates were incubated aerobically at 35°C for 48 ± 2 h. There were significant differences ($P \leq 0.05$) in aerobic and antibiotic resistant bacteria counts due to processing seasons. Differences in standard plate count could partly be attributed to the culturing season and processing conditions while the differences in the antibiotic resistant bacteria could be attributed to survival and growth conditions in the ponds and seasonal variations which affect the nature and number of antibiotic resistant bacteria. Both aerobic and antibotic resistant bacterial plate counts were significantly lower (P <0.05) during the colder weather and significantly higher (P <0.05) during warm weather.

(P34) EFFECT OF PRODUCTION SYSTEM ON THE INDICATIVE AND PATHOGENIC MICROBIOLOGICAL QUALITY OF AQUACULTURED FINFISH

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The nature and number of indicative and pathogenic microbes in fish reared using pond and recirculating systems were compared. For each system, 20 samples of rainbow trout (*Oncorhynchus mykiss*), tilapia (*Tilapia nilotica*), hybrid striped bass (*Morone saxatilis* ¥ *M. chrysops*), and pacu (*Piaractus mesopotamicus*) were randomly selected, gutted, and microbial analyses performed using AOAC procedures. Five fish were subsampled and analyzed for indicative microbial quality using 3M™Petrifilm™. The general microbial quality differed significantly (P <0.05) among the treatments, except for total coliform counts. Rainbow trout cultured in pond and recirculating systems had lower counts for aerobes (2.00-3.11 log CFU/g) (P <0.05), where-as those reared in a recirculating system had significantly lower psychrotrophic numbers (0.86-1.85 log CFU/g). Pacu had the highest fecal coliform counts (2.74-3.70 log CFU/g), whereas hybrid striped bass and rainbow trout grown in ponds had lower fecal coliform counts (0.00-1.39 log CFU/g). Rainbow trout grown in ponds had significantly higher *E. coli* counts (0.00-2.11 log CFU/g). No human bacterial pathogens – *L. monocytogenes*, *Y. enterocolitica*, *E. coli* O157:H7 and *Salmonella* spp. were isolated. All the samples, except pacu, met the ICMSF criteria for freshwater fish and hence were considered to be of good quality. Pacu had fecal coliform counts higher than 400 CFU/g and were concluded to be of unacceptable quality.

(P35) EFFECTS OF VITAMIN E SUPPLEMENTATION AND HIGH VERSUS LOW INITIAL MICROBIAL LOADS ON RETAIL DISPLAY LIFE OF BEEF MUSCLE

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Dietary supplementation of cattle fed with vitamin E (VE; a-tocopherol)provides a natural antioxidant in postmortem muscle that delays metmyoglobin formation, prolongs beef retail caselife and increases the opportunity for sale. In a split-split plot design, this study evaluated lean color and psychrotrophic aerobic plate counts (APC) during retail display (0, 2, 4 or 6 d) of beef strip loin steaks (*longisimus dorsi*; tray-overwrapped and displayed at 0°C under fluorescent lighting) produced from beef cattle either supplemented (500 IU/d for 100 d; n=18) or not

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supplemented (n=18) with VE and processed to create steaks with either low (LOW: 1.7 to 1.9 log CFU/cm^2) or high (HI: 6.4 to 7.1 log CFU/cm^2 by inoculating 1 ml of 7.9 log CFU/mL psychrotrophic broth) initial APC loads, respectively, and industry-normal control steaks (CON). Psychrotrophic APC differed by processing treatment at 2, 4 and 6 d of display (HI > CON > LOW; P <.05), but not by level of VE. By 4 d of display time, spectrophotometric a* and b* values, percent discoloration and consumer acceptability scores all were lower for HI and higher for LOW steaks; CON steaks were intermediate (P < .05). High levels of contamination eliminated benefits to beef lean color from VE supplementation. Among LOW and CON steaks, VE improved (P <.05) a*, b*, percent discoloration, and consumer acceptance scores at 4 and 6 d of display.

(P36) RAPID CATALYTIC ACTIVITY METHOD FOR MEASUREMENT OF ENDPOINT TEMPERATURE IN COOKED BEEF AND SAUSAGE

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Verification of endpoint temperature (EPT) is needed in cooked meat products due to recent outbreaks of *E. coli* O157:H7. USDA and FDA have issued cooking requirements for hamburger patty-type products allowing a series of temperature-time processes to produce pathogen-safe consumer products. However, no testing methods are currently available. Catalase (CAT) activity was determined on 1 g samples of ground round (4% fat), and commercial pork sausage (38% fat) cooked to 65, 68.3, and 71°C then removed every 15 s and quick-chilled (0-1°C). Samples retained high CAT activity through 90, 60, and 45 s at 65, 68.3, and 71°C, respectively before showing rapid decrease in activity. Hamburger (24% fat), was cooked at four USDA-FSIS approved meat patty heating processes (66.1°C/41 s, 67.2°C/26 s, 68.3°C/ 16 s, and 69.4°C 10 s) and analyzed for CAT activity. Meat state (non-frozen vs. frozen) prior to cooking caused slightly lower (P<.05) CAT activity in frozen meat. CAT activity decreased (P<.05) among 66.1°C/41 s, 67.2°C/26 s, 68.3°C/16 s, but 68.3°C/ 16 s was not different (P<.05) from 69.4°C/10 s. Results show this rapid (20-25 min) test could be used for verifying EPT by FSIS inspectors and by food processors in quality assurance/HACCP programs.

(P37) SHELF LIFE OF GROUND BEEF PATTIES TREATED BY GAMMA IRRADIATION

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Irradiation has been reported to reduce the level of spoilage bacteria in meat products extending product shelf life. This study investigates the effects of low-dose gamma irradiation on the microbial population in ground beef patties vacuum packaged and irradiated frozen at target doses of 0, 1, 3, 5 and 7 kilograys (kGy). Irradiated samples were stored at 4°C or -18°C for 42 days. Mesophilic aerobic plate counts (APC/g) were determined by plating the samples on standard methods agar incubated at 35°C for 48 h. Fresh ground beef, 10^2 CFU/g, treated with 3, 5, and 7 kGy was acceptable for 42 days at 4°C. The 1 kGy beef samples were acceptable microbiologically after 42 days, but developed an unacceptable off-odor after 21 days. Shelf life diminished in fresh ground beef patties with an initial microbial count of 10^4 CFU/g. Only beef patties treated with 7 kGy were found to be acceptable at 42 days. Beef patties treated at 1 and 3 kGy reached spoilage levels by day 14, whereas patties treated at 5 kGy did not spoil until 42 days. The control samples for both batches of ground beef spoiled within 7 days. However, ground beef patties stored at -18°C did not decrease in microbial counts. This study indicates that shelf life of ground beef patties stored at 4°C may be extended with low-dose gamma irradiation, especially at 5 and 7 kGy. Initial microbial load in ground beef was an important shelf life factor.

(P38) SENSORY CHANGES OF IRRADIATED GROUND BEEF THROUGH SIX WEEKS OF STORAGE

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Low dose gamma irradiation has been shown to be a safe method for elimination of pathogenic bacteria. Thus, the purpose of this study was to evaluate sensory aspects of irradiated ground beef patties over a six-week storage period. Ground beef patties were irradiated at low dose levels of 1.0, 3.0, 5.0 and 7.0 kGy. Non-irradiated patties were used as a control. Sensory evaluation was completed weekly by 10 to 12 experienced panelists on samples stored at -18°C for a period of six weeks. Patties were flame broiled and evaluated for odor, taste and texture. No significant differences in odor were noted for up to four weeks among the irradiated and non-irradiated samples. After the fifth week there was a difference between the non-irradiated beef patties and those irradiated at 7.0 kGy. Only after six weeks of storage with 7.0 kGy of irradiation was there a significantly strong aftertaste noted among samples. No significant differences were noted by the panelists in dryness among samples. Increased irradiation resulted in a trend toward a more tender product through week four. Overall acceptability of the samples was not significantly lower except at week six for the 7.0 kGy of irradiation. Storage combined with irradiation appears to yield an effect only after five weeks of storage.

(P39) THE EFFECT OF GROWTH MEDIUM AND HEATING MENSTRUUM ON HEAT RESISTANCE OF *PEDIOCOCCUS* SP.

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Pediococcus sp. (formerly, *Micrococcus freudenreichii*) is a spoilage non-pathogenic organism that was isolated from milk and milk utensils. This bacterium is a recognized marker for milk pasteurization due to its heat resistance. These characteristics made this bacterium an attractive test organism in studying the mode of bacterial destruction by microwave energy. We studied the effect of growth medium on the thermal D-value of this organism in different heating menstruums (skim milk, whole liquid

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eggs, 10% glucose solution, pineapple juice, apple juice, tomato juice and water). The D-value (60°C) of exponential phase cells grown at 28°C in tryptone glucose yeast extract (TGY) ranged from 0.15 min in pineapple juice to 7.92 min in skim milk. The D-value (60°C) range of tryptic soy broth (TSB) grown cells was from 0.70 min in pineapple juice to 12.49 min in 10% glucose solution. Preliminary membrane fatty acid data suggested that the increase in the heat resistance of the TSB grown cells was due to an increase in the ratio of saturated to unsaturated fatty acid chains.

(P40) EVALUATION OF CHANGES IN MICROBIAL POPULATIONS ON BEEF CARCASSES RESULTING FROM STEAM PASTEURIZATION

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Naturally occurring bacterial populations on 20 beef carcasses immediately before and after commercial steam pasteurization (6.5 s steam exposure at 82.2°C) were determined. Total aerobic mesophilic bacterial (APC) populations were reduced ($P \leq 0.01$) from 1.84 $\log_{10}$ CFU/cm^2 before treatment to 0.84 $\log_{10}$ CFU/cm^2 after. Randomly selected isolates (100) were identified from APC plates before and after pasteurization. Microflora before pasteurization primarily consisted of *Tetragenococcus* (26.4%), *Staphylococcus* (23.0%), *Aeromonas* (20.8%), and *Streptococcus* (6.6%). After treatment, the microflora was *Bacillus* (45.8%), *Staphylococcus* (20.8%), *Corynebacterium* (9.4%), and *Tetragenococcus* (6.3%). *Enterobacteriaceae* populations (-0.39 $\log_{10}$ CFU/cm) prior to pasteurization - 0.39 $\log_{10}$ CFU/cm^2 were reduced to -1.22 $\log_{10}$ CFU/cm^2 by steam treatment. Identification of 25 isolates before pasteurization indicated *Escherichia coli* (41.7%), *Enterobacter* (25.0%), *Citrobacter* (6.3%), *Klebsiella* (4.2%), and *Aeromonas* (4.2%) presence. After pasteurization, micro-flora was composed of *Enterobacter* (44.0%), *Citrobacter* (16.0%), and *Klebsiella* (8.0%). Steam pasteurization results in carcass microflora almost exclusively comprised of Gram-positive spore-forming rods and cocci with virtual elimination of Gram-negative bacteria.

(P41) COMPARISON OF METHODS FOR BEEF CARCASS DECONTAMINATION

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Different cleaning and sanitizing treatments of beef carcass surfaces for reduction of *Salmonella typhimurium*, *E. coli* O157:H7, and different indicator organisms were compared in model laboratory conditions. Carcass surface regions were removed from hot carcasses and inoculated with bovine feces containing 10^6/g each of *S. typhimurium* and *E. coli* O157:H7. Inoculated surfaces were subjected to water wash, trimming and steam vacuum cleaning treatments alone and followed by hot water, lactic acid and combinations of these two sanitizing interventions. An identical number of carcass surface regions was inoculated with feces without pathogen inoculation and treated by the same cleaning and sanitizing interventions to determine the effect of these treatments on aerobic plate count, *Enterobacteriaceae*, *E. coli*, thermotolerant coliforms and total coliforms. Regardless of the preliminary cleaning treatment, combination of hot water and lactic acid produced the greatest log reduction of *S. typhimurium* and *E. coli* O157:H7 or indicator organisms such as *E. coli* or thermotolerant coliforms. During in-plant evaluations, a combined treatment consisting of hot water followed by lactic acid spray showed ability to reduce significantly APCs, coliform and *E. coli* counts. From the data collected in this study, it is possible to choose an effective but inexpensive treatment to decontaminate beef carcasses and to select indicators to verify the selected interventions used as CCPs in a HACCP plan.

(P42) EFFICACY OF TRISODIUM PHOSPHATE FOR DESTRUCTION OF *SALMONELLA* ON CANTALOUPE

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This study was undertaken to determine the effectiveness of trisodium phosphate (TSP) dip solutions for destruction of *Salmonella* on cantaloupe skin. A factorial arrangement of six TSP concentrations (0, 1, 5, 10, 12, or 15% wt/vol), two temperatures (37 or 45°C), and three contact times (2, 6, or 10 min) was used. Excised areas (2.5 cm^2) of cantaloupe skin were inoculated with a three-strain mixture of *Salmonella*, air dried, then submerged in water (0% TSP) and TSP solutions according to the experimental design. Morphology of treated and control cells was evaluated by scanning electron microscopy (SEM). Numbers of *Salmonella* on the skin were 5.30 log 10 CFU/cm^2 before dipping into the control (0%) and TSP solutions. *Salmonella* was completely inactivated after exposure to 10, 12, or 15% TSP (45°C) for 10 min. Numbers were significantly ($P < 0.05$) reduced by about 2 logs on skin dipped in 1% TSP (37 or 45°C) for 10 min or in 5-15% TSP (37 or 45°C) for 2 or 6 min. TSP-treated cells appeared wrinkled and showed signs of lysis when observed by SEM. The use of TSP as a sanitizer for uncut cantaloupe seems to have good potential.

(P43) GROWTH AND ADHERENCE ON STAINLESS STEEL BY *ENTEROCOCCUS FAECIUM*

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In Brazil, refrigerated pasteurized milk is frequently spoiled by thermoduric psychrotrophic lactic acid bacteria. One such isolate from raw milk was identified as *Enterococcus faecium* using API System Strep 20. For growth temperature studies, *E. faecium* was inoculated into 10% RSM (Reconstituted Skim Milk) and MRS broth, incubated at 6.5° and 9°C for 10 d and at 30°, 42° and 45°C for 48 h. Generation times (g) and growth rate (R) were determined in MRS broth at 30°C for 24 h. Cells were enumerated by spread-plating samples onto MRS-Agar incubated at 30°C

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for 48 h. The ability of *E. faecium* to adhere to stainless steel chips (6 × 6 mm), 304, finish #4 was investigated. MRS broth containing stainless steel chips was inoculated with 10^3 or 10^6 CFU/mL of *E. faecium* and the adherent cells were enumerated by epifluorescence microscopy using acridine orange stain. *E. faecium* grew between 6.5°C and 42°C in MRS and between 9° and 40°C in RSM. In MRS broth, samples with 10^6 or 10^3 CFU/mL, the g values were 1.38 and 0.89 h^{-1} and R values were 0.72 and 1.12 h^{-1}. Values of g = 0.85 and R = 1.12 h^{-1} were determined for *E. faecium* growing in RSM with 10^3 CFU/mL. In MRS broth, samples with a starting inoculum of 10^6, adherence to stainless steel chips was first observed at 2 h. In contrast, adherence was first observed at 4 h in samples with an initial inoculum of 10^3 cells. After 10 h of exposure the number of adherent cells was similar for all samples regardless of initial inoculum. These results indicate the *E. faecium* readily adheres to stainless steel. It also underscores the need to control of *E. faecium* by using appropriate low storage temperatures and adequate sanitizing practices in the dairy industry.

(P44) SCANNING ELECTRON MICROSCOPY OF CHANGES IN HIGH DENSITY POLYETHYLENE (HDPE) CONVEYOR SURFACES DURING NORMAL PROCESSING IN MEAT PLANT OPERATIONS

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Conveyor systems in food processing facilities have advanced from stainless steel contact surfaces to complex integrated polyethylene modular systems. Polyethylene and polypropylene of various molecular weights and densities are the most common plastics in the food industry for direct contact, such as conveyors, cutting boards and tubs. Extensive research has been conducted on stainless steel surfaces used in the food industry, but minimal research has been reported of the effects of soiling, cleaning or normal wear on the deterioration of plastic food contact surfaces. New surface features of plastic polymers are important for product selection only if the surface remains stable for long periods of time under conditions found in food processing environments. Conveyor surfaces in a meat plant environment are affected by many different factors. These can include products impacts, abrasions from knives, and friction against other components of the conveyor complex. Each of these factors may actively degrade the surface texture. Processes used during cleaning, such as scrubbing and pressure washing coupled with the chemical influences from high acid and alkaline components may all induce varying degrees of surface damage. Scarring and abrasion of the plastic contact surfaces is inevitable, but to what extent it occurs was one of the objectives of this study. Using scanning electron microscopy, this study examined 1) the unused surfaces of polyethylene plastic links from three different manufacturers, polypropylene, acetyl and stainless steel; 2) changes that occurred between new samples links with those that were from conveyors exposed to normal processing conditions and 3) polyethylene surfaces from a conveyor receiving extensive knife work followed by extreme chemical and physical cleaning.

(P45) DELAMINATION IN POLYETHYLENE STRUCTURES AND THE INFLUENCE OF MULTILAYERED UPPER SURFACES ON DETERIORATION PROCESSES

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Polyethylene, a thermoplastic discovered in 1933, is widely used in a variety of equipment contact surfaces. The easily molded, light polymer has many useful properties and allows the production of complex molded parts which would be impossible or expensive to produce from stainless steel. Previous observations of in-use samples indicate that delamination after initial interface incision is an important factor in the deterioration of the food contact surface. This deterioration can lead to a food safety risk, since it is not possible to completely clean and sanitize such a surface. The molecular structure of polyethylene is a regular oriented crystal lattice, but if the molecules lose energy quickly during molding, the interconnected lattice does not form to the required degree. The reason for the deterioration pattern observed may be directly tied into the molecular configuration of the crystal matrix. The objectives of this study were 1) to search for the presence of multilayered structures in the contact interfaces of polyethylene surfaces that may be contributing to rapid deteriorative changes of surface structures; 2) to evaluate the effects of various cleaning procedures following initial knife cuts into the upper surface layers; and 3) to compare different samples with that of a refurbished sample (removal of the upper layers of the plastic) to gain insight into the multilayered structure's influence on surface delamination. Understanding and verifying the link between the delamination phenomenon and the multilayered appearance of upper surface layers may allow a means of controlling the deterioration effect by refurbishing the plastic links before use. These results could play a significant role in the food safety analysis of plastic food contact surfaces.

(P46) MICROBIAL SPOILAGE OF CHUB-PACKED GROUND BEEF FROM FOUR PROCESSING PLANTS IN THE UNITED STATES

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Ten pound chubs of coarsely ground beef at two different lean:fat specifications (73:27 and 81:19) and coarsely ground chuck (81:19) were stored at 1°C and 7°C to monitor the effects of storage temperature on microbial spoilage of the product and to determine the bacteria responsible for the accumulation of gas under the packaging film. Ground beef was tested from 4 processing plants in the United States (2 trials each), and microbial analyses were conducted (days 0, 6, 10, 14, 18) using 9 different media to estimate total aerobic and anaerobic counts, lactic acid bac-

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(P50) BACTERIAL POPULATIONS OF DIFFERENT SAMPLE TYPES FROM POULTRY

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Bacterial populations associated with three sample types from poultry carcasses in the dirty area of an abattoir, as well as rubber fingers, were enumerated and characterized. Sample types included neck skin only, feathers only and a neck skin/feather combination from pre- and post-scalded carcasses. The neck skin of carcasses after defeathering was also sampled. Neck skins sampled before and after scalding consistently exhibited the lowest aerobic plate counts and feathers the highest. Scalding resulted in decreases of bacterial numbers by at least 1.5 log CFU g^{-1}, which was reflected by all three sample types. However, neck skins after defeathering exhibited increased bacterial numbers by 1.1 log CFU g^{-1}. Isolates (751) from Yeast Extract supplemented Tryptone Soya agar (aerobic plate count) plates of all samples were characterized. Bacterial populations from plates of all three sample types and from pre- and post-scalded carcasses were dominated by Gram-positive bacteria, while Gram-negative isolates predominated on plates of neck skins from carcasses sampled after defeathering. Isolates from plates of rubber fingers were dominated (94.4%) by *Micrococcus* and *Staphylococcus*. *Listeria* was found at a low prevalence (3/18) on feather-associated samples, while *Staphylococcus aureus* was isolated from neck-skin-associated samples (5/25). Presumptive *Salmonella* was isolated from almost all product (24/29) and rubber finger (1/3) samples.

(P51) MICROBIAL ECOLOGY OF SOUTH AFRICAN RETAIL SORGHUM BEER

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The microbial ecology of 52 sorghum beer samples, representing six commercial brands marketed by two local manufacturers, was investigated. Aerobic plate counts, lactic acid bacteria counts and yeast counts were determined by conventional and Petrifilm™ methods. Conventional methods recovered the highest microbial numbers. Yeast counts of 7.8 log CFU ml^{-1}, lactic acid bacteria counts of 6.5 log CFU ml^{-1} and aerobic plate counts of 6.0 log CFU ml^{-1} were obtained. The Petrifilm™ method also recovered yeast counts of 7.8 log CFU ml^{-1}, but lactic acid bacteria counts of 5.0 log CFU ml^{-1} and aerobic plate counts of 5.3 log CFU ml^{-1}. Aerobic plate counts and lactic acid bacteria counts obtained on Petrifilm™ were significantly ($P<0.05$) lower than those obtained by conventional methods. Predominant colonies from Standard One Nutrient agar (aerobic plate count) plates and MRS agar (lactic acid bacteria count) plates and equivalent Petrifilm™ plates of all samples were isolated. Of the 419 isolates, 369 (88.1%) were lactic acid bacteria. Lactic acid bacteria populations consisted of homofermentative lactobacilli (48.8%), heterofermentative lactobacilli and leuconostoc-like organisms (30.3%), as well as pediococci (19.0%). The conventional MRS agar plates used for lactic acid bacteria counts recovered higher proportions of heterofermentative lactobacilli compared to the corresponding Petrifilm™ procedure.

(P52) MICROBIOLOGICAL QUALITY OF CREAM FILLINGS FROM DOUGHNUTS SOLD AT BULAWAYO, A ZIMBABWEAN CITY

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Thirty-eight cream samples from retail doughnuts from Bulawayo outlets were assessed microbiologically for compliance with the City Council's bacteriological standards and to identify probable contaminants. Analysis was by dilution-plating on nutrient, mannitol/milk salt and MacConkey agars. Aerobic plate counts (APC), staphylococcal and coliform counts per gram of 25 samples ranged from 1.6×10^2 to 9.2×10^3, 1.1×10^2 to 5.6×10^4 and 1.1×10^2 to 1.2×10^4, respectively. APC from 14 (56%) samples were acceptable but presence of coliforms made all the samples unsatisfactory bacteriologically. The remaining 13 samples were unsatisfactory as judged by their APCs which ranged from 4.1×10^4 to 2.5×10^6.

Enterobacter aerogenes (11), *E. cloacae* (3), *Citrobacter freundii* (3), *Arizona* (1) were among 21 (52.5%) coliforms which fermented lactose at 44.5%. *Staphylococcus aureus* comprised over 80% of 48 staphylococci. *Micrococcus luteus* was found. Contamination of doughnut cream fillings by *Micrococcaceae* and coliforms suggest inadequate pasteurization or unhygienic handling of cream and necessitate regular microbiological monitoring.

(P53) MICROBIAL QUALITY OF KOSHARI, ONE OF THE MOST FAMOUS FLOKSY MEALS COMMON IN EGYPT

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Eighty samples of the popular koshari meal obtained from 20 local restaurants from Egypt, Cairo and Assuit, were examined microbiologically, in summer, autumn, winter and spring, to determine the number and types of the microorganisms. Reference koshari samples were prepared seasonally by the investigator under complete hygienic conditions. Microbiological evaluation included determination of aerobic plate count (APC), lactic acid bacteria (LAB), fecal coliform, *Escherichia coli*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus* and *Bacillus cereus* in additions to yeast and mold; pH and moisture were also measured. The means of the APC, LAB and coliform counts were 2.3×10^8, 1.7×10^8 and 6.1×10^3 CFU/g, respectively, in summer. These counts were significantly higher (<0.05) than those determined in autumn ($1.4 ¥ 10^5$, 7.3×10^6 and 1.8×10^3), winter (1.4×10^5, 7.3×10^4 and 3.1×10^1) and spring (1.7×10^5, 3.7×10^3 and 2.9×10^1). *E. coli* and *S. aureus* counts were $<10^2$ CFU/g in all koshari samples. *E. coli* O157:H7 was isolated from two samples, and *L. monocytogenes* isolated from one sample; however, their numbers were $<10^1$ CFU/g. *B. cereus* was detected in 13

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teria (LAB), Gram-negative bacteria, H_2S producers and *Clostridium* spp. Initial aerobic and anaerobic counts and initial LAB counts were 3.5 to 4.5 log CFU/g meat, with growth at 7°C in all meat types reaching 7 to 8.5 log CFU/g at day 10. At 1°C, the number of days for these counts to reach similar levels of growth varied between plants and meat types; however, counts from all meats from plant 2 and ground chuck from plant 1 only reached 5.5 to 6.5 log CFU/g at day 18, while counts from all meats from plants 3 and 4 reached 7 to 8 log CFU/g at day 10. Regardless of meat types, counts varied greatly among the selective agars. Gas- producing isolates were identified as *Citrobacter*, *Hafnia*, *Serratia*, *Aeromonas* and *Enterobacter* species. Results substantiate that gas-producing facultative anaerobes grow in low O_2-packed ground beef and that a lower refrigeration temperature can delay microbial spoilage.

(P47) SIMULATION OF *BACILLUS* SPOILAGE IN A MODEL FOOD SYSTEM

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Microbiological concerns in the food industry have influenced the rapid development of the field of predictive food microbiology. Large inocula have usually been used in the study of the germination and growth rates of spore populations. Real food systems may contain only a small number of spores so randomness and biological variability become much more apparent. The objective of this research was to characterize the variability inherent in microbial spore populations and to model the time to spoilage of a model food system with a low initial spore count.

Phase contrast microscopy was used to study the germination time of spores. Change of color experiments were carried out in 96-well ELISA plates. A simulation was written using Excel with @risk software. @risk performs simulations using the Monte Carlo technique. Input parameters included the initial population, germination probability, growth rate, and number of cells to cause spoilage. Simulation results agreed with experimental results.

(P48) DEVELOPMENT OF AN EXPERIMENTAL MODEL FOR MICROBIAL CROSS-CONTAMINATION AND EVALUATION OF THE EFFICIENCY OF AN ANTIBACTERIAL KITCHEN DISINFECTANT

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Contamination of foods with pathogenic microorganisms can occur during food preparation in the kitchen through cross-contamination from a variety of sources, including hands, the cutting board and knives. *Enterobacter aerogenes* B199A, an indicator bacterium with similar attachment characteristics as that of *Salmonella* spp. and *E. coli* O157:H7 was used. Chicken meat inoculated with 10^6 CFU of *E. aerogenes* B199A/g was placed on a sterile cutting board and cut into small pieces to determine the extent of cross-contamination occurring from meat to the cutting board and from the cutting board to vegetables (lettuce and cucumbers). Bacteriological analysis of swab samples from the surface of the cutting board and hands and from lettuce and cucumbers recovered approximately 10^5 CFU of *E. aerogenes*/cm^2 from the board and hands and approximately 10^3 to 10^4 CFU of *E. aerogenes*/g from the lettuce and cucumbers. Studies also were done to evaluate the efficacy of a commercially available antibacterial kitchen disinfectant in reducing bacterial contamination. The surface of the cutting board and hands were sprayed with the antibacterial agent after cutting the meat, and counts of *E. aerogenes* on the cutting board and vegetables (lettuce and cucumbers) were determined. Results revealed that application of the disinfectant reduced the population of *E. aerogenes* to almost nondetectable levels. The average count after treatment was <20 CFU per sample of vegetable, with counts ranging from <20 to 200 CFU/g on the cutting board and subsequently on the vegetables. These results indicate that bacteria with attachment characteristics similar to two major foodborne pathogens can be readily transferred to cutting boards during food preparation and then cross-contaminate fresh vegetables if the boards are not cleaned. Application of an antibacterial kitchen cleaner can greatly reduce bacterial contamination on cutting boards.

(P49) EFFICACY OF THREE SANITIZERS AGAINST FOOD SPOILAGE BACTERIA

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In vitro efficacies of chlorhexidine gluconate (CG), iodophor (I) and peracetic acid/ hydrogen peroxide (PAH) sanitizers were evaluated against planktonic and sessile *Pseudomonas fluorescens* and *Bacillus subtilis* attached to stainless steel and polyurethane test surfaces. *P. fluorescens* and *B. subtilis* attached to stainless steel and polyurethane were less susceptible to treatment with all three sanitizers than their planktonic counterparts. Planktonic and sessile *P. fluorescens* were more susceptible to treatment with all three sanitizers than *B. subtilis*. Cell numbers of planktonic *P. fluorescens* and *B. subtilis* were significantly reduced ($P<0.05$) compared to control cell numbers after exposure to PAH, I and CG. Similarly, cell numbers of attached *P. fluorescens* on polyurethane test surfaces were significantly lower ($P<0.05$) than numbers of untreated control cells after exposure to all three sanitizers. By contrast, cell numbers of attached *P. fluorescens* on stainless steel test surfaces were significantly lower ($P<0.05$) than numbers of untreated control cells after exposure to PAH only. Cell numbers of *B. subtilis* on polyurethane test surfaces were significantly reduced ($P<0.05$) after exposure to PAH, but not significantly ($P>0.05$) reduced on stainless steel test surfaces after treatment with all three sanitizers compared to numbers of untreated control cells.

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samples with counts of $<10^2$ CFU/g. *Salmonella* was not isolated from any samples. Average yeast count was $5.2 ¥ 10^2$ CFU/g. The pH and moisture content of koshari was 6.2 and 66.12%, respectively, in addition to presence of certain inhibitory substances, which may explain the predominance of such types of microorganisms. In addition, the plant of koshari raw material could contribute to the presence of the low number pathogens.

(P54) SURVIVAL OF *L. MONOCYTOGENES* IN REFRIGERATOR DILL PICKLES

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Recent concern related to the potential growth of *L. monocytogenes* in chilled, brined foods has prompted the question of the safety of the refrigerator dill method for making pickles in the home. Yet home preservationists still request information on this procedure. There is a lack of information on this process related to the potential danger for *L. monocytogenes* growth. To determine if concern is warranted, cucumbers inoculated with *L. monocytogenes* were prepared as refrigerator dills. They were prepared using varying NaCl levels to evaluate the potential for growth or survival of *Listeria* if less than advised levels of NaCl were used. Pickling cucumbers were inoculated with approximately 10^5 *Listeria*/g, added to one of 3 different brine formulations (3.8, 3.1, or 2.3%), held 1 wk at 25°C and then 3 wk at 4°C. Total aerobic and *L. monocytogenes* populations did not increase at any point of the pickling process. In most cases, the *Listeria* populations decreased by approximately 1.5-2.0 logs after the first week and by 3.5-4.0 logs after 4 wk, even for the brine with the least salt concentration. The concern about *Listeria* exposure through refrigerator dills may not be warranted.

(P55) FATE OF GAMMA IRRADIATED *L. MONOCYTOGENES* ON RAW OR COOKED TURKEY BREAST MEAT DURING REFRIGERATED STORAGE

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Gamma irradiation was investigated as a way to control *L. monocytogenes* that may contaminate cooked poultry products and cause listeriosis. Raw and cooked turkey breast meat nuggets (25g) or ground turkey samples were inoculated with a mixture of *L. monocytogenes* ATCC 7644, 15313, 43256 and 49594. Each sample was vacuum packaged in an oxygen-permeable pouch. Gamma-radiation D-values for *L. monocytogenes* were significantly different on raw and cooked nuggets, 0.55 ± 0.03 kGy and 0.63 ± 0.06 kGy, respectively. When a high inoculum (~10^9 CFU/g) was used, the CFU of *L. monocytogenes* on raw ground turkey declined during 14 d of storage at 4°C in both irradiated and non-irradiated samples. In contrast, on cooked turkey depending on the radiation dose, the CFU either remained the same or increased during storage. A moderate inoculum (10^3 CFU/g) did not survive a radiation dose of 3 kGy, and a dose of 2 kGy greatly reduced the CFU on either raw or cooked ground turkey. During 21 days of storage of the meat at either 2 or 7°C, the CFU increased in cooked samples that had received radiation doses of 1 or 2 kGy. On samples inoculated before cooking, the order of irradiation and cooking did not significantly affect the D-value.

(P56) EFFECTIVENESS OF TWO COOKING SYSTEMS IN DESTROYING *E. COLI* O157:H7 AND *L. MONOCYTOGENES* IN GROUND BEEF PATTIES

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Minimizing the transmission of *E. coli* O157:H7 and *L. monocytogenes* through cooked ground beef patties is one of the major challenges of the meat industry. Cooking to the FDA recommended temperature of 68.3°C ensures destruction of pathogens but reduces the palatability of the hamburger patty. Effectiveness of the rapid, high temperature commercial "clam shell" griddle in reducing microbial numbers and retaining the palatability of the patties was investigated against the conventionally used open-hearth Farberware broilers. Thermocouples were inserted into uniform ground beef patties (110 g each) which contained either *E. coli* O157:H7 or *L. monocytogenes* (10^6 to 10^7/g). These were cooked to internal temperatures of either 60°C or 68°C. Endpoint internal temperature, position on the grill, degree of doneness, after-cook weight, cook-time and texture of the patties were monitored. Pre- and post-cook bacterial counts were made on general purpose and appropriate selective media. In comparing the clam shell with the Farberware cooker, *E. coli* O157:H7 populations were decreased by 4 and 3 log greater magnitudes in patties cooked to 60°C and 68°C, respectively; *L. monocytogenes* similarly were decreased by 3.7 and 2.1 log greater magnitudes at 60°C and 68°C, respectively. Thus, the clam shell griddle was more effective in destroying pathogens in ground beef patties, even at lower temperatures of 60°C.

(P57) FATE OF *E. COLI* O157:H7, *L. MONOCYTOGENES*, AND *SALMONELLA* SPP. IN REDUCED SODIUM BEEF JERKY

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Interest in low-sodium food products necessitates re-examination of home preservation processes relying in part on salt for antimicrobial effects. The fates of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. in reduced sodium beef jerky were determined. Beef loin strips or ground beef, with approximately 1 or <0.1% salt, were inoculated with the pathogens (10^6 CFU/g). Samples were either dried at 60°C (140°F) in a dehydrator or heated to 71.1°C (160°F) prior to drying at 60°C (140°F). Populations were determined at 0 and at 2-h intervals until dry.

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Reductions of the pathogens were 1-2 logs greater in ground beef jerky with higher salt levels compared to that with reduced levels, and in most cases, with a greater reduction (1.5 logs) when heated prior to drying. Heating before drying resulted in a decrease of 0.5-1.5 logs more than dehydrator drying alone for *E. coli* and 2.0 logs more for *Salmonella* in whole meat strips. Reductions were similar for *Listeria* regardless of heating prior to drying. For the whole strip jerky, there were no differences in *Salmonella* and *Listeria* populations after drying regardless of the salt level. *E. coli* populations exhibited a slightly greater decrease in whole strip jerky with the higher salt level when heated prior to drying. The antimicrobial role of salt is more notable in ground beef jerky than in whole strip jerky.

(P58) THE IMPACT OF COLD SHOCKING ON THE MINIMUM GROWTH TEMPERATURE FOR *ESCHERICHIA COLI* O157:H7

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Escherichia coli O157:H7 has been identified as an important human pathogen, particularly in undercooked ground beef and raw milk. Many physical treatments used in food processing are designed to kill or decrease pathogenic and/or spoilage microorganisms. When sublethal treatments are used, surviving populations may contain pathogens. It is possible that these treatments may also enhance microbial survival with further processing. For example, stress adaptations may occur in response to an abrupt decrease in temperature, resulting in the possible induction of cold shock proteins. The purpose of this project was to determine if *E. coli* O157:H7 elicits a cold shock response and whether the response affects the minimum growth temperature of the organism. In preliminary studies, cells grown exponentially at 37°C were rapidly shifted to 10°C and kept at this temperature for 1 h. The presence of cold shock proteins was demonstrated using SDS polyacrylamide gel electrophoresis and autoradiography. Results indicate that the process of cold shocking does impact the minimum temperature of growth for *E. coli* O157:H7. Factors contributing to the minimum growth temperature for *E. coli* are important since low temperature preservation products constitute a primary reservoir.

(P59) INFLUENCE OF PACKAGE ATMOSPHERE ON GROWTH AND SURVIVAL OF UNINJURED AND SUBLETHALLY HEAT-INJURED *ESCHERICHIA COLI* O157:H7

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Escherichia coli O157:H7 is capable of survival and may exhibit enhanced growth under modified atmospheric conditions. The purpose of this investigation was to determine the effect of atmospheric composition on growth and survival of uninjured and sublethally heat-injured (56°C, 10 min.) *E. coli* O157:H7. Test organisms were inoculated (10^3 to 10^5 CFU/ml) onto brain heart infusion agar supplemented with 0.3% beef extract, packaged in barrier bags in air, 100% CO_2, 100% N_2, 20% CO_2/80% N_2, and vacuum and stored at 37, 10, and 4°C for up to 20 days. Package atmosphere and inoculum status (i.e., uninjured or heat-injured) influenced ($P<0.01$) growth and survival of *E. coli* O157:H7 stored at all test temperatures. Growth of heat-injured *E. coli* O157:H7 was slower ($P<0.01$) than uninjured *E. coli* O157:H7 stored at 37°C. At 37°C, uninjured *E. coli* O157:H7 reached stationary phase growth earlier than heat-injured populations. Uninjured *E. coli* O157:H7 grew during 10 days of storage at 10°C, while heat-injured populations declined during 20 days of storage at 10°C. Uninjured *E. coli* O157:H7 stored at 10°C reached stationary phase growth within about 10 days in all packaging atmospheres except CO_2. Populations of uninjured and heat-injured *E. coli* O157:H7 declined throughout storage for 20 days at 4°C. Survival of uninjured populations stored at 4°C, as well as heat-injured populations stored at 4 and 10°C, was enhanced in CO_2 atmosphere. Survival of heat-injured *E. coli* O157:H7 at 4 and 10°C was not different ($P>0.05$). Results of this investigation indicate that uninjured and heat-injured *E. coli* O157:H7 are able to survive at low temperatures in the modified atmospheres used in this study. Therefore, packaging treatments commonly applied to fresh beef may inadequately inhibit growth and survival of this pathogen.

(P60) FATE OF SELECTED PATHOGENS IN VACUUM-PACKAGED DRY-CURED (COUNTRY-STYLE) HAM SLICES AT 2°C AND 25°C

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Whole dry-cured (country style) hams from six manufacturers were sliced and the slices randomly allotted into five treatment groups per manufacturer. One treatment group served as a control and slices in the four other treatment groups were inoculated with approximately 10^5/g of either *E. coli* O157:H7, *L. monocytogenes*, a mixture of three *Salmonella* spp. (*S. typhimurium*, *S. enteritidis* and *S. choleraesuis*), or *S. aureus*. All ham slices were vacuum-packaged. Half of the packages in each treatment group was stored at 25°C, and the rest of the packages was stored at 2°C. Two packages from each manufacturer for each treatment and storage temperature were examined after storage for 0, 7, 14, 21 and 28 days. *S. aureus* was detected in 2 of 60 control slices, *Salmonella* in 2 of 120, *L. monocytogenes* in 4 of 120 and *E. coli* O157:H7 was not detected in any of the 120 control ham slices analyzed before or after storage. The aerobic (26°C and 35°C) and staphylococcal populations of the control vacuum-packaged hams slices increased ($P<0.05$) with storage time and the increase in populations was greater ($P<0.05$) in vacuum-packaged ham slices at 25°C than at 2°C. The extent of the decreases in populations of the inoculated pathogens during storage of the vacuum-packaged dry-cured ham slices varied with manufacturer ($P<0.05$) and storage temperature ($P< 0.05$). Decreases in *Salmonella* and *E. coli* O157:H7 populations were greater ($P<0.05$) in slices at 25°C than at 2°C, while

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decreases in *L. monocytogenes* were similar at both storage temperatures. *S. aureus* enterotoxin was not detected in either *S. aureus*-inoculated or control ham slices after storage for 28 days. Survival of these pathogens in vacuum-packaged dry-cured ham slices suggests that contaminated hams may pose a safety risk to consumers if consumed without adequate cooking.

(P61) FATE OF *L. MONOCYTOGENES* ON SMOKED FISH COATED WITH SORBATE-CONTAINING CELLULOSE-BASED EDIBLE FILMS

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Potassium sorbate incorporated into an edible coating (mixture of hydropropyl methylcellulose and methyl cellulose) for smoked rainbow trout was evaluated for its ability to inhibit *Listeria monocytogenes*. The trout fillets were coated either before or after inoculating the fish surface with *L. monocytogenes* (either 4 or 7 logs/50 cm^2) and stored at 4°C or 10°C for 30 d. In the product held at 4°C, *Listeria* population decreased or remained constant on samples coated, while the population increased on uncoated samples. The coating was more effective if applied before the trout was inoculated with *Listeria*. In the product held at 10°C, *Listeria* populations remained constant up to 12 d on samples coated but increased by ca. 3 logs on uncoated samples by day 7. The combination of coating and low storage temperature can effectively control the growth of *L. monocytogenes* on smoked fish.

(P62) EFFECT OF ACIDULANT IDENTITY ON THE ACID TOLERANCE RESPONSE OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI*

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The effect of acidulant identity on the acid inactivation and acid tolerance (AT) of enterohemorrhagic *E. coli* was studied using citric, lactic, and acetic acids. Six O157:H7, one O111:H-, and one biotype 1 reference strain of *E. coli* were used throughout the study. The strains were cultured individually for 18 h in TSB+dextrose and TSB-dextrose to yield AT induced and non-induced cells, respectively. These cultures were then used to inoculate test tubes containing 10 mL of sterile BHI that had been supplemented with 0.5% citric, lactic, or acetic acid and adjusted to pH 3.0 with HCl. The initial level of cells was 10^6 - 10^7 CFU/ml. All tubes were incubated at 37°C for 7 h, samples removed after 0, 2, 5 & 7 h, viable counts done using BHI agar and MacConkey agar, and the results compared to data previously obtained using HCl only. At varied greatly among the four acids, with resistance being HCl = citric>acetic>lactic for TSB + dextrose grown cells and HCl>citric>acetic>lactic for TSB-dextrose grown cells. Inducing acid tolerance increased the resistance of *E. coli* to acid inactivation, with the increase in resistance being dependent on both acid identity and strain. The extent of injury also varied with acid and strain with as much as a 5 log cycles differential in BHI agar and MacConkey agar counts.

(P63) EFFECT OF pH AND ACID TOLERANCE ON RADIATION RESISTANCE OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI*

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Seven enterohemorrhagic (six O157:H7 and one O111:H-) and one reference strain of *Escherichia coli* were individually grown in TSB with and without 1% dextrose to produce cells that were or were not preadapted to acidic conditions, respectively. The cultures were then used to inoculate prechilled (2°C) test tubes containing BHI broth adjusted to pH 4.0, 4.5, 5.0, or 5.5 using HCl. The cultures were then irradiated at 2°C with a series of doses up to 1.0 kGy. Viable counts were performed using BHI and MacConkey agars to assess both survival and injury. One set of cultures was examined immediately after irradiation and another was examined after storage for 7 d at 2°C. Comparison of irradiation D-values indicated that there was only a small enhancement of irradiation inactivation of *E. coli* resulting from pH depression. However, comparison of survival rates after 0 and 7 days indicated that low dose irradiation potentiated the acid inactivation of the pathogen during refrigerated storage. The greatest effect observed was as much as a doubling of irradiation D-values when strains were induced to acid tolerance by prior exposure to a pH of approximately 4.6. This cross-protection effect would have to be considered to accurately calculate irradiation processes for the elimination of enterohemorrhagic *E. coli* from acidic foods.

(P64) ACID TOLERANCE AND ACID SHOCK RESPONSES OF *E. COLI* O157:H7 AND NON-O157:H7 STRAINS IN THE PRESENCE OF ARGININE, LYSINE, AND METHIONINE

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E. coli O157:H7 and non-O157:H7 survival due to an enhanced acid tolerance response (ATR) or acid shock response (ASR) to lactic acid exposure in the presence of selected amino acids was studied. *E. coli* O157:H7 isolates (932 and E009) and a non-O157:H7 strain (23716) were used to determine if the addition of arginine, lysine, or methionine could enhance the inducible acid resistance ATR or the general stationary-phase dependent acid resistance ASR. Cells grown to stationary phase at 32°C were either acid shocked by exposing the cells to lactic acid at pH 4.0 or by acid adapting cells by first exposing them to a pH of 5.5 and then an acid challenge of pH 4.0. Arginine, lysine, or methionine was added to a minimal glucose medium at one of five times at time of inoculation, before acid shock treatment, before acid adaptation treatment, after 1 h of acid shock treatment, or after acid adaptation treatment, depending on the treatment. Treated cells were

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incubated at 32°C, and survival of the strains was monitored at 0, 3, and 24 h. The addition of each of these amino acids to the minimal glucose medium enhanced the ATR in comparison to the ASR for isolate E009. Differences between ATR and ASR in the presence of the amino acids were not observed for isolates 932 and 23716. The presence of certain free amino acids in foods might enhance acid adaptation of some strains of *E. coli* O157:H7.

(P65) CHARACTERIZATION OF ACID SHOCK AND ACID TOLERANCE RESPONSE IN *L. MONOCYTOGENES* STRAINS V7, V37, AND CA

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Foodborne pathogens are capable of tolerating and surviving extreme stress conditions including extreme acidity. One possible reason for this survival may be the production of protective stress proteins. The acid shock response (ASR) and acid tolerance response (ATR) of *L. monocytogenes* strains V7, V37, and CA in tryptic soy broth without dextrose acidified with lactic acid were studied. The strains were cultivated overnight at pH 6.8-7.2, pelleted by centrifugation, and were either directly challenged at pH 4.0 and 3.5 to study their ASR or initially adapted at pH 5.5 for the equivalent of 1 generation before challenging at pH 4.0 and 3.5 to study their ATR. In both cases, viability was determined by enumeration at 0, 1, 2, 3, 6, and 10 h after challenging by plating onto brain heart infusion agar. The production of stress proteins in both cases was analyzed by 2-D gel electrophoresis. There were some differences in the survival responses for each strain; however, the acid adapted cells of each strain survived to a greater degree than unadapted cells at both pH 4.0 (at least 10-fold) and 3.5 (at least 100-fold). A greater understanding of the molecular mechanisms of *L. monocytogenes* in acidic conditions will aid in developing better preventive and control measures for the food industry.

(P66) COMPARISON OF CHLORINE AND A PRODUCE RINSE FOR KILLING PATHOGENS ON FRESH PRODUCE

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Based on the current literature, washing whole and cut produce in chlorinated water has a sanitizing effect, although reduction in microbial populations is minimal, usually less than 100-fold. A study was undertaken to evaluate the efficacy of a produce rinse comprised of food-grade ingredients in killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, yeasts and molds, and total aerobic microorganisms on whole apples, tomatoes, and lettuce leaves. Inoculated produce was treated with water (control), 200 or 2,000 ppm chlorine, or the produce rinse for 0, 1, 3, 5 or 10 min. rinsed with sterile water, and analyzed for populations (CFU/cm^2) of target organisms. Compared to the control treatment, additional reductions in pathogens of 0.35 to 2.03 $\log_{10}$ CFU/cm^2, equivalent to 90 to nearly 100% reductions of the inoculated pathogens, were achieved using chlorine and the produce rinse. Chlorine was generally more effective at 2,000 ppm than at 200 ppm. Treatment with the produce rinse was as effective as, or had greater lethality than chlorine in reducing populations of pathogens on the inoculated produce. These reductions are significant relative to potential levels of these pathogens that may be present on produce.

(P67) INHIBITION OF *LISTERIA INNOCUA* IN MANCHEGO CHEESE BY BACTERIOCIN-PRODUCING *ENTEROCOCCUS FAECALIS*

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The inhibitory effect of enterocin 4, a bacteriocin produced by *Enterococcus faecalis* INIA 4, on *Listeria innocua* was investigated. Raw ewe's milk was inoculated with ca.10^5 CFU/mL of *L. innocua* and with 1% of a commercial lactic starter, 1% of a *E. faecalis* INIA 4 culture, 1% of each culture or with no culture, and Manchego cheese was manufactured. After 24 h, *L. innocua* counts had increased by 0.26, 0.35 and 1.57 log units in cheese from milk inoculated with INIA 4 culture, with commercial starter or with no culture, respectively, whereas *L. innocua* decreased by 1.57 log units in cheese from milk inoculated with INIA 4 and commercial starter. After 60 d of ripening, the respective *L. innocua* counts in cheeses made with INIA 4, with commercial starter, with both cultures or with no culture were 1.63, 1.22, 2.30 and 0.45 log units lower than in the inoculated milk.

(P68) INHIBITION OF *L. MONOCYTOGENES* ON FRESH PORK LOIN USING A NISIN-BASED TREATMENT

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The inhibitory activity of a nisin-based formulation (NCF) against *L. monocytogenes* Scott A (LM) on fresh pork loin was evaluated. Pork loin samples (25 g) were inoculated with an antibiotic-resistant strain of LM (2.9 log CFU/g of pork), packaged separately in Whirl Pak® bags containing 5 mL of either the NCF (100µg/mL nisin, 5 mM EDTA, 0.5% Tween 20, pH 3.5 - HCl) or distilled water (pH 3.5, control) and stored at 4°C for 24, 48, 72, or 96 h. Following storage, surviving LM were enumerated on BHI agar containing 10 and 5µg/mL of chloramphenicol and erythromycin, respectively. The study was replicated three times. Compared to the control, meat treated with the NCF resulted in reductions in LM populations averaging 1.3 log cycles over the 96 h. In a second study, LM-contaminated pork loins were treated as outlined above except that pork loins were first dipped for 30 min in either treatment solution and packaged in Whirl Pak bags containing the 5 mL cover solutions. Significant reductions in the LM population of 3.3 and 3.1 log cycles, were achieved with the NCF after 24 and 96 h, respectively. In summary, a nisin-containing formulation was effective in reducing the population of LM on fresh pork loins during refrigerated storage.

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(P69) CONTROL OF *L. MONOCYTOGENES* BY USE OF LYSOZYME, LACTOFERRICIN-ß AND EDTA

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The presence of *L. monocytogenes* in various foods including poultry and egg products has become a concern in recent years. *Listeria* can be transferred from the poultry to eggs transovarianly and may survive the current liquid egg (LE) pasteurization temperatures (55.6-63.3°C for 3.5-6.2 min). As LE products are heat sensitive, an antimicrobial intervention strategy may be needed to effectively control *Listeria*. The two natural substances, lysozyme and lactoferricin-ß, were studied to determine their role in the destruction of *L. monocytogenes*.

The effects of lysozyme, lactoferricin-ß, EDTA and combination of lysozyme and lactoferricin-ß on *L. monocytogenes* were studied in TSB (Tryptic Soy Broth) and LE white (LEW) at temperatures of 37°C and 20°C. Standard plating was performed to evaluate the antimicrobial effect of lysozyme and lactoferricin-ß. Both combinations of lactoferricin-ß with EDTA and lysozyme with EDTA produced a 2 log reduction in population at 37°C and a 5 log reduction at 20°C. However, the antimicrobial treatments were more effective in TSB compared to LEW. Pretreatment of liquid egg products with lysozyme and lactoferricin-ß before heat pasteurization could enhance destruction of *L. monocytogenes* and improve safety and shelflife of LE products.

(P70) ANTIMICROBIAL ACTIVITIES OF LYSOZYME AND LACTOFERRICIN-ß AGAINST *SALMONELLA*

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The concern with *Salmonella* contamination of food products and especially poultry has increased significantly in recent years. *Salmonella* accounts for 57% of all bacterial foodborne diseases in the United States and its control in foods, especially poultry and eggs, is of prime importance. Generally about one in 10,000 eggs are contaminated with *Salmonella*. Therefore their prevalence in liquid egg (LE) products cannot be discounted. The LE products are heat sensitive and are pasteurized at temperatures not exceeding 55.6-66.3°C for 3.5-6.2 min. to eliminate *Salmonella*. Such temperature constraints may allow survival of *Salmonella* and warrant additional antimicrobial treatments. We studied natural substances, namely lysozyme and lactoferricin-ß for their added role in destruction of *Salmonella*.

Four treatment combinations, a control, lysozyme, lactoferricin-ß, and lysozyme plus lactoferricin-ß were compared in Tryptic Soy Broth (TSB) and liquid egg white (LEW) at temperatures 37°C and 20°C. A differential growth reduction in population was determined by surface plating on Tryptic Soy Agar (TSA). In the combination treatment of lactoferricin-ß and lysozyme, a 3 log reduction was observed at 20°C in TSB. Addition of EDTA further enhanced the antimicrobial effect, resulting in a 5 log reduction in population. In LEW, the reduction in population was to a lesser degree. However, in conjunction with heat, lysozyme and lactoferricin-ß could play an important role in reducing risk of *Salmonella* in liquid egg products.

(P71) INCIDENCE OF *SALMONELLA* ON BEEF CARCASSES AT VARIOUS STAGES OF THE SLAUGHTERING PROCESS

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One provision of new regulations for meat and poultry inspections issued by the Food Safety and Inspection Service (FSIS) is performance standards for *Salmonella* incidence in raw meat products. FSIS plans to test carcasses for *Salmonella* and when an establishment fails to meet the performance standard (based on 1% positive for steers/heifers; and 2.7% positive for cows/bulls) more than once, it will be required to take immediate action. This study determined baseline data for *Salmonella* incidence through sampling of beef carcasses during slaughtering in seven plants during both dry and wet seasons. Thirty samples were removed from each carcass site (brisket, flank, rump) at each of three locations in the slaughtering chain (pre-evisceration, final washing, 24-h chilling) and analyzed (3,780 total samples) for *Salmonella* by standard methods. *Salmonella* incidence differed among plants and seasons with average incidence, after 24-h chilling, for all plants of 0.6% and 1.7% in the dry and wet seasons. After 24-h chilling, for all plants combined *Salmonella* on the brisket, flank and rump, respectively, was 1.4% to 2.4%, 0.5 to 1.0% and 0 to 1.9%, respectively. The results of these studies are timely and useful to the meat industry in its efforts to operate under the new inspection regulations.

(P72) PROBABILITIES OF PASSING *E. COLI* PERFORMANCE CRITERIA IN SEVEN BEEF SLAUGHTERING PLANTS

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In new meat and poultry inspection regulations, *E. coli* Biotype I counts will serve as performance criteria for slaughter process control verification. A 3-class attribute sampling plan, applied in a moving window, will use values for m, M, c and n of 5 CFU/cm^2, 100 CFU/cm^2, 3, and 13, respectively, for beef carcasses. This study evaluated probabilities of passing *E. coli* performance criteria in seven U.S. beef slaughtering plants. Sampling (100 cm^2, brisket, flank, rump) and analysis for *E. coli* of carcasses was done in four steer/heifer and three cow/bull slaughtering plants, during two seasons, before evisceration, after final washing, and after overnight chilling. Results (CFU/cm^2) were used to calculate (using a USDA formula) probabilities of passing or failing the performance criteria. Depending on plant, and for chilled carcasses, the overall probabilities of passing the regulatory requirement were

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0.748 to 1.00; after final washing were 0.698 to 1.00; and for individual chilled carcass sites were 0.597 to 1.00 (brisket), 0.471 to 1.00 (flank) and 0.485 to 1.00 (rump). If the sampling was changed from chilled carcass to finally washed carcass, the criteria would be more stringent for the meat industry. The results indicate that there will be substantial variation among plants and seasons in ability to meet the *E. coli* performance criteria.

(P73) INCIDENCE OF *EDWARDSIELLA, SALMONELLA* AND *SHIGELLA* ON FRESH CATFISH FILLETS

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Twenty fresh channel catfish (*Ictalarus punctatus*) fillets were randomly selected from catfish processing plants in southeastern United States and analyzed for the presence of *Edwardsiella*, *Shigella* and *Salmonella*. Fillets were collected four times, at 3-month intervals, during the study to encompass the potential effects that climatic conditions might have on bacterial incidence. At each sampling period, five of the 20 fillets were analyzed for total aerobic and facultative anaerobic bacteria (standard plate count) by the 3M™ Petrifilm™ method. Pathogens were detected using procedures described in the Compendium of Methods for Microbiological Examination of Foods. There was significant difference ($P < 0.05$) in the standard plate counts (3.00 to 6.03 log CFU/g) due to differences in the unit processing operations and processing seasons (e.g., fall, winter, spring, summer). *Edwardsiella* was observed during all four seasons, whereas *Shigella* and *Salmonella* were not detected during the fall but were present during winter, spring and summer sampling. The frequency of isolation of *Salmonella* and *Shigella* was about 20% of fillets examined. According to ICMSF criterion, presence of *Salmonella* in fresh catfish fillets is considered as case 10, a moderate hazard, and cooking would reduce the degree of hazard.

(P74) INCIDENCE OF *GIARDIA LAMBLIA* IN FINISHED POTABLE WATER SAMPLES IN HERMOSILLO SONORA, MEXICO

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It is estimated that 9 million of the Mexican population are infected by *Giardia*, which represents a public health problem. In the last decade water has been reported as an important transmission vehicle. Therefore our objective was to investigate if finished water samples in Hermosillo, Mexico, were carriers of *Giardia*. Five hundred liters of water were filtered by polypropylene filter, eluted, centrifuged, and screened by immunofluorescence antibodies to detect *Giardia* cysts. There are 10 tanks supplying the city of which three samplings were carried out as well at water faucets of the nine city sectors. In addition, analytical parameters were analyzed such as pH, free and total chlorine, turbidity, temperature and hardness. Only one tank during the second sampling yielded positive results for *Giardia*; six tanks revealed positive results in the third sampling. Concerning the water faucets, two samples were positive in the first sampling. During the second and third sampling, four and six samples yielded positive results. The average of the analytical parameters were pH = 7.5±0.22, turbidity = 1.17±0.7, total chloride=1.33±0.11%, free chloride= 0.241±0.11%, temperature = 24±5°C, hardness = 259.2. It can be concluded that *Giardia lamblia* is present in finished potable water, which implies a potential health risk.

(P75) OCCURRENCE OF *VIBRIO* SPP. IN GUACUCO CLAMS (*TIVELA MACTROIDES*) AND CHIPI-CHIPI CLAMS (*DONAS DENTICULATUS* AND *DONAS STRIATUS*) FROM VENEZUELA

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This study determined the occurrence of human pathogenic vibrios in Guacuco (18 samples) and Chipi-chipi (4 samples) clams, using the 3-tube most probable number (MPN) procedure with Alkaline Peptone Water and Thiosulphate Citrate Bile Salts Sucrose Agar. The isolates were confirmed biochemically by individual tests and commercial API 20E assay and the APILAB Plus software. All samples were positive for *Vibrio* spp., and ranged from <3 MPN/g to 43 MPN/g. Out of all the isolates, *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *Vibrio* spp. were identified representing 55.1%, 28.2%, 8.9% and 7.8%, respectively, when the identification was performed for an individual biochemical test. The API 20E assay permitted a better separation of the species. Out of all isolates, *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *Vibrio* spp. and other genera were identified at 26.9%, 16.7%, 3.8%, 6.4% 38.7% and 42.4%, respectively. In conclusion the incidence of toxigenic *Vibrio* species was high in clams available in Venezuela. These results should prompt us to pay more attention to the role of these vibrios in local foodborne diseases that are emerging at present.

(P76) REVISED MODEL FOR AEROBIC GROWTH OF *SHIGELLA FLEXNERI* TO EXTEND THE VALIDITY OF PREDICTIONS AT LOW TEMPERATURES

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Although *Shigella* is a major fooborne pathogen, its growth in foods has received little attention. Growth of *S. flexneri* 5348 inoculated into commercially available sterile foods (canned broths, meat, fish; UHT milk; baby foods) was studied at 10 to 37°C. *S. flexneri* was enumerated by surface-plating on Tryptic Soy Agar, and growth curves were fitted by means of the Gompertz equation. Observed growth kinetics values and values calculated using a previously developed response surface model compared favorably for growth at 19 to 37°C, but not at < 19°C. To refine the model, additional data were collected for growth at 10 to 19°C. A total of 844 cultures in BHI broth, representing 197 variable combinations of temperature (10-37°C), pH (5.0-7.5), NaCl (0.5-5.0%) and $NaNO_2$ (0-1000 ppm) was used for the revised response surface model. The revised model, developed in BHI, gave significantly better

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agreement of calculated growth kinetics values with those observed in foods at 10 to 19°C.

(P77) LAG PHASE DURATIONS OF *L. MONOCYTOGENES* CELLS IN DIFFERENT PHYSIOLOGICAL STATES TO CHANGES IN ENVIRONMENT

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L. monocytogenes cells were grown at 4, 8, 15, 28 or 37°C in BHI to the exponential or stationary phase, were starved (2% BHI), or desiccated for 2 d, and then transferred to fresh BHI at these five temperatures. The lag times and exponential growth rates were determined in the second broths by fitting the data to a two-phase linear model. Exponential phase cells had the shortest lag times, stationary and starved cells had longer times, and desiccated cells had the longest lag phases. Cells transferred to fresh medium at the same temperature as they were grown in (e.g. 8 to 8°C) had the shortest lag times. Cells shifted from higher to lower temperatures had increased lag times as the temperature shifts increased. With temperature shifts from lower to higher temperatures, the lag times also increased with increased temperature shifts, but the overall reduction in lag times at higher growth temperatures made differences in this transition much shorter. Regardless of original state or temperature, after the lag phase was completed, the growth rates were dependent only on the growth temperature. Similar behavior was observed for shifts in pHs (7.0 to 5.0) and, to a lesser extent, for salt additions (0.5 to 5.0%). This information will permit more accurate modeling of a series of growth stages as a food moves through successive processing and storage steps.

(P78) UPDATED MODELS FOR THE EFFECTS OF TEMPERATURE, pH, NaCl, AND $NaNO_2$ ON THE AEROBIC AND ANAEROBIC GROWTH OF *L. MONOCYTOGENES*

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Data from several studies in our laboratories were appended onto aerobic and anaerobic data sets that had been previously used to develop response surface models describing the growth kinetics of *L. monocytogenes* (Buchanan & Phillips, 1990). These expanded data sets included 709 aerobic and 358 anaerobic growth curves, representing 189 and 150 unique combinations of the four variables (temperature, pH, NaCl, $NaNO_2$), respectively. Models were developed for both the Gompertz B and M terms and the lag phase durations (LPD) and generation times (GT). Models were also developed using calculated water activity as a variable in place of NaCl. A number of data transformations were evaluated in an attempt to better utilize the no-growth data. Full quadratic models of the natural logarithm transformation of the data were selected as the most effective. The assignment of GT = 50 h and LPD = 600 h (the approximate maximum duration of experiments) for the conditions that did not support growth proved to be the most effective means of making use of those data. Matrices were developed for the LPD and GT models to calculate 95% confidence intervals. The agreement between observed and predicted growth kinetics was excellent, and the models provided reasonable predictions of the growth of *L. monocytogenes* in foods. These updated models will be incorporated into the version of the USDA Pathogen Modeling Program.

(P79) A COMPUTER MODEL DESCRIBING THE COMPETITIVE GROWTH OF *LISTERIA MONOCYTOGENES* AND *LACTOCOCCUS LACTIS* IN CUCUMBER JUICE

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Current mathematical models by food microbiologists have been used to predict the effects of environmental variables on the growth of individual bacterial pathogens in foods, but do not address the subject of competitive growth of bacteria. We have developed a computer simulation program which is based on a system of nonlinear differential equations describing the changes in two or more cell populations and acid production by the competing bacteria, when growth is limited by the concentration of protonated acid in the growth medium. In a model system (cucumber juice, 10°C, initial pH 5.8), the growth rate and maximum cell numbers of *Listeria monocytogenes* were suppressed by the presence of a non-nisin-producing *Lactococcus lactis* strain, although the limiting concentration of protonated lactic acid was similar (5 mM, at pH 4.1). The computer model has been used to predict these results and the values of parameters affecting the growth and death of the competing populations. The effects of bacteriocins and other factors may be incorporated into the model to broaden the scope of biocontrol modeling.

(P80) MODULATION OF LAG PHASE AT 5°C OF *LISTERIA MONOCYTOGENES* SCOTT A BY OSMOLYTES

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Plant- and animal-derived osmolytes were evaluated for their effects on the lag phase duration (LPD) of *L. monocytogenes* at 5°C in minimal media. The LPD of untreated lag phase cells was ca. 120 h, while LPD was shortened by 10 mM *L*-carnitine HCl, 1 mM glycine betaine, and 0.1 mM betaine aldehyde to 42, 65, and 53 h, respectively. Stationary phase cells exhibited LPDs of 140 and 105 h for control and 1 mM glycine betaine treatment, respectively. Two-dimensional gel electrophoresis and autoradiography of lysates, after treatment with ^{35}S-methionine and cysteine after a 37°C to 5°C shift, showed a threefold protein synthesis decrease within 30 min after temperature downshift. Sixty-five proteins were not synthesized compared to controls and six proteins were either newly synthe sized or their levels increased 1.5-fold. Thus, naturally occurring compounds can shorten lag phase duration of *L. monocytogenes* at a cold temperature. This has implications for predictive microbiology and for the development of food preservation systems.

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TECHNICAL SESSIONS

(T1) EFFECTS OF CULTURE TEMPERATURE, INOCULUM CONCENTRATION, AND CONTACT TIME ON ATTACHMENT OF *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* TO CHICKEN SKIN

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Attachment of *Escherichia coli* O157:H7 and *Listeria monocytogenes* to chicken skin as affected by culture temperature (23 or 37°C), inoculum level (10^4, 10^5 or 10^6 CFU/skin), and contact time (10, 20, or 30 min postinoculation) was determined. Breast skin pieces were obtained from freshly processed broilers, irradiated (10 kGy) and inoculated. At the given contact times, skins were transferred to diluent (20 ml), agitated (2 min.), then transferred to fresh diluent (20 ml), and blended for 2 min. Populations in initial rinse and blended samples were enumerated to determine the number of unattached and attached bacteria, respectively. When 10^4 CFU of *E. coli* O157:H7 were applied, cells cultured at 37°C attached at a higher rate than those grown at 23°C, while at the other inoculum levels, culturing at 37 vs 23°C decreased attachment. When applied at 10^4 or 10^5 CFU, *L. monocytogenes* attached at higher rates when grown at 37 vs 23°C, and at 10^6 CFU/skin, attachment rates were equal for each culture temperature. At 10^4 CFU, skin attachment was greatest after 20 min of contact, whereas at 10^5 and 10^6 CFU, attachment was greatest at 30 min. These data are needed to develop models for evaluating antimicrobial treatments for processed poultry.

(T2) FACTORS AFFECTING INHIBITORY ACTIVITY OF LACTATES AGAINST *E. COLI* O157:H7 AT 10°C

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Effects of lactates on growth and survival of *E. coli* O157:H7 in brain heart infusion broth (BHI) and chicken meat at 10°C as affected by salt form [sodium(SL) or potassium(KL)], concentration [0 (control), 4 or 7%, wt/wt], and manufacturer (Purac or Trumark) were determined. Media, BHI or chicken meat (irradiated to eliminate background microflora), were supplemented with the appropriate treatment, inoculated to provide an initial population of 3.6-3.8 $\log^{10}$ CFU/ml or g of *E. coli* O157:H7, and held for 21 d at 10°C. At seven sampling periods, *E. coli* O157:H7 were enumerated. In the BHI control, the population increased by >5 $\log_{10}$ CFU/ml within 10 d. Addition of 7% Trumark SL completely inhibited growth, whereas 7% Purac SL and 4% Trumark SL moderately inhibited growth. Growth in all other treatments was similar to that in the control. Growth of *E. coli* O157:H7 in control chicken meat and control BHI as equal. However, lactates were more inhibitory in chicken meat than in BHI. When added to chicken meat, 7% SL or KL completely inhibited growth. With the exception of 4% Purac SL and 4% Trumark KL, all treatments resulted in significantly lower populations. The inhibitory activity of lactates against *E. coli* O157:H7 can be affected by salt form, concentration, manufacturer, and medium.

(T3) A SENSITIVE 24-H VERO CELL TISSUE CULTURE ASSAY FOR CYTOTOXINS OF EHEC O157:H7 STRAINS

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Of all the cell lines tested, including mouse myeloma NS1 and Ped-2E9 hybridoma cell lines or CHO, HEp-2, and HeLa cells, Vero cells were found to be the most sensitive indicator of cytotoxins from pathogenic EHEC O157:H7 strains. The cell-free cytotoxins were prepared by filtering 24 h shaking cultures of EHEC O157:H7 with 0.45 µl filters. The Vero cell monolayer was extensively damaged by the presence of cell-free culture supernatant of pathogenic EHEC O157:H7, showing signs of cell lysis and extensive release and floating up of rounded cells which were completely lysed or granulated. By challenging the Vero cell monolayer with a higher dose of EHEC O157:H7 cell-free culture supernatant (exposure ratios of 2:1 vol/vol; 200 µl culture of Vero cell monolayer in 96 well microtiter wells with 100 µl EHEC supernatant) and by using fresh monolayers (3-4 days old) of the Vero cells, the pathogenic strains of EHEC O157:H7 causing cytotoxic effects on Vero cells were detected within 24 h versus 96 h previously reported by FDA. Cell-free cytotoxic supernatants of different EHEC O157:H7 strains tested (932 – human isolate; 505B and 933 - beef isolates; C7929 – apple cider isolate; 204P - pork isolate, and 301C – chicken isolate) proved to be pathogenic in these assays. Conversely, verocytotoxin negative EHEC strains or non-EHEC strains did not cause any changes in the appearances of the Vero cell monolayers over 96 h.

(T4) STIMULATION OF GROWTH AND SURVIVAL OF *E. COLI* O157:H7 AT SUBOPTIMAL TEMPERATURES BY SODIUM LACTATE

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The response of *E. coli* O157:H7, when held at suboptimal temperatures in the presence of sodium lactate (SL), was assessed. Treatments were no added SL (control), 4% (wt/wt) Purac SL, 4% Trumark SL, 7% Purac SL, and 7% Trumark SL. Sterile BHI (100 ml) amended to provide the given SL treatments was inoculated with *E. coli* O157:H7 to an initial population of 3.0-3.2 $\log_{10}$ CFU/ml and held statically for 38 d at 8 or 6°C. At 12 times during the holding period, triplicate samples were taken from each treatment to enumerate *E. coli* O157:H7. At 8°C, *E. coli* O157:H7 exhibited a 23 d lag phase and increased by 2.9 $\log^{10}$ CFU/ml during the remaining period. In contrast, addition of 4% Purac SL or 4% Trumark SL resulted in 6.0 and 4.1 $\log_{10}$ CFU/ml increases, respectively. No net change in population occurred in the 7% Purac SL treatment, whereas addition of 7% Trumark SL resulted in a 1.0 $\log_{10}$ CFU/ml reduction. At 6°C, *E. coli* O157:H7 was unable to

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grow in any of the treatments. However, survival was greater (higher populations recovered) in SL treatments than in the control. Populations decreased below the detection limit (1.3 $\log_{10}$ CFU/ml) in the control within 18 d, whereas detectable populations persisted 28 d in SL treatments. Data indicate that SL can stimulate growth and survival of this pathogen at suboptimal temperatures.

(T5) A SMALL OUTBREAK OF LISTERIOSIS LINKED TO THE CONSUMPTION OF IMITATION CRAB MEAT

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A small outbreak of listeriosis involving two previously healthy adults occurred in Ontario. Food samples obtained from the refrigerator of the patients included imitation crab meat, canned black olives, macaroni and vegetable salad, spaghetti sauce with meatballs, mayonnaise, and water. All of the samples except for the water contained *Listeria monocytogenes*. The three most heavily contaminated samples were the imitation crab meat, the olives, and salad which contained 2.1×10^9, 1.1×10^7 and 1.3×10^6 CFU/g, respectively. *L. monocytogenes* serotype 1/2b was isolated from the patients and from the opened and unopened crab meat. Molecular typing of the isolates by both randomly amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) demonstrated the crab meat and clinical strains to be indistinguishable. Challenge studies done with crab meat and olives showed that *L. monocytogenes* grew only when using a relatively high inoculum. It is evident that a refrigerated product which has a long (>30d) shelf life must have additional safety factors built in to prevent the growth of and disease caused by foodborne pathogens such as *L. monocytogenes*.

(T6) THERMAL DESTRUCTION OF *L. INNOCUA* IN GROUND BEEF PATTIES WITH 5, 25, OR 50% FAT

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Ground beef lots were mixed with soy, bread crumbs, water, phosphates, and NaCl to yield final products with 5, 25, or 50% fat, simulating commercial products. The three products were formed into 10 cm diam. chubs and frozen at -26°C. Chubs were tempered to 0°C and sliced into 1-cm thick patties. Patties were thawed and allowed to equilibrate at 5°C before testing. *Listeria innocua* M1 cells gelled in 0.4 cm alginate beads (about 10^5 CFU/bead) were placed in the geometric center on the surface of patties. A recording thermocouple was placed with the tip in the same position. A second identical patty was placed over the first, creating a 2-cm thick patty. The meat was massaged to bind the two patties into a unit. Patties were cooked in a multi purpose oven at 204.4°C dry bulb and 87.8°C wet bulb. Heating rates for centers of patties were 5.0, 3.3 and 3.2°C/min, respectively for 50, 25, and 5% fat level patties, and maximum internal temperatures were 75-76°C. After cooking, two beads were immediately retrieved at random from each of 5 patties per treatment and placed in WhirlPak bags (4 oz) with 0.5 ml sterile peptone water. Beads were crushed manually, and the bag was stomached for 1 min. Three tubes each of TSB with 0.6% YE and of *Listeria* Recovery Medium (LRM) were each inoculated with a 10 µl loop, vortexed, and incubated at 35°C for 48 h. After 4 h, 50 µl of *Listeria* Selective Enrichment Supplement as added to LRM, and the tubes were vortexed. Tubes were read as growth/no growth. Total integrated heat treatments (°C-min) of products were calculated using center temperatures above 50°C. The minimal mean heat treatment required to produce no detectable survivors was 93.1°C-min. For all 3 fat levels, fat content did not affect the thermal death; apparently, the use of beads eliminates the protective effects of fat.

(T7) ACCELERATED RECOVERY OF INJURED *SALMONELLA* THROUGH MEDIA MODIFICATION

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When *Salmonella* are sublethally injured and present in low numbers, it is difficult to achieve the minimum of 10^4 to 10^6 cells of *Salmonella*/ml needed for detection by current ELISA, genetic probe, or PCR technologies within 24 h. To minimize lag time and accelerate growth rates, the Bactometer® impedance system and conventional growth curves were used to demonstrate the length of lag phase of 4 strains of heat injured *Salmonella* compared to uninjured *Salmonella*. Significant differences among strains were observed with *S. bredeney* being most fragile, *S. lanka* and *S. london* most vigorous, and *S. typhimurium* in the middle. Universal preenrichment broth was found to give better recovery of injured cells compared to buffered peptone. Preliminary studies using glucose, catalase, cysteine, and a combination of the three have demonstrated that lag phase time of injured cells can be reduced. Further studies are being conducted with these and other amendments to optimize enrichment media for recovery of injured *Salmonella*.

(T8) *SALMONELLA* CONTROL IN POULTRY

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Significant reductions in salmonellae prevalence from processed broiler carcasses were observed in field trials in mucosal competitive exclusion treated flocks, as compared to control flocks. Some salmonellae contamination of treated flocks occurred as a result of the contamination present in the hatcheries. When this happens, the beneficial effect of a treatment can be minimized or overridden. Therefore, in an attempt to eliminate hatchery influence and produce salmonellae free poultry, a multifaceted approach will be required, involving application of the most effective chemical disinfectant to the fertile egg as soon as possible after the egg has been laid, disinfection of the circulating air in hatching cabinets during pip, application of a

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yeast culture to protect the gut of the chick from any salmonellae that survived above treatments, and then application of an effective mucosal competitive exclusion culture.

(T9) FACTORS AFFECTING GROWTH AND TOXIN PRODUCTION BY *CLOSTRIDIUM BOTULINUM* IN PEANUT SPREAD

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Growth and toxin production by *Clostridium botulinum* in peanut spread with a_w of 0.98, 0.96, 0.94, or 0.92 stored under aerobic or anaerobic conditions for 16 weeks at 30°C were investigated. The pH of samples stored under anaerobic or aerobic conditions decreased from pH 6.0 to 4.3 with increase in a_w within 3 or 1 week, respectively. Under aerobic conditions, pH of samples with a_w of 0.96 and 0.98 increased from 4.8 to 7.0 during subsequent storage for 16 weeks. Growth of *Penicillium* and *Mucor* spp. likely caused the increase in pH. Decreases in redox potential (Eh) with increase in a_w of samples were observed within 3 weeks of storage under anaerobic conditions. Lower Eh values in samples with a_w of 0.98 or 0.96 stored under aerobic conditions were observed within 1 week and/or 9 weeks compared to samples with a_w of 0.94 or 0.92. None of the samples stored under anaerobic conditions were toxic after storage for 16 weeks. All samples with a_w of 0.98 and two of three samples with a_w of 0.96 became toxic after 9 and 16 weeks of storage under aerobic conditions, respectively. *C. botulinum* also grew to populations of 10^6 and 10^5 CFU g^{-1} in samples with a_w of 0.96 and 0.98. However, samples were judged inedible due to mold growth and off-aromas before toxicity developed, thus greatly minimizing the likelihood of their consumption.

(T10) RESPONSE TO ACID CHALLENGE BY *YERSINIA ENTEROCOLITICA* DEPENDS ON PHYSIOLOGICAL STATE AND STRAIN

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Survival by *E. coli* or *Salmonella* on transfer to strong acid (pH 3.3) is enhanced in cultures grown in mild acid medium (pH<7.0) compared with those grown at higher pH. We examined responses to acid challenge by several *Y. enterocolitica* (YE) strains under varied environmental conditions. Distinct responses were seen. After acid challenge of exponentially growing cultures two strain-dependent survival patterns were observed. Some strains cells grown in mild acid had increased survival compared to cells grown in mild base (pH 7.5). Other strains survived acid challenge at high levels after growth in acid or base. Lower survival was observed after acid challenge at high temperature (37°C), whereas at low temperatures (10°C), prolonged survival was seen after acid challenge in all strains regardless of pH during growth. Stationary phase cultures were highly acid-resistant. While a functional *rpoS* gene was required for mild acid-induced responses in other enteric bacteria, an *rpoS* mutant of YE exhibited an inducible acid tolerance response. In conclusion, both physiological and genetic factors affect the ability of YE strains to survive challenge with strong acid.

(T11) A QUANTITATIVE RISK ASSESSMENT OF *VIBRIO VULNIFICUS* IN GULF OF MEXICO OYSTERS CONSUMED IN CANADA

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Vibrio vulnificus infections from consumption of raw oysters have not been recorded in Canada. Yet, Gulf of Mexico oysters, which have been implicated in serious illnesses and deaths in the United States, are being imported throughout the year (majority during October to May). These oysters, comprising less than 3% of the total oyster consumption in Canada, are consumed mostly in Quebec. A model has been developed to consider the prevalence, numbers, and seasonality of *V. vulnificus* in imported oysters, including the potential for growth during transport and storage and the influence of meal sizes. Assumptions have been made that: (i) the infectious dose per person is $10^{8\text{-}10}$ cells for healthy and $10^{5\text{-}7}$ cells for high-risk populations, and (ii) only 30% of strains are virulent enough to cause illness. From this model it would appear that the high-risk individuals are prone to infection throughout the year, but the highest risk comes from oysters imported in September to November, because the levels of the organism are still high.

(T12) COMPARISON OF *STAPHYLOCOCCUS AUREUS* DETECTION BY CONVENTIONAL AND NEW PETRIFILM™ METHODS

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Detection of *S. aureus* in food samples is an important indication of food quality and safety. Many isolates produce heat-stable enterotoxins that, when present, may result in staphylococcal food poisoning. A 28-h, 3M™ Petrifilm™ (PSC) system has been developed which gives a presumptive identification of *S. aureus* and a confirmatory test uses a reactive disk to detect thermonuclease. The PSC system result was compared to the Baird-Parker agar (BPA) result confirmed by the tube, rabbit-plasma, coagulase method for the detection and differentiation of enterotoxigenic *S. aureus* and other species found in food samples. One hundred ninety-nine strains of gram positive bacteria were run in parallel, including 93 *S. aureus* strains. Bacteria were identified using the API™ STAPH system. The BPA/coagulase methods gave sensitivity and specificity of 82.8% (77/93) and 96.2% (10^2/10^6), respectively, whereas the PSC system had sensitivity of 96.8%(90/93) and specificity of 91.5% (97/106). All 93 *S. aureus* strains were evaluated for the production of enterotoxins by EIA. Seventy-one of 93 isolates were EIA positive. BPA/coagulase detected 57 strains (80.3%); the PSC system detected 69 strains (97.1%). In addition, two strains of *S. hyicus* and three strains of *S. intermedius* that

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were BPA/coagulase negative and PSC system positive produced detectable levels of enterotoxin. These data suggest that the PSC system may provide better detection of *S. aureus* strains, including enterotoxigenic *S. aureus* isolates, than the conventional method. Furthermore, the PSC system gives results in approximately one-third the time (28 h versus 72 h). Performance comparisons with food samples are in progress.

(T13) A SINGLE TEST UNIT FOR QUANTITATING COLIFORMS, *E. COLI*, AND *SALMONELLA* IN WATERS AND FOODS

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Coligel®, an easy and safe to use single unit screening method for quantitation of coliform and *E. coli* in drinking water, is compared to membrane filter, LTB, BGLB and ECMUG determinations in waste water spiked drinking water as part of the EPA-ATP procedure. In addition the method is easily adapted for use in foods and can detect *Salmonella* simultaneously. The Coligel-S was studied using various dilutions of juices and carcass rinses. The purpose of the experiment was to determine the maximum sample addition that would yield proper 28 h development without adverse affect on colorogenic and fluorogenic indicators. Maximum juice addition that could be added without adverse effect was 3 ml. Certain juices required a neutralization step prior to juice addition. Fish, poultry, swine, and beef carcass rinse solutions resulted in defined colonies and good simultaneous detection of *E. coli* and *Salmonella*. Various control charts demonstrating Coligel-S as a microbial control monitor for HACCP are presented.

(T14) ENSURING THE MICROBIOLOGICAL QUALITY OF A LOW PROOF BEVERAGE

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To ensure the production of high quality, microbiologically stable products, sensitive and rapid techniques are required. In the high acid beverage of interest, control of preservative resistant yeast contamination was of utmost concern. Three key factors to maintain product quality included ingredients, sanitation, and product testing. Rapid sanitation monitoring was completed by bioluminescent swabs. Product and ingredient testing were carried out using a microbial detection system consisting of a fully automated, instrument employing unique culture bottles equipped with colorimetric sensors allowing for CO_2 detection. The use of this system allowed for more sensitive and rapid detection of potential spoilage organisms in the ingredients and products. One yeast cell could be detected in a 25 mL sample in 3.5 days, compared to 10 cells per mL in 5 days using standard pour plate techniques. Successful production of a microbiologically stable beverage was achieved by employing these rapid techniques.

(T15) ASSESSING SURFACE CLEANLINESS—AN INTEGRATED APPROACH USING ATP BIOLUMINESCENCE AND MICORBIOLOGICAL ANALYSIS

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No one ideal method exists with which to assess food contact surface cleanliness. ATP bioluminescence and microbiological swabbing are two commonly used techniques. Despite the speed with which ATP bioluminescence provides results, the technique cannot yet identify specific organisms. Therefore, the need for swabbing still exists in identifying and assessing specific pathogens. This study evaluated the use of both techniques in the development of an integrated approach to hygiene monitoring. Sanitized stainless steel surfaces inoculated with *E. coli* (NCTC 10418) and a raw milk suspension were used to evaluate the sensitivity and reproducibility of direct and indirect measurements of ATP from surfaces. One luminometer detected 0.5 femtomoles of ATP with results being significant at the 5% level. One luminometer detected 10^2 cells of *E. coli* from inoculated swabs, while with surface inoculations, the lowest level detected by any luminometer was 10^4 cells. Direct measurements from surfaces gave greater sensitivity over indirect measurements. No significant difference in operator reproducibility was found for eight assay systems under test conditions. Surfaces inoculated with *E. coli*, *S. aureus* (NCTC 6571) and an environmental isolate of the genus *Staphylococcus* were used to assess the effects of selected variables on bacterial recovery rates using swabbing. A range of factors influenced bacterial recovery rates. Of particular importance were recovery medium and diluent type. The results are considered in relation to a proposed three-stage hygiene monitoring protocol.

(T16) THE USE OF BIOLUMINESCENCE FOR EVALUATING PLANT CLEANLINESS IN A BAKING FACILITY

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Standard surface swabbing techniques were compared with commercial adenosine triphosphate (ATP) bioluminescence hygiene monitoring kits to determine their adequacy as a rapid method for evaluating the sanitation program in a baking facility. Samples were collected from stainless steel equipment surfaces and nonfood contact surfaces, both before and after sanitation. Two different baking facilities were tested on three occasions. The numbers of microbiological contaminants collected using standard surface monitoring techniques were compared to the ATP recovered with the ATP bioluminescence kits. The rates at which the techniques passed or failed a surface were in good agreement. It was concluded that the ATP bioluminescence hygiene monitoring systems could be used in a baking facility to evaluate cleaning and sanitation effec-

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tiveness. The technique was used successfully to identify potential sources of contamination in the production of a shelf-stable product.

(T17) RAPID MOLECULAR METHOD FOR DETECTION OF HUMAN ENTERIC VIRUSES IN PREPARED HAMBURGER AND LEAF LETTUCE

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A universal method to extract and concentrate human enteric viruses from food commodities for detection using reverse transcription-polymerase chain reaction (RT-PCR) and confirmation by internal oligoprobe hybridization (OP) was developed. Using prepared hamburgers and lettuce as model foods, 50-gram samples were seeded with 10^3-10^5 plaque-forming units (PFU) of poliovirus type 1 (PV1) or hepatitis A virus (HAV) and processed for virus concentration using the sequential steps of elution, filtration, and polyethylene glycol (PEG) precipitation/elution. Virus recoveries after elution and filtration averaged 50% and 15% for PV1 and HAV, respectively. Both viruses were effectively precipitated at PEG concentrations of 6-8%, regardless of food commodity, although virus elution from PEG pellets was less than optimal. An additional processing step using the virus-precipitating agent Viraffinity® enabled further removal of inhibitory compounds with recovery of 75-100% of input virus. When followed by extraction of viral RNA using a guanidinium thiocyanate approach, final sample concentrates were of low volume (<100 µl) and compatible with viral nucleic acid amplification using RT-PCR. Initial detection levels have been established at < 10^3 PFU per 50-gram food sample.

(T18) IMMUNOMAGNETIC SEPARATION AND FLOW CYTOMETRY FOR RAPID DETECTION OF *E. COLI* O157:H7

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A rapid detection method for *E. coli* O157:H7 combining immunomagnetic beads (IMB) and flow cytometry was evaluated. Labeling antigens separated by IMB with fluorescent antibody enabled the detection of 10^3 CFU bacteria/ml in pure and mixed culture. The optimum concentration of magnetic beads for flow cytometry was lower (ca.10^5 particles/ml) than that of conventional IMB assay (more than 6-8 × 10^6 particles/ml). Immunomagnetic separation and flow cytometry (IMFC) were evaluated for detecting *E. coli* O157:H7 in the presence of a competing microorganism and for detecting antibodies. The total assay time from separating antigens with IMB to analyzing with flow cytometry was about 1 h. The detection limit of IMFC was not decreased significantly by competing organism and ground beef matrices in 6-h ground beef preenrichment broth. The 6-h ground beef preenrichment broth artificially inoculated with 2-6 cells/g of *E. coli* O157:H7 was positive with IMFC. The new assay system produces another approach to separation and detection of low populations of pathogens and low concentration of toxins directly from food in a short time.

(T19) HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP) IMPLEMENTATION OF FOODSERVICE OPERATORS

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The purpose of this research was to determine Hazard Analysis Critical Control Point (HACCP) implementation by the 105 foodservice operators who attended HACCP training in five locations in Kansas and 400 healthcare foodservice directors who responded to a national survey. Respondents were asked if they would implement a complete HACCP system or in stages using the seven steps of HACCP. The respondents used a Likert scale to rate their perception of HACCP implementation from 1 – will not implement to 5 – will implement immediately. Ninety-nine (94%) of the training participants completed the questionnaire, and 40% of the mailed surveys were returned. Mean ratings were highest for implementing a monitoring system to check temperatures (3.42±1.20), while the lowest mean ratings were for developing flow charts for every recipe in the operation (2.37±1.00). Most food service operators are interested in implementing a HACCP system. However, lack of time, training, and support will prevent most respondents from implementing the seven-step HACCP program.

(T20) HANDWASHING VS. GLOVING FOR FOOD PROTECTION

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There have been a number of situations where food handlers have been implicated as a primary vector in contributing to foodborne illness. The most effective method to break the contamination vector between food handlers and consumers is intensely debated. One view holds that food servers must eliminate bare hand contact with ready-to-eat food (by use of gloving) to insure protection, while the other position is that a well managed hand washing and sanitizing program is sufficiently effective to insure protection. This presentation explores this wide difference of opinion via literature review and discussion of laboratory studies performed to investigate the effectiveness of gloves vs. handwashing to prevent the transfer of microbes from food to food handler and from food handler to food.

In one phase of the study, human volunteer's hands were gloved immediately after the hands were contaminated with *Escherichia coli*. In the majority of cases, the gloved hands demonstrated significant bacterial counts on the outside glove surface, suggesting that the *E. coli* were easily transferred from the hands through pre-existing holes

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in the gloves. In addition, it was demonstrated in another portion of this study that gloves were unable to prevent contamination of hands from artificially contaminated food after three hours. Of the numerous configurations examined, bare hands with hourly washing followed by utilization of a hand sanitizer showed significantly better overall hand sanitation levels.

These studies indicate that if gloves are worn to prevent microbial contamination of food by food handlers, the gloving should be preceded by an effective handwash. Additional studies should be conducted in food service settings to validate the most effective hand sanitation regimen for protection of public health.

(T21) FOODBORNE DISEASE IN THE HOME

Elizabeth Scott, Consultant in Food and Environmental Hygiene, 98 Ridge Avenue, Newton, MA 02159

The subject of foodborne disease in the home is one of growing interest. The occurrence of foodborne disease is on the increase in the developed world, and data from several countries indicate that the majority of cases of salmonellosis and campylobacteriosis arise within the home, often as a result of inadequate food hygiene procedures in the domestic kitchen. This paper will discuss the role of cross-contamination as a factor in foodborne disease and the risk significance of contaminated kitchen surfaces. Examination of this information together with a review of disinfection methods in the home enables the development of an advisory to consumers on food hygiene in the home, including the prevention of cross-contamination.

(T22) STATEWIDE TRAINING FOR ENVIRONMENTAL HEALTH SPECIALISTS

Bibby Moore, Division of Environmental Health, P.O. Box 29596, Raleigh, NC 27626-0596

The Initial Internship Training program, begun in 1993, is required for all new environmental health specialist interns to: 1) provide the knowledge and skills to implement North Carolina Environmental Health laws & rules; 2) promote uniform application of the laws and rules, 3) provide up-to-date information on other relevant programs. The program covers 19 sets of rules in five areas of authorization: Food, Lodging & Institutions; On-site Wastewater; Childhood Lead Poisoning Prevention, Public Swimming Pools, and Tattoos. The 6½ week training program, offered twice a year, is taught by over 100 practicing professionals from state government, universities and county health departments. The training program provides a uniform understanding of the rules and knowledge of who to contact for problem solving. The participants have the knowledge level of someone practicing for a year. Their skill level is further developed by supervised practice at the county health department. Of the 950 registered environmental health specialists, the 200 newly trained interns are making an impact by upgrading the knowledge of their colleagues when they return from training and by providing more uniform interpretation of the rules. Over time, the anticipated outcome is that there will be fewer litigations, better communication with the public and industry and increased educational efforts to support good public health practices.

(T23) RECIPE HACCP

O. P. Snyder, Jr., Hospitality Institute of Technology and Management, 830 Transfer Rd., Suite 35, St. Paul, MN 55114

In the case of all raw and pasteurized fruits, vegetables, meat, poultry, fish, dairy products, and grains and cereals pre-prepared in retail food operations and in the home, it is the cook who assures that the food is safe when it is consumed. The question is, "Where does the cook find the hazard control rules to follow in order to assure that the food is safe when consumed?" Recipe books have inadequate information. Recipes, the document cooks use to prepare food, rarely have safety control information. If the cook is to assure the safety of food produced with a recipe, the recipe must be written with hazard control procedures and standards.

This presentation will present research-validated hazard control procedures and standards that cooks must know to make raw food safe and to keep pasteurized food safe. Then, it will show how to flow diagram recipes to assure that they are logically sequenced. Finally, it will show how to turn the flow diagram into a user-friendly recipe form that all cooks can use and will assure the safety of the food when it is consumed.

(T24) A QUANTITATIVE RISK ASSESSMENT FOR HUMAN ILLNESS ARISING FROM *SALMONELLA ENTERITIDIS* IN EGGS IN CANADA

E. Todd,* W. Ross, T. Gleeson, K. McIntyre, P. Sockett, R. Irwin, A. Muckle, C. Poppe, J.-Y. D'Aoust and R. Medaglia, Bureau of Microbial Hazards, Health Protection Branch, Health Canada, Banting Reserach Centre, 2204A2, Ottawa, Ontario, Canada K1A 0L2

Salmonella enteritidis (S.E.) has not been a major problem for egg producers in Canada as it has been in Europe, the United States and other countries. However, three recent phage-type 4 incidents involving shell eggs in Canada might be the first indication that its prevalence is on the increase in breeder and layer flocks. Accordingly, a team of government microbiologists, epidemiologists, egg specialists and bio-statistitians constructed a quantitative risk assessment model for S.E. in table eggs from the multiplier breeders, through the egg-laying flocks, to the eggs and their consumption. Transovarian and egg surface sources of contamination, egg collection, washing, storage, preparation and types of egg meals were considered in this model. The dose-response curve was based on outbreak data. With current data put in the model, the average probability of illness is low, about 1 in a million, with consumption of raw or lightly cooked eggs being a major factor. If the scenario changes to where 1 in 3,000 shell eggs are transovarially infected, however, an unacceptable situation would occur.

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(T25) VERIFICATION OF A QUANTITATIVE RISK ASSESSMENT FOR *E. COLI* O157:H7 IN HAMBURGERS

E. Todd,* W. Ross, M. Cassin, A. Lammerding and R. Khakhria, Bureau of Microbial Hazards, Health Protection Branch, Health Canada, Banting Reserach Centre, 2204 A2, Ottawa, Ontario, Canada K1A 0L2

The Canadian Federal Interdepartmental Committee for Food Safety Risk Analysis considered that five pathogens in raw foods of animal origin were the cause of major health impacts. One of these is *E. coli* O157:H7, which affects children more severely than adults. In Canada, during 1995 there were 1,277 laboratory isolations of this pathogen. From historic outbreak data, about 30-50% of cases arise from consumption of contaminated ground beef hamburgers. A model had previously been developed to simulate the probability of illness from hamburger meals, with an average predicted value of about 1,100 illnesses, 111 hemorrhagic uremic syndrome cases and 11 deaths in Canada each year. These estimates may be slightly conservative if laboratory isolations are underestimated by a factor of 10 to 50. Simulations were run with scenarios corresponding to reported outbreaks with levels ranging from 0.3 to 100 CFU/g in the ground beef before cooking. Uncertainty in the specification of a dose-response model was also assessed.

(T26) RAPID DESICCATION WITH HEAT IN COMBINATION WITH WATER WASHING FOR REDUCING BACTERIA ON BEEF CARCASS SURFACES

C. N. Cutter,* W. J. Dorsa, and G. R. Siragusa, USDA-ARS, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE 68933

A series of experiments was conducted to determine the effectiveness of rapid desiccation with heat at one or two points in the slaughter process to reduce bacterial contamination on beef carcass surfaces. In the first experiment, beef surfaces were inoculated with bovine feces and water washed (A; 125 psi, 15 s, 35°C); desiccated (400°C, 15 s) before inoculation and subjected to a water wash (B); inoculated, water washed and desiccated for 30 s (C); or desiccated, inoculated, water washed, and desiccated for 30 s (D). Remaining bacterial populations of samples treated with D exhibited the fewest populations of APC, coliforms, and *Escherichia coli*. When *E. coli* O157:H7, *Salmonella typhimurium*, *Listeria innocua*, and *Clostridium sporogenes* were monitored following treatments with D, none of the organisms were detected. An additional set of experiments was conducted with less heat (300°C) for shorter times to minimize surface discoloration. When desiccation (300°C) was conducted for 10, 12, or 15 s prior to fecal contamination and followed by a water wash, it was demonstrated that none of the treatments were significantly different from the others for reducing APC from shortplates; however, the 10 s treatment was preferred for its shorter time. When desiccation for 10 s was combined with water washing and followed by a second desiccation step (300°C) for 15, 20, or 25 s, populations of APC, coliforms, and *E. coli* were reduced to the greatest extent when the second desiccation step was applied for 25 s. In all cases, the desiccation step(s) and water wash combinations were more effective than water washing alone for reducing bacterial contamination on beef surfaces.

(T27) A PURGE SAMPLING METHOD TO DETECT TOTAL AEROBIC BACTERIA AND *E. COLI* O157:H7 IN RAW BEEF COMBOS

W. J. Dorsa* and G. R. Siragusa, USDA-ARS, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE 68933

The purge from model beef combos (a palleted box of beef trimmings used to make ground beef) was tested as a means of representatively sampling the microbial content of this raw product. Purge was sampled from model beef combos that had been inoculated with bovine feces (Study 1) or inoculated with an antibiotic resistant *E. coli* O157:H7 (Study 2). The purge from both studies was assayed for bacteria using culture methods. Data from Study 1 indicated a strong correlation ($r = 0.94$) between the total aerobic bacteria counts derived from the purge samples of a model combo of beef and the total aerobic bacteria present in a rinse sample of the entire model combo of beef. In Study 2, the marked *E. coli* O157:H7 was retrievable after 24 h regardless of the location of the inoculated pieces of meat within the 75-cm meat column, demonstrating that bacteria do migrate vertically downward into the purge of a model beef combo. Consequently a third study was conducted where 90 beef combos at the receiving dock of a commercial grinding facility were randomly sampled using both purge and concurrent 25 g grab samples. Purge samples from these combos recovered significantly greater numbers of mesophilic and psychrotrophic aerobic bacteria, coliforms, and *E. coli* than grab samples from the same combos. Additionally, coliforms and *E. coli* were recoverable from 100 and 80 percent, respectively, of the purge samples taken, while grab samples were only able to recover 60 and 40 percent, respectively, from the same combos. These findings indicate that a purge sample from a beef combo is a more efficacious sampling method for determining the general bacterial profile and identifying the presence of specific bacteria than randomly taken grab samples.

(T28) EVALUATION OF THE USDA SPONGE SAMPLING TECHNIQUE FOR BEEF CARCASSES FOR ENUMERATION OF *E. COLI*

N. A. Kotrola,* J. S. Kotrola, R. K. Phebus, J. L. Marsden and C. L. Kastner, Kansas State University, Dept. of Animal Sciences and Industry, 210 Call Hall, Manhattan, KS 66506

Survival of *E. coli* in Butterfield's phosphate buffer (BPB) during chilled storage in sterile sponge-containing Whirl-Pak® bags was evaluated to determine the effect of sample storage on cell recovery in the USDA-FSIS's new Pathogen Reduction/HACCP *E. coli* criteria. Sponge-containing bags with 25 ml BPB were used to sample 20 beef carcasses per USDA guidelines. Bags were then inoculated with 60 or 600 *E. coli* cells/ml and stored at 4°C for 0 and 5 min, and 6, 12, 18, 24, 36, and 48 h. Popula-

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tions were enumerated on *E. coli* Petrifilm plates. *E. coli* recovery was reduced ($P \leq 0.05$) during the first 5 min of storage by 81.7 and 65.7% from initial levels of 60 and 600 *E. coli*/ml, respectively. After 24 h, counts for these inoculum levels were reduced by 99.1 and 96.8%, respectively. To ascertain the effects of the sponge in the Whirl-Pak bag on *E. coli* recovery, bags containing sponges were filled with diluent, the sponge was removed, and the diluent was inoculated with 66 or 660 *E. coli*/ml. After storage, *E. coli* populations were reduced by 21.2 and 4.5% for 5 min. for the two inoculum levels, respectively. After 12 h storage, no *E. coli* was detected at either inoculum level. In BPB contained in glass tubes, *E. coli* populations remained stable during 12 h storage at 4°C. The sponge sampling method may not provide adequate *E. coli* recoverability for monitoring HACCP programs.

(T29) REDUCTIONS IN MICROBIAL POPULATIONS AT FIVE ANATOMICAL LOCATIONS ON STEAM PASTEURIZED BEEF CARCASSES

A. L. Nutsch,* R. K. Phebus, J. Kotrola, T. Brown, M. J. Riemann, and R. C. Wilson, Rm. 139, Call Hall, Kansas State University, Manhattan, KS 66502

The effectiveness of a patented steam pasteurization treatment for reducing naturally occuring bacterial populations at five anatomical locations on commercially slaughtered beef carcasses was evaluated. Before and after pasteurization treatment (6.5 s exposure time), a sterile sponge was used to sample 300 cm^2 at one of five locations (inside round, loin, midline, brisket, or neck). Eighty carcasses (40 before and 40 after treatment) were sampled per anatomical location. Before treatment, aerobic plate counts (APCs) were found to be highest ($P \leq 0.01$) at the midline (2.5 $\log_{10}$ CFU/cm^2), intermediate at the inside round, brisket, and neck (approx. 1.8 $\log_{10}$ CFU/cm^2), and lowest at the loin (1.4 $\log_{10}$ CFU/cm^2). After treatment, APCs at all locations were significantly reduced ($P \leq 0.01$). The inside round, loin, and brisket had the lowest ($P \leq 0.01$) APCs (approximately 0.5 $\log_{10}$ CFU/cm^2), while the midline and neck had APCs of 1.1 and 1.3 $\log_{10}$ CFU/cm^2, respectively. Generic *Escherichia coli* populations were low at all locations before treatment, with populations on 32% of all carcasses sampled <0.05 CFU/cm^2 (detection limit of study). After pasteurization treatment, generic *E. coli* populations on 85% of all carcasses sampled were <0.05 CFU/cm^2, with a maximum population of 0.25 CFU/cm^2 detected.

(T30) CHARACTERIZATION OF LACTIC ACID BACTERIA FROM A SOW, A HEALTHY PIGLET, AND AN ILL PIGLET

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A sow, one healthy piglet, and one sick piglet that died before weaning, were sampled vaginally and/or rectally on days 3, 10, and 22 for the presence of intestinal lactic acid bacteria (LAB). A loopful of each sample, without prior dilution, was streak-plated onto Rogosa SL agar plates and incubated anaerobically at 37°C overnight. From each sample site on each sampling day, 15 colonies were selected at random from each of the animals. These isolates were characterized phenotypically, as well as biochemically using API CH 50 sugar fermentation tests. From 60 of 180 isolates tested to date, the predominant LAB associated with the sow included *Lb. brevis* (23 isolates; 38%), *Lb. fermentum* (21 isolates; 35%), and *Lb. plantarum* (16 isolates; 27%). In contrast, *Lb. fermentum* (6 isolates; 20%), *Lb. brevis* (1 isolate; 3%) and *Lb. salivarius* (1 isolate; 3%) predominated among 30 isolates obtained from one of the surviving piglets. From the 30 isolates obtained from the piglet that died on day 28 after birth, *Lb. salivarius* (11 isolates; 37%) and *Lb. fermentum* (4 isolates; 13%) predominated. In related studies, prefatory experiments using pulsed-field fingerprinting revealed 37 restriction endonuclease digestion profiles (REDP) among the 60 isolates examined. Further molecular comparison of LAB from both sick and healthy piglets from this litter will be useful for exploiting these organisms as biopreservatives and biotherapeutics.

(T31) THERMOTOLERANCE OF *ENTEROBACTER SAKAZAKII* IN AN HTST PASTEURIZER

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Enterobacter sakazakii, a gram-negative peritrichous rod, designated a unique species in 1980, has been implicated in a rare but severe form of neonatal meningitis, with dried-infant formula being implicated as the mode of transmission. The high mortality rate (40-80%) and the lack of information about this organism led to a study of the heat resistance of *E. sakazakii*. Ten Canadian *E. sakazakii* strains were used to determine the heat resistance of this organism at 52, 54, 56, 58 and 60°C in reconstituted dried-infant formula. D-values of 54.8, 23.7, 10.3, 4.2 and 2.5 min. were obtained for each temperature, respectively. The calculated z-value was 5.82°C. In a comparison of the D-values of several members of the *Enterobacteriaceae* in dairy products, *E. sakazakii* appeared to be one of the most thermotolerant organisms. In order to validate this finding, and more realistically simulate processing conditions, data was also obtained on the inactivation of *E. sakazakii* in whole milk in a high-temperature short-time (HTST) pilot scale pasteurizer. Using a computer program designed at the Center for Food and Animal Research, Agriculture and Agri-Food Canada, the integrated lethal effect, or pasteurization effect (PE) was determined. Times and temperatures in each section of the pasteurizer were integrated for holding times and temperatures of 3-60 s and 60.5-69.5°C. A linear model was derived from 5 trials which related values of PE to log % residual counts. Risk analysis simulations were performed using the Lotus 1, 2, 3 add-in @RISK to determine the probability of achieving a 4-log reduction of *E. sakazakii*. These results confirm and extend the previous findings that *E. sakazakii* is thermotolerant and the importance of process control during manufacture and the

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use of aseptic procedures during the preparation, use and storage of dried-infant formula must be emphasized.

(T32) REDUCING CONDITIONS AND SERYL AND SULFHYDRYL INHIBITORS ON AFLATOXIN B_1 DEGRADATION BY *F. AURANTIACUM*

D. H. D'Souza* and R. E. Brackett, CFSQE, University of Georgia, Griffin, GA 30223

This study was undertaken to determine the effects of reducing conditions and the addition of seryl (phenylmethyl-sulfonyl fluoride (PMSF)) and sulfhydryl (Cd^{2+}) group inhibitors on aflatoxin B_1 (AFB_1) degradation by *Flavobacterium aurantiacum*. HPLC was used to determine AFB_1 concentrations in 72-h cultures of *F. aurantiacum* that had been washed and resupsended in phosphate buffer (0.1 M, pH 7.0). The addition of 0.1, 1, or 10 mM L-cysteine to these cultures did not have a significant effect ($P<0.05$) on AFB_1 degradation after incubation at 30°C for 4, 24 or 48 h. The addition of 0.1 mM PMSF did not significantly decrease AFB_1 degradation, but 1 mM PMSF significantly decreased the degradation of AFB_1 after 4, 24 and 48 h of incubation. No significant difference in AFB_1 degradation was obtained with 0.1 mM Cd^{2+}, but 1 and 10 mM Cd^{2+} significantly decreased the degradation of AFB_1 after 4 and 24 h. The chelators, 1 mM EDTA and 1 mM 1, 10-phenanthroline, did not counter the inhibition of AFB_1 degradation observed with 1 and 10 mM Cd^{2+}. This suggests that seryl and sulfhydryl groups may be involved in the active site of the AFB_1 degradative enzyme system of *F. aurantiacum*. Future research on the crude enzyme preparations using these inhibitors is essential in order to purify and characterize this enzyme system.

(T33) EFFECT OF PREBIOTICS ON *BIFIDOBACTERIUM*

S. Tsai* and J. B. Luchansky, Food Research Institute, University of Wisconsin-Madison, Madison, WI 53706

This study evaluated the potential of selected oligosaccharides for use as prebiotics. Commercially-available oligosaccharides, 2 fructo-oligosaccharides (FOS-1 and FOS-2) and a xylo-oligosaccharide (XOS), as well as inulin, were tested at levels of 0.1% to 4.0% (w/v) for the ability to enhance the growth of *Bifidobacterium* spp. in a basal synthetic media. Thirteen bifidobacteria strains were evaluated, including 8 fecal isolates from a newborn and 4 fecal isolates from an attendant sibling, as well as *B. breve* ATCC 15698. All 13 bifidobacteria showed enhanced growth at 37°C in 30 hours in the presence of 1.5% of FOS-1 and FOS-2, whereas 6 of the 13 bifidobacteria strains (2 strains from the infant and 4 strains from the sibling) showed enhanced growth at 37°C/30 h temp/time in the presence of 1.5% XOS. All strains tested did not display enhanced growth in the presence of 1.5% inulin. Moreover, increasing the levels of inulin or XOS to 4.0% did not result in enhanced growth of strains that grew poorly at levels of 1.5% inulin or XOS. Studies are ongoing to validate the prebiotic capabilities of FOS-1, FOS-2, and XOS in model food systems using 3 of the 13 strains displaying the greatest response in synthetic media.

SPECIAL POSTER SESSION

(SP1) UPDATE OF WASHING AND SANITIZING OF MILK TANK TRUCKS AND DAIRY PLANT EQUIPMENT

Tom Bowman,* FDA, 60 - 8th St., N.E., Atlanta, GA 30309

The dairy industry was one of the first to implement mandatory washing and sanitizing of Grade A raw milk tank trucks. These regulations are described in the Pasteurized Milk Ordinance (PMO). An update of these PMO requirements will be presented. Illustrations of the clean-in-place system in the tank trucks and dairy plant are shown. The prescribed sanitizers for use in the dairy plant will be given.

(SP2) AN ASSESSMENT OF THE CLEANING AND DISINFECTION OF POULTRY TRANSPORT CONTAINERS AND TRUCK BEDS

Sam Joseph,* Lewis Carr, and Christos Rigakos, University of Maryland, Dept. of Microbiology, College Park, MD 20742

Processors are being encouraged to provide wholesome and *Salmonella*-free poultry in the marketplace. Provisions of clean and decontaminated transport units should enable significant reduction of the transmission of disease organisms between farms. However, many transporters of live poultry do not properly clean and decontaminate their transport systems between loads of live poultry. Much of the washing activity is cosmetic without proper attention to viral and bacterial agents that may be transported from farm to farm. Results for a twelve-plant international study conducted by our laboratories at the University of Maryland showed that units prior to washing and post-washing were positive for *Salmonella* in the range of 0% to 100% for both situations and coliform ranged from 79% to 100% and 67% to 100%, respectively. Truck beds prior to washing and post-washing were positive for *Salmonella* in the range of 0% to 100% and 20% to 100%, respectively and coliform ranged from 90% to 100% and 75% to 100%, respectively. Recycled water samples from five of the eight plants were positive for *Salmonella* (63%) and eight of eight samples were positive for coliform (100%). Ineffectiveness of decontamination procedures suggests that inefficient cleaning and disinfection systems as well as intrinsic bacterial factors, such as the ability to produce biofilms, may play an important role in the inability of processors to clean and decontaminate their poultry transport units satisfactorily.

(SP3) EFFICACY OF HOLDING PEN WASHING TO REDUCE BACTERIAL LEVELS

Kathleen T. Rajkowski, USDA-ARS-ERRC-MFS, 600 E. Mermaid Lane, Wyndmoor, PA 19038

In a previous study, the floors and bedding material from swine-hauling trailers were shown to be contaminated

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with *Salmonella* and *E. coli*. At least 15 different serotypes of *Salmonella* were identified. The hog slaughtering plant's washing-sanitizing regime was effective in eliminating *Salmonella* and reducing *E. coli* levels. As part of the continuing study, the hogs from contaminated haulers were followed into the holding pens. The hogs remained in the pens for 2 to 4 hours before slaughter. These pens were sampled for bacterial levels after the hogs were removed. The *Salmonella* serotypes isolated from the holding pen floors were compared to those isolated from the trailers and were found to be the same. After the pens were washed with water, there was a reduction of *Salmonella*. These results demonstrate that washing the pens between loads reduces the incidence of *Salmonella*, which could ultimately improve food safety by reducing the incidence and level of *Salmonella* in the final product.

(SP4) NEW METHODS FOR SANITIZATION OF EGG SHELLS

S. D. Worley, Dept. of Chemistry, Auburn University, Auburn, AL 35768

A novel series of biocidal compounds known as organic N-halamines will be described which could be useful in the sanitization of egg shells. The compounds were tested against *Salmonella enteritidis* on the surfaces of egg shells. Some experiments involved spraying inoculated egg shells with aqueous solutions of the compounds, and free chlorine for comparison. Others involved suspending the most stable of the compounds in mineral oil which was then coated onto the surface of the egg shells and inoculated with *Salmonella*. In both types of experiments the N-halamine compounds were found to be effective in inactivating *Salmonella*. The rates of diffusion of the compounds through the egg shells were also measured and found to be insignificant. It is postulated that the new compounds will be useful alternatives to corrosive-free chlorine in egg shell sanitization.

(SP5) BIOFILMS IN AQUATIC FOOD PROCESSING

Douglas L. Marshall, Dept. of Food Science and Technology, Mississippi State University, Box 9805, Mississippi State, MS 39762

Ready-to-eat seafood products are routinely recalled from the marketplace due to either the presence of pathogenic bacteria or to gross sanitation problems within processing plants. Primary sources of these microbes in the processing environment are contaminated raw material, personnel, and poorly cleaned and sanitized equipment. The predominate pathogen responsible for these recalls has been *Listeria monocytogenes*, which can attach readily and quickly to food and equipment surfaces. Given ample time and nutrients, attached cells can proliferate to form multicellular microcolonies called biofilms. In general, older biofilms are more difficult to remove than younger biofilms. Simple water rinsing techniques are not effective biofilm control measures. Other dominant bacteria present on processing equipment are *Pseudomonas* and *Aeromonas*. Presence of aeromonads on surfaces indicates poor sanitation, since they are quite sensitive to heat or chlorine treatments. However, pseudomonads are relatively more resistant to chlorination than other gram-negative bacteria. Hand picking and shucking of shellfish present a major problem in controlling personnel hygiene. Many ethnic workers have language barriers that make enforcement of personal cleanliness a challenge for supervisors. Thus to control contaminating microbes in seafood processing plants, careful attention to both personnel hygiene and equipment sanitation is needed.

(SP6) WASHING FRESH FRUITS AND VEGETABLES

Jerry A. Bartz, University of Florida, Dept. of Plant Pathology, Gainesville, FL 32611-0680

Fresh fruits and vegetables are usually not intentionally washed except to remove soil from root crops and contaminants on product surfaces such as sooty mold from citrus intended for fresh market. Washings, however, usually accompanies the primary purpose of using water which includes moving, cushioning, or cooling produce. Soft fruits such as strawberries, raspberries, etc. are not handled in water due to concerns that residual water will promote postharvest decays. Since most produce handling requires large volumes of water, the water is usually recirculated and, as a result, must be treated with chlorine gas or a hypochlorite salt to prevent the widespread inoculation of produce with decay pathogens. Water chlorination can prevent microbes washed into the water from accumulating, eliminate transfers of microbes among products in the system, and reduce populations of microbes on products. However, microbes inside produce or embedded in wounds or matrices are not affected by chlorine. For example, diseased tissues continue to shed pathogen propagules until the tissues are completely dispersed or removed from the system. Plant pathogen populations in fleshly inoculated wounds are reduced but not eliminated, whereas the status of clinical pathogens in wounds is unknown. The use of surfactants to enhance the contact of chlorinated water with fresh produce is usually not recommended because the tendency of water to infiltrate internal spaces in fresh produce increases as the surface tension of the water decreases. The movement of microbes into produce can accompany infiltration despite the presence of adequate free chlorine in the water.

SYMPOSIA

(S1) NEW PRODUCT OPPORTUNITIES, WHAT ARE CONSUMERS SEEKING?

Christine M. Bruhn, University of California, Center for Consumer Research, Davis, CA 95616-8598

Consumer's flavor, health, and convenience needs are not fully met in today's marketplace. A 1996 market evaluation found dairy lags behind other categories in new product introductions. This presentation reviews the forces shaping consumer demands and identifies areas where market niches may be found.

Today's lifestyle creates an environment conducive to new product acceptance. Most households are looking for convenience items. Supermarkets use multiple techniques to facilitate consumer buying but dairy products are

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not included. Purchase of prepared foods has increased with away-from-home purchases going from about 33% of the food dollar in 1970 to 43% in 1994. Innovative presentations by chefs impact current and future sales when consumers recreate dishes at home.

Taste is the primary reason for food selection. Ethnic flavor combinations could influence dairy-based foods. Trends in beverage consumption also point to newer flavor combinations the dairy industry has not explored.

Nutrition, important to both men and women, can focus on what to avoid or what to emphasize. The industry has led in responding to dietary fat concerns, but has not effectively communicated the nutrient content of dairy products. Special health benefits may also be perceived from use of beneficial cultures.

Some consumers can be reached through appeals to style and philosophy of production. This may be one factor driving the newly developing organic milk market.

(S2) ABSTRACT NOT AVAILABLE

(S3) SQUARE PEGS IN ROUND HOLES

Scottie Mayfield, Mayfield Dairy Farms Inc., P.O. Box 310, Athens, TN 37371-0310

To address the rationale for choosing a new beverage container for milk, three areas need to be explored: availability, perception, and their combination.

There is a phrase used by some folks called "share of stomach." The term is used to refer to the share a product or product category might have of all things people eat or drink. As we look at the opportunity for single-service beverages, one area we should consider is how well we compete for the "share of availability" at different purchase occasions. In evaluating purchase occasions, we must be aware of the predisposition of people's participation in specific occasions. What beverage are they most likely to prefer? If the occasion is a baseball game, milk may not be at the top of the list. If the occasion is a baking contest, with samples of chocolate chip cookies, milk would be very close to the top of the preferred beverage list.

What purchase occasions exist where milk is in the top ten preferred beverages and it is not available? The answer to this question leads straight toward opportunity for sales. Before we can answer this question, we must first understand the effects of perception on purchase behavior.

(S4) HOW DO IDF, CODEX, AND TRADE AGREEMENTS IMPACT THE DAIRY FARMER?

Duane R. Spomer, Dairy Division, Agricultural Marketing Service, USDA, Stop 0230, Room 2750-S, P.O. Box 96456, Washington, D.C. 20090-6456

The signing of the General Agreements on Tariffs and Trade (GATT) and the establishment of bilateral and multilateral trade agreements has heighten interest in international trading of dairy products. The International Dairy Federation (IDF) and the Codex Alimentarius Commission each play vital roles in establishing international standards and guidelines. In order to compete, dairy products produced in the United States must meet these standards. Milk quality requirements impact our ability to compete in international trade. In order to become a reliable exporter of dairy products, current milk quality requirements must be revaluated. The U.S. dairy industry has a great potential for market expansion. In order to realize this potential the dairy farmer, processor, consumers, and government representatives must be actively involved in establishing international standards that best position our industry for the global market.

(S5) FEDERAL MILK MARKETING ORDER REFORM

Aggie Thompson, Dairy Division, Agricultural Marketing Service, USDA, Room 2968, P.O. Box 96456, Washington, D.C. 20090-6456

Section 143 of the 1996 Farm Bill, signed by President Clinton on April 4, 1996, requires consolidation and reform of federal milk marketing orders not later than April 4, 1999. The Bill requires that the Secretary limit the number of orders to not less than 10 nor more than 14 from the current 32 orders and the Secretary is directed to designate the State of California as a federal milk order if California dairy producers petition for and approve such an order. USDA is authorized to use informal rulemaking procedures to implement these reforms. In order to accomplish the requirements of the 1996 Farm Bill within the allotted time frame, a detailed plan of action was developed and implemented to utilize maximum public and industry input and expertise. To ensure completion of the reform, all interested parties are invited to actively participate in the exchange of ideas, suggestions, and comments with USDA to develop viable reforms to the federal milk marketing order program.

(S6) QUANTITATIVE MICROBIAL RISK ASSESSMENT

Robert Buchanan, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038; Harry Marks, USDA-FSIS, 300 12th St., S.W., Washington, D.C. 20250; Suzanne van Gerwen, Food and Bioprocess Engineering Group, Wageningen Agricultural University, P.O. Box 8129, Wageningen, The Netherlands; Margaret E. Coleman, USDA-FSIS, Office of Public Health and Science, Rm. 3718 FCB, 1400 Independence Ave., S.W., Washington, D.C. 20250; Michael Cassin, Decision-analysis Risk Consultants, 85 Waterloo St., Kitchener, Ontario N2H 3V3, Canada; and Roberta Morales, USDA-FSIS, Office of Public Health and Science, North Carolina State University, Raleigh, NC 27695-8109

The development of quantitative microbial risk assessments (QMRA) will have a major impact on our conceptualization of food safety and its regulation. QMRA can provide an important linkage between HACCP and public health. The purpose of this symposium is to illustrate how QMRA relates to the National Academy of Science's paradigm for risk assessment. Specific components of QMRA to be discussed will include: the prevalence of contamination in raw ingredients; growth, survival and thermal death modeling; human response to the consumption of microbial pathogens; and simulation modeling — Monte Carlo techniques for risk characterization. Subsequent decision making and economic analyses for risk management will also be explored using specific examples. This symposium is designed to cover the basic concepts as well as to provide specific applications in the food safety arena, with the goal of providing information and guidance for those creating and/or interpreting food safety QMRA in the future.

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(S7) SAFETY OF REFRIGERATED FOODS—AN UPDATE

Thomas Schwarz, FDA, 200 C. St. S.W., Washington, D.C. 20250; Donald Zink, Nestle USA Inc., 800 N. Brand Blvd., Glendale, CA 91203; Martin Cole, Nabisco Biscuit Company, 200 DeForest Ave., P.O. Box 1944, East Hanover, NJ 07936-1944; Eric Johnson, Food Research Institute, University of Wisconsin-Madison,1925 Willow Drive, Madison, WI 53706; E. Jeffery Rhodehamel, Applications Development and Support, Cryovac North America, P.O. Box 464, Bldg. A, Rogers Bridge Rd., Duncan, SC 29334-0464; Robert Brackett, Center for Food Safety and Quality Enhancement Laboratory, University of Georgia, Georgia Experiment Station, Griffin, GA 30223

In response to consumer demand for ready-to-eat foods, industry has developed a generation of foods with extended refrigerated shelf-life. Foods may receive mild preservation treatments or may be packaged under modified atmospheres to retard spoilage. In this class of foods, pathogenic microorganisms such as *Clostridium botulinum, Bacillus cereus, Listeria monocytogenes, Escherichia coli* O157:H7, and *Salmonella* may survive, and in some cases grow in these foods prior to spoilage. Refrigeration is often the sole barrier to pathogen growth and toxin production. Safety is compromised when strict refrigeration is interrupted during the chain of manufacture, retail, or at the consumer level. Food safety risks associated with extended shelf-life refrigerated foods can be reduced by providing additional hurdles to inhibit microbial growth and toxin production. Approaches include use of packaging films containing antimicrobials, novel alternatives to heat pasteurization such as high-pressure pasteurization and electrocution, antimicrobial peptides (bacteriocins), natural antimicrobials, as well as traditional chemical preservatives. By using predictive models, ranges and combinations of formulation parameters can be established. This symposium will discuss the issues and present strategies to assure the safety of minimally-processed refrigerated foods.

(S8) FRESH-CUT FRUITS—PITFALLS AND CHALLENGES FOR THE FUTURE

Edith Garrett, IFPA, 1600 Duke St., Suite 400, Alexandria, VA 22314; Adel Kader, University of California-Davis, Dept. of Pomology, Davis, CA 95616-8631; Devon Zagory, Devon Zagory & Associates, 759 N. Campus Way, Davis, CA 95616; Bill Conway, USDA, Horticultural Crops Quality Lab, Bldg. 002, 10300 Baltimore Ave., Beltsville, MD 20705; and Jeffrey Farber, Health Canada, Health Protection Branch, Microbiology Research Division, Banting Bldg., Tunney's Pasture, Ottawa, Ontario K1A 0l2, Canada

Fresh-cut fruits are a growing group of products with great market potential. However, fresh-cut fruit processing, although similar to fresh-cut vegetable processing, will require processors to function at a higher technical level. Among other things, this will include having a thorough understanding of the physiology and ripening characteristics of various fruit products. From a microbiological standpoint, whole fruits have traditionally been considered as a safe group of products. However, with the further processing of these products by cutting, chopping or peeling and placement into a sealed environment, various microbial hazards can be introduced. These hazards can lead to the survival and/or growth of foodborne pathogens and possibly to serious foodborne illness, especially if the products are temperature-abused. In this symposium, the fresh-cut fruit area will be covered from gate to plate. There will be a general introduction to the market potential of fresh-cut fruits in both the foodservice and retail arenas, as well as a discussion of what companies have to do to get fresh-cut fruits off the ground. Following, there will be a discussion on which fruit commodities are suitable for the fresh-cut area, as well as some of the quality factors which affect the storage life of these products. Also included will be discussions on the effects of farm management practices on the quality of fresh-cut fruits. An overview of the microorganisms responsible for fruit spoilage will be presented, along with some novel chemicals being used to delay spoilage. Finally, general microbiological principles dealing with the fresh-cut fruit industry will be presented, along with some research dealing with the survival and/or growth of foodborne pathogens on these products. The major organisms of concern in fresh-cut fruit will be discussed, along with an overview of some of the current and future control mechanisms that can be used to reduce the microbiological hazards associated with these products.

(S9) MICROBIOLOGICAL SAMPLING ASPECTS OF THE "MEGA-REG"

Gary R. Acuff, Texas A & M University, Dept. of Animal Science, College Station, TX 77843-2471

The presence of various pathogenic bacteria on raw meats and poultry is primarily a result of their incidence in the live animal rather than as a result of inferior hygiene. The occurrence of these pathogens in raw meat and poultry cannot be entirely prevented by the application of strict sanitary hygienic principles. In addition, the distribution of pathogens in raw products is extremely variable, severely limiting the degree of confidence of a sampling plan to accurately indicate the absence or level of a particular pathogen in a lot.

Microbiological testing is most effectively used to verify that a specific critical control point is in control. End-product testing may also be conducted as part of a HACCP verification program; however, when pathogens are only occasionally present and at low levels, end-product testing is very inefficient at detecting the presence of the organism. Higher confidence is obtained by verifying that critical control points are maintaining control of the process as designed.

(S10) *E. COLI* TESTING AND PROCESS CONTROL

Michael C. Robach, Continental Grain Company, 340 Jesse Jewel Parkway, Suite 200, Gainesville, GA 30501

On January 27, 1997 large meat and poultry slaughter operations were required to begin testing for *E. coli* biotype I, non-specific to species, on chilled carcasses to verify that their processes were under control and preventing fecal contamination. The reason for this testing was the implementation of USDA FSIS's Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems final rule. This microbial testing program is designed

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as a verification tool of the HACCP plan. The effectiveness of microbiological criteria for foods of animal origin has been debated for many years. In 1985 the National Research Council stated that it is impractical to set microbiological criteria for raw meats and poultry. The level of *E. coli* on raw meat and poultry carcasses will be dependent on the condition of the live animals entering the plant, not the effectiveness of a slaughter HACCP program. The 1996 Codex Principles point that microbiological criteria "are not normally suitable for monitoring Critical Control Points..." The information is not timely and is not indicative of process control. Data will be presented that reflect industry experience for the first six months of 1997 and how plants can cope with violation of the USDA imposed "guidelines." The implementation of HACCP principles throughout the entire process should be used to produce the safest product possible.

(S11) *E. COLI* AND *SALMONELLA* LEVELS ON BEEF CARCASSES—SURVEY RESULTS COMPARED TO MEGA-REG REQUIREMENTS

John N. Sofos, Colorado State University, Dept. of Animal Sciences, Fort Collins, CO 80523-1711

In anticipation of establishment of microbiological performance criteria and standards by the new United States meat and poultry inspection regulations (Mega-Reg), we conducted a study designed to determine the microbiological status of carcasses in United States beef slaughtering operations. The project involved sampling and analysis of individual (brisket, flank, rump) excised (100 cm^2) samples (30 each) at each of three locations (pre-evisceration, final washing, 24-h carcass chilling) in each of four steer/heifer and three predominantly cow/bull slaughtering plants during the "wet" (November to January) and "dry" (May to June) season of 1996. The samples (n=3,780) were analyzed for aerobic plate counts, total coliform counts, *E. coli* counts, and for *Salmonella*. The *E. coli* and *Salmonella* results have been evaluated according to the criteria (m, M, c) published in the Mega-Reg, and probabilities of passing the *E. coli* performance criteria have been determined. Contamination was reduced by carcass washing and did not change greatly by chilling. Depending on season, at 24-h chilling, *E. coli* counts of <5 CFU/cm^2 were found, on the average, in 85.9% to 100%, 84.2% to 99.2% and 91.1% to 100% of brisket, flank and rump samples, respectively. Corresponding average *Salmonella* incidence was 0.8% to 4.2%, 0% to 2.2% and 0% to 2.5%. Variation in probabilities of passing the criteria was high among plants. Individual establishments will have to evaluate their plant designs and processing operations as they attempt to operate under HACCP and in order to meet any microbiological performance criteria or standards.

(S12) THE IMPORTANCE OF THE FEEDBACK LOOP IN HACCP: THE CONSUMER PERSPECTIVE

Caroline Smith DeWaal, Center for Science in the Public Interest, 1875 Connecticut Ave., Washington, D.C. 20009

Microbial testing is important to provide feedback that the HACCP system is actually working to produce a microbially safer product. Ongoing microbial verification testing is especially critical for raw meat and poultry products, where HACCP systems are designed to minimize, rather than eliminate, the food safety hazards. Mandatory *E. coli* testing by industry will provide important feedback to assure the effectiveness of HACCP plans in meat plants. However, due to the high prevalence of *Salmonella* on poultry products, this pathogen would have been a more accurate indicator of HACCP's success in poultry plants. USDA should have mandated that the poultry industry do verification testing for *Salmonella* rather than *E. coli*. The paper will discuss additional strengths and weaknesses of the testing system mandated by the USDA and the chosen performance standards.

(S13) INTERNATIONAL PERSPECTIVE OF THE "MEGA-REG" MICROBIOLOGICAL TESTING REQUIREMENTS

Peter Miller, Australian Embassy, Washington, D.C. 20036

The publication by FSIS of the "Mega-Reg" marked a crossing of the Rubicon for meat inspection regulation in the USA and for countries exporting meat to the USA. For the first time microbiological criteria were included explicitly as part of the evaluation process to determine acceptable regulatory standards of slaughter and processing hygiene for fresh meat and poultry. Countries exporting meat and poultry to the USA are required to put in place equivalent microbiological monitoring programs to enable continuing access for their product to the USA market. Regulatory authorities in exporting countries have to decide how they will institute programs which will be judged "equivalent" by FSIS, but will also be technically relevant to the conditions and risks present in the domestic processing industries they control. The performance criteria for both *E. coli* and *Salmonella* spp. specified in the Mega-Reg were statistically derived from a baseline survey data collected by FSIS from the U.S. domestic processing industry in circumscribed sampling windows during 1993 for beef and during 1994 for poultry respectively. The internal and external validity and value of the use of microbiological data to evaluate process control and pathogen reduction within and between meat and poultry processing systems have still to be rigorously tested. This talk will examine the approach used by the Australian Quarantine and Inspection Service (AQIS) to institute microbiological monitoring in the Australian export meat industry to meet the requirements of the "Mega-Reg" for meat (beef and sheepmeat) exported to the USA, the problems encountered and the means by which they have been addressed.

(S14) MICROBIOLOGICAL PERFORMANCE STANDARDS AND HACCP

Dane T. Bernard, National Food Processors Association, 1401 New York Ave. N.W., Washington, D.C. 20005

The FSIS Mega-Reg states that HACCP-based process control, combined with appropriate food safety performance standards, is the most effective means available for controlling and reducing harmful bacteria on raw meat and poultry products. For any performance standard for microorganisms in food there should be an underlying food safety objective. FSIS claims that performance criteria for *E. coli* and *Salmonella* are intended to address the food safety objective of reducing foodborne illness to the maximum extent possible. While this is a laudable goal, the microbiological testing program for raw prod-

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ucts will potentially shift the focus from ensuring control through HACCP to meeting a microbiological target level. As USDA continues to change from "command-and-control" requirements to HACCP-based regulations, FSIS will propose the further use of performance standards that specify the ends to be achieved but not the means to achieve those ends. In May of 1996 FSIS issued the first proposed performance standards for cooking, cooling and handling of certain cooked meat and poultry products. Such performance standards must be scientifically based and aimed at safety. For example, we believe sound science supports a 5-D reduction in *Salmonella* as adequate for achieving the desired level of food safety for cooked meat and poultry products. However any performance standard should also be flexible in allowing alternate processes based on a lower level of lethality as long as the food safety objectives are met (e.g., a similar probability of survival of the pathogen of concern). Under HACCP it is the responsibility of the company to determine not only the plant-specific procedures but the appropriate science-based lethality, cooling and handling requirements as well. Performance standards should have sufficient flexibility to allow this.

(S15) FOOD ALLERGIES AND INTOLERANCE

Robert K. Bush, University of Wisconsin, William S. Middleton Memorial Veteran Hospital; 2500 Overlook Terrace, Madison, WI 53705; Susan Hefle, University of Nebraska, Dept. of Food Science & Technology, Lincoln, NE 68583; Steve Gendel, FDA, Center for Food Safety & Technology, 6502 S. Archer St., Summit, IL 60501; Ann Munoz-Furlong, The Food Allergy Network, 10400 Eaton Place, Suite 107, Fairfax, VA 22030; Lydia Midness, General Mills, Inc., P.O. Box 1113, 7BT, Minneapolis, MN 55440; and Janis Oliver, FDA/CFSAN, 200 C St., S.W., Washington, D.C. 20204

Food allergy can pose a serious health threat to certain "at risk" individuals. With the apparent increase recently in the number of reports concerning consumers who have experienced adverse reactions following exposure to an allergenic substance in foods, has come an increased awareness of the potential scope of the problem, and a reemphasis of the already recognized need to manage the risk concern in a systematic, logical, and comprehensive way. To this end, this symposium will serve to provide a vehicle where experts in the field will employ an integrated, systematic approach in their topic presentations, which are phases of the HACCP approach to food safety assurance. The ultimate goal, to assure that a sound and transparent science-based rationale is advanced that provides a clear understanding of (a) the clinical nature of the threat, (b) the population scope and associated risk/severity issue(s), (c) potential "new food protein" emerging risk concerens, (d) the consumer information need and how it might best be served, (e) industry strategies and intervention approaches that serve to address the concern and (f) the regulatory perspective on the concern and its management.

(S16) NO ABSTRACT AVAILABLE

(S17) HYGIENIC DESIGN ON A WORLD STAGE: ISSUES AND HARMONY

John Holah, Food Hygiene Dept., Campden & Chorleywood Food Research Association, Chipping Campden, Glos GL55, 6LD, UK

The intention of this paper is to set the scene on hygienic design, how this issue is addressed in a number of countries throughout the world and how international harmonization is being developed to ensure that barriers to both trade and food product safety are not permitted. Hygienic design will be explained in relation to its effect on food product, quality and safety. The organizations throughout the world who are providing guidance on good hygienic design e.g., 3-A, NSF, EHEDG, IDF will be briefly described. The mechanisms for incorporation of this guidance into standards, advisory or otherwise, on a national level in the U.S. (3-A, NSF), Europe (CEN), and Japan (JSA) will be outlined. Since early 1995 ISO committee TC/199/WG 2 has been working to create a harmonized international standard that will consider hygienic design at the highest level for all industries in which hygiene is an issue (e.g., food, cosmetics, biotechnology, and pharmaceuticals). In order to produce a draft standard, the committee has had to reach agreement on three key issues; equipment design concepts, risk assessment (hazards and risks associated with the equipment/product interaction) and equipment conformity validation by visual assessment and test methodology. The development of these issues will be disussed.

(S18) THE MEANING OF THE 3-A SYMBOL

Warren S. Clark, Jr., American Dairy Products Institute, 130 N. Franklin St., Chicago, IL 60606

This paper reviews the historical founding, transformation and current meaning of the 3-A sanitary standards program. The development of sanitary dairy processing equipment standards was first conceived in the 1920s, and the first uniform standards were developed for fittings used on milk pipelines. With the U.S. Public Health Service endorsement of the concept and the addition of its support to the development of uniform equipment standards in 1944, the program has grown and today over 60 Sanitary Standards and Accepted Practices have been adopted.

The 3-A Symbol Administrative Council, which came into existence in 1995, accepts applications from equipment manufacturers and distributors for authorization to display the registered 3-A Symbol trademark on equipment fabricated to meet the specifications of an individual 3-A Standard. The voluntary compliance program, administered by the 3-A Symbol Council, has a 40-year history of successes. The composition and operation of the Council and its authorization program are discussed.

(S19) REGULATORY AND INSPECTION BODIES INVOLVED

Rocklyn R. Bates and F. Tracy Schonrock, USDA/AMS/Dairy Division, Dairy Grading Branch Stop 0230, Room 2750-S, P.O. Box 96456, Washington, D.C. 20090-6456

The Dairy Grading Branch offers a variety of voluntary, user-fee funded services to the dairy industry. One of these services is our Equipment Sanitary Design Review. This service is available to processing equipment designers, fabricators, and users. We utilize and support the 3-A Sanitary Stan-

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dards and Accepted Practices when available. Equipment that is not covered by 3-A Sanitary Standards or Accepted Practices is evaluated according to the *USDA Guidelines for the Sanitary Design and Fabrication of Dairy Processing Equipment*. These USDA guidelines were developed to support the 3-A criteria and general principles for nonstandardized equipment. The USDA evaluations are conducted in association with our plant survey program. The service provides the purchasers of equipment a means by which they can assure themselves that the equipment will meet either 3-A or USDA requirements. Equipment evaluations are available throughout the design, fabrication and installation process. The evaluations can be conducted at USDA, at the designer's or fabricator's facilities or at the user's facilities. Users of the service are encouraged to initiate the evaluations as early in the process as possible in order to more easily correct nonconformances.

(S20) ABSTRACT NOT AVAILABLE

(S21) ABSTRACT NOT AVAILABLE

(S22) IS THE SYSTEM WORKING?

(S23) Vince Mills, Evergreen Packaging, Division of International Paper, 2400 - 6th St., S.W., Cedar Rapids, IA 52406; and Richard Smith, 3-A Sanitary Standards, 329 Huntington Lane, Elmhurst, IL 60126

The End Users of Dairy equipment have long appreciated the value of the 3-A Symbol applied to the equipment that we purchase. We know that if a piece of equipment meets the 3-A Standard for that equipment that it will most likely meet our own company standards for the sanitary design and fabrication of the equipment. It will be cleanable and inspectable which are the main criteria for sanitary design.

It makes our job easier in describing to the manufacturers, the sanitary requirements for our equipment. It also allows the manufacturers to standardize their design and offer standard pieces of equipment to us. The economics of this are obvious. One can imagine how expensive it would be if each User and each local sanitarian required a special design.

We can also buy standard dairy equipment with assurance that the equipment will satisfy the requirements of our local control authority as well as federal authorities like USDA and USPH. Without the 3-A program it might require purchasing a piece of equipment and then obtaining "approval" or to submit plans for each new piece of equipment to Washington for approval. We think using the 3-A Symbol is a much more efficient process, saving time and money for all of us.

(S24) THE SAFETY OF NOVEL FOOD BIOTECHNOLOGIES AND GENETICALLY MODIFIED ORGANISMS

Peter R. Day, Rutgers, The State University of New Jersey, New Brunswick, NJ 80903-0231; Pat Sanders, Monsanto Corp., 700 Chesterfield Parkway N., GG4J, Chesterfield, MO 63198; Steve Gendel, FDA, Center for Food Safety and Technology, 6502 S. Archer St., Summit, IL 60501; Christine Bruhn, University of California, Center for Consumer Research, Davis, CA 95616; Barbara Petersen, Novigen Sciences, Inc., 1730 Rhode Island Ave., N.W., Washington, D.C. 20036; H. Michael Wehr, National Milk Producers Federation, 1840 Wilson Blvd., Arlington, VA 22201; and Doug Powell, University of Guelph, Guelph, Ontario N1G 2W1 Canada

This symposium will address issues associated with the development of novel foods and genetic modification of foods. Basic principles of genetic modification techniques will be presented, followed by an industrial perspective on food safety issues. Critical factors relating to the assessment of safety of genetically modified foods will be considered. An objective evaluation of the issues from the consumer's perspective will be presented. One of the main arguments charged against novel foods has been their alleged effects on nutritional properties. This will be explored in depth. In the international arena, the *Codex Alimentarius* has considered the safety of bioengineered foods at length. These issues, and their impact on the maintenance of free trade in global markets, will be discussed. Finally, the effective communication of issues relating to the safety of novel foods, and ways to remove barriers to their acceptance, will be deliberated.

(S25) INTERNATIONAL TRENDS IN MICROBIOLOGICAL METHODS

Russell S. Flowers, Silliker Laboratories Corp., 900 Maple Road, Homewood, IL 60430; Paul Teufel, Federal Institute for Health Protection of Consumers and Veterinary Medicine, P.O. Box 330013, 14191 Berlin, Germany; John R. Lupien, Food Policy and Nutrition Division, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, 00100 Rome, Italy; Wallace H. Andrews, Center for Food Safety and Applied Nutrition, FDA, 200 C St., S.W., Washington, D.C. 20204; and Roy Betts, Campden & Chorleywood, Food Research Association, Chipping Campden, Glouchestershire, GL55 6DL United Kingdom

Recent trends toward globalization of the food supply will likely continue and will present ongoing challenges to food safety experts. Although HACCP (Hazard Analysis Critical Control Point) will increasingly be used to prevent microbiological hazards, microbiological examination of foods will continue to be a central component of trading foods internationally, especially as a verification of HACCP reliability. Methods for which the accuracy, reproducibility, and inter- and intra-laboratory variability have been established, will be required. During this symposium, a renowned panel of experts will address many of the factors that must be considered when determining international standards for laboratory accreditation, microbiological methods, and validation programs. The symposium will conclude with a roundtable discussion.

(S26) FOODBORNE *CYCLOSPOROSIS*: WIDESPREAD OUTBREAK CAUSED BY IMPORTED RASPBERRIES

Barbara Herwaldt, Division of Parasitic Diseases, Centers for Disease Control, Mailstop F22, 4770 Bufuord Highway N.E., Atlanta, GA 30341-3724

Cyclospora cayetanensis was recently demonstrated to be a coccidian parasite. Much about this parasite, which causes protracted episodes of gastroenteritis, is unknown (e.g., host range, viability under various conditions). An outbreak of cyclosporiasis occurred in the United States and Canada in the spring and summer of 1996. A total of 1,465 cases

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Two types of water supplies are needed on berry farms. One for drip irrigation and another that is potable. The potable water is necessary for drinking; spray irrigation; spraying with solutions of fungicides, insecticides and other products; hand washing; humidifying; cleaning sorting-and packaging-room tables and other berry-contact equipment; and other hygienic purposes. The potable portion is a relatively small volume of the total water compared to that needed for irrigation, and, therefore, manageable.

The farm-implemented HACCP system will need to be designed to include water as a critical control point. This will require critical limits for location, construction and maintenance of water supplies and sewage disposal facilities. Monitoring must be done on the farms by farm personnel. Verification will need to include periodic monitoring for fecal coliform bacteria in samples of the water and on-site observations of the source, storage and distribution.

(S32) POPULATION SUBGROUPS REQUIRING SPECIAL FOOD SAFETY ATTENTION

Morris E. Potter, Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Atlanta, GA 30333; Thomas Cebula, FDA CFSAN, 200 C St., S.W., Washington, D.C. 20204; Robert Buchanan, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038; Richard Belzer, Office of Information and Regulatory Affairs, MEOB, Rm. 1202, Washington, D.C. 20503; Don Zink, Nestle, USA, Inc., 800 N. Brand Blvd., Glendale, CA 91203; and Martha R. Roberts, Florida Dept. of Agriculture & Consumer Services, The Capitol, Tallahassee, FL 32399-0810

As we move forward with food safety assurance, hazard intervention systems and strategies like HACCP, risk analysis will play a critical role in industry and regulatory food safety decisions. In this context, it has become increasingly apparent that one key element of the food safety risk analysis, albeit risk assessment, aquation will be identifying consumers requiring special food safety consideration. Issues that, ostensibly, will need to be considered include (a) the scope and extent of the population subgroups involved, (b) the pathogen/host relationship and the severity of the hazards to which these population subgroups may be exposed, (c) the nature of the "emerging" pathogen and the genetic/evolutionary pressures that may impact on the same, (d) situations where a degree of risk may be viewed as acceptable, and (e) how best to communicate potential risks to the special consumer. This symposium will serve to give an overview of those principal elements and issues that constitute the "special consumer" risk concern, and provide an opportunity to discuss and advance strategies to deal with the same.

(S33) THE BENEFITS AND PITFALLS OF HACCP FOR THE SEAFOOD INDUSTRY

Donn R. Ward, North Carolina State University, Dept. of Food Science and Technology, North Carolina State University, Raleigh, NC 27695

The seafood industry is in the process of unprecedented change. The promulgation and eventual implementation of the seafood HACCP regulation mark a fundamental change in the Food and Drug Administration's oversight of the seafood processing industry. As a consequence, there are many who anticipate great benefits to the industry and the consumer. However, with implementation of the HACCP regulation several months away, we can only speculate as to the actual benefits, while most see HACCP as a positive change, those suggest that numerous problems should be resolved before the actual benefits are realized. These too must be gauged in light of industry's actual implementation of the regulation. This paper will attempt to summarize the potential positive and negative implementations of HACCP to the seafood processing industry.

(S34) EXPERIENCES IN IMPLEMENTATION OF HACCP IN A SEAFOOD PROCESSING PLANT

Michael Mondragon, Tyson Seafood Group, Pier 91, Bldg. 392, Box C 119, Seattle, WA 98119

During the last decade, the Tyson Seafood Company has had to deal with three different sets of HACCP-type requirements. The presentation is to discuss our experience on the implementation and transition of these programs. (1) Prelude to implementation of HACCP for the Tyson Seafood offshore facilities (1985 to 1991): we experienced the State of Arkansas Plan of Operations (AKPOP), Quality Assurance Programs, and the commonalities between NMFS/USDC HACCP and AK POP; (2) Differentiating between NMFS and FDA HACCP programs (1991 to 1993): we experienced the implementation of NMFS/USDC program and found the pros and cons of the program. The pros include (i) increase in awareness level for at sea personnel and (ii) putting into perspective sanitation requirements. The cons include (i) cost of certifying personnel, (ii) quality and economic fraud issues, (iii) some proprietary quality info, (iv) over burdened HACCP program — cumbersome, and (v) cost and space for personnel needed to maintain records; (3) Implementing FDA HACCP program (1994 to present): we have been separating the quality issue from safety, conducting hazard analysis, and managing multiple plans. The company has been establishing management commitment awareness at the offshore facilities level and conducting training programs.

(S35) ABSTRACT NOT AVAILABLE

(S36) EXPERIENCES IN IMPLEMENTATION OF HACCP IN THE SEAFOOD FOODSERVICE INDUSTRY

Ed. R. Reichel, DARDEN Restaurants, Inc., P.O. Box 593330, Orlando, FL 32859-3330

In the 1960s, Pillsbury developed the first HACCP system for NASA to ensure that all critical food safety checkpoints were identified, monitored, verified, and documented during the production, packaging, transport, and use of all foods. The purpose was to prevent foodborne illness while the astronauts were in space. This successful system is not only used in food production and manufacturing industries, but in the food-service industry as well. In the restaurant industry, HACCP helps ensure food safety and prevent foodborne illness. It also results in less food waste, labor hour savings, higher food quality, and overall cost constraints. At DARDEN Restaurants, Inc., hazards were identified by product type, food storage practices, production techniques, cooking methods, and cooling/reheating pro-

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(S44) RAPID HYGIENE MONITORING—A NEW LIGHT

Anne M. Davies, S. J. Powell, and D. L. Hurry, Celsis-Lumac, Cambridge Science Park, Milton Road, Cambridge CB4 4FX, United Kingdom

A range of currently available portable hygiene monitoring systems was compared for technical performance. The recent trend for the design of hygiene monitoring systems has been towards ease of use. The availability of these systems has raised a question over the reliability of the results they produce, compared to the original cuvette-based tests. The evaluation revealed that the parameters of most significance to the performance of these systems were reproducibility and sensitivity. Performance of a cuvette-based assay by five different operators showed the lowest overall %CV, at only 18%, compared with values as high as 69% from an "integrated swab" system. The sensitivity of the same cuvette-based system was < 1 fmol (<550 fgram) ATP per assay. The four other systems tested did not detect below 40 fmol ATP per assay with statistical significance. Additional performance features included stability of the light signal and the percentage of "out of the box" failures of swabs.

(S45) SCIENCE-BASED STRATEGIES FOR PROTECTING OUR GLOBAL FOOD SUPPLY

Michael P. Doyle, Center for Food Safety and Quality Enhancement, University of Georgia, Griffin Station, Griffin, GA 30223-1797; Kurt Deibel, Medallion Laboratories, General Mills, 9000 Plymouth Ave. North, Minneapolis, MN 55427; Yoshifumi Takeda, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162, Japan; Ernesto Salinas Gomez-Roal, Nestle Mexico, Mexico City, Mexico; and H. Russell Cross, Institute of Food Science and Engineering, Texas A & M University, 120 Rosenthal Center, College Station, TX 77843-2259

The demand to provide consumers with fresh, wholesome, and nutritious foods has led to the globalization of food production and distribution. As these practices continue, the need to provide safe foods will become an increasing global concern. The challenge of maintaining the safety of our worldwide food supply has become more complex, and must take into account new food processing and packaging technologies as well as new consumer demands and trends. The purpose of this symposium is to further the understanding of these food safety issues and to present and discuss various international perspectives on science-based approaches to food safety protection. Initially, presenters will focus on how emerging foodborne pathogens develop and on potential control strategies. During the second portion of this symposium, panelists from several countries will address the integration of science and food safety standards. A discussion period with the panelists will conclude the symposium.

(S46) ISSUES OF CONCERN TO THE JUICE INDUSTRY

Cameron R. Hackney, Virginia Tech, Food Science and Technology, Blacksburg, VA 24061-0418; Isabel Walls, National Food Processors Association, 1401 New York Ave., N.W., Washington, D.C. 20005; Susan S. Sumner, Virginia Tech, Food Science and Technology, Blacksburg, VA 24061-0418; Jan Narcisco, 426 Lanier Lane S.E., Winter Haven, FL 33884; and Richard Smith, Pepsico, Inc., 100 Stevens Ave., Valhalla, NY 10595

Fruit juice products, especially pasteurized products, have a good safety record. Traditionally, mycotoxins such as patulin have been the agents of most concern. However, acid-resistant bacteria and acid-resistant sporeforming bacteria, parasites, viruses, and molds in paperboard are moving to the forefront as important issues. Several outbreaks of *Escherichia coli* O157:H7 and *Cryptosporidium parvum*, have been associated with drinking unpasteurized apple juice. *Alicylobacillus* spp. are sporeforming microorganisms which have been shown to survive a typical pasteurization process then germinate and grow in products with a pH as low as 3.0. There have been a number of incidents of spoilage of juices by these organisms, both in the U.S. and Europe. Current and alternative technologies for eliminating microorganisms are being developed and implemented to reduce or eliminate some of the microbial food safety concerns in fruit juices. Extended shelf-life and aseptic packaged products have unique problems. Paperboard microbial standards may not be adequate for extended shelf-life products. Cartons from four packaging manufacturers, both blank (never filled) and juice filled, were studied. More than 40 species of 15 genera and six *Mycelia sterilia* organism were isolated from the paperboard portion of the carton material. A bottler's perspective on the concerns facing the juice industry will also be presented. Case studies will be presented to outline a number of uncommon, but costly, process failures and some of the misguided (but amusing) approaches employed to deal with the problem at the time. Hard-earned learning will be shared in the hope that it might benefit other technologists, operators, and engineers towards avoiding unnecessary pitfalls in the science and art of aseptic packaging of juice. The issues discussed in this symposium represent new challenges to the juice industry and potentially to all processors of acid and acidified foods.

(S47) OVERVIEW OF THE VIRAL FOODBORNE DISEASE ISSUE: NEW YORK STATE PERSPECTIVE

John J. Guzewich, New York State Dept. of Health, II University Place, Room 404, Albany, NY 12203-3399

In the period 1980 to 1995, the New York State Dept. of Health reported 1,903 foodborne disease outbreaks involving 41,075 cases of illness. There were 458 outbreaks of viral etiology involving 13,394 cases of illness of which 74.2% were nonspecific viral gastroenteritis, 17% Norwalk agent, 5% hepatitis A, 2.8% rotavirus, and 0.9% Snow Mountain virus. Shellfish were the specific ingredient most often implicated. Of the identified factors, the cost often reported contributing factors were: contaminated ingredients, 62%; consumption of raw food of animal origin, 61.3%; unapproved source, 61.3%; infected person, 29.7%; and hand contact with implicated food, 10%.

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(S53) HARNESSING THE POTENTIAL OF DNA-RFLP SUBTYPING METHODS FOR FOODBORNE PATHOGENIC BACTERIA

Bala Swaminathan, Centers for Disease Control and Prevention, Atlanta, GA 30333

Molecular subtyping methods based on genomic DNA restriction fragment length polymorphisms (RFLP) and random amplified polymorphic DNA analysis (RAPD) have become indispensable tools for unraveling the epidemiology of foodborne diseases and for tracing the sources of contamination of foods. However, much of the potential of these molecular subtyping methods remains unutilized due to the lack of standardization of methods and the consequent inability to compare DNA-RFLP patterns between laboratories. Also, there is no universally accepted nomenclature system for the different RFLP patterns for each foodborne pathogen. During the past three years, significant efforts have been made to standardize subtyping methods for *Listeria monocytogenes* and *Escherichia coli* O157:H7. The Foodborne and Diarrheal Diseases Branch at CDC has played a catalytic role in standardizing molecular subtyping of foodborne pathogenic bacteria and is setting up the first electronic database of DNA-RFLP patterns for *E. coli* O157:H7.

(S54) MOLECULAR TYPING SYSTEMS FOR *CAMPYLOBACTER*

Irving Nachamkin, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-4283

Campylobacter jejuni is one of the most common foodborne causes of bacterial gastroenteritis in humans with an estimated 2 million cases or more each year in the United States. In order to study the epidemiology and pathogenesis of *Campylobacter* infection, numerous typing systems have been developed that range in complexity and ability to differentiate strains. The most common method used for typing *Campylobacter* is serotyping and includes the O (Penner) and HL (Lior) systems. Few laboratories, however, have the ability to perform serotyping and the availability of typing sera is almost non-existent. Several molecular-typing systems have been developed for *Campylobacter* and offers the advantage of standardization, relatively low reagent cost, and is less labor intensive than current serotyping methods. We have developed a molecular-typing system, called flagellin gene typing, based on polymorphisms in the flagellin gene, flaA, of *C. jejuni* and *E. coli* and have identified over 100 types to date. This system has already been shown to correlate with serotyping methods in an analysis of epidemiologically-related strains, yet can distinguish among strains within a particular serotype. In an effort to standardize the typing system, a multicenter evaluation at the University of Pennsylvania, USDA and Minnesota Dept. of Health has shown good correlation of typing results. With further refinements, flagellin gene typing should become a useful epidemiologic tool for studying *Campylobacter*.

(S55) FATTY ACID ANALYSIS AND RANDOMLY POLYMORPHIC DNA FOR EPIDEMIOLOGICAL TYPING IN FOOD MICROBIOLOGY

Heidi Schraft, Dept. of Food Science, University of Guelph, Guelph, Ontario, NIG 2W1, Canada

Analysis of bacterial fatty acid composition has been used traditionally to identify microorganisms at the genus and species level. The development of an automated system for fatty analysis, the microbial identification system (MIS), has brought this technique to be applied in many fields of microbiology. Beyond identification, fatty acid profiles can be used for epidemiological typing of microorganisms. Thus, the MIS has been employed successfully as a typing tool for dairy *Bacillus* spp. Fatty acid profiles from over 500 *Bacillus cereus* isolated from milk processing lines were analyzed to determine sources of *B. cereus* contaminating and spoiling pasteurized milk. Results from these studies demonstrated the presence of plant- specific *B. cereus*. They also indicated that these plant-specific strains may originate from certain dairy farms. Fatty acid analysis has thus proven to be a suitable tool for epidemiological typing of microorganisms. However, in many cases it is preferred to rely on genetic information for microbial typing. Randomly amplified polymorphic DNA (RAPD) is a rapid and sensitive nucleic acid-based typing technique that uses the enteric bacterial genome as a template for generating a DNA profile. This technique has been reliably used for typing of *B. cereus*. However, for its application in extensive epidemiological investigations, more sophisticated systems for data capturing and analysis need to be developed.

(S56) AUTOMATED RIBOTYPING FOR CHARACTERIZATION AND IDENTIFICATION OF PATHOGENS AND FOOD SPOILAGE ORGANISMS

Scott J. Fritschel, Qualicon™, Route 141 & Henry Clay Road, Wilmington, DE 19880-0357

Until recently, food microbiologists have been frustrated by the lack of appropriate tools to facilitate identification and characterization of pathogenic and food spoilage organisms. The difficulty associated with using classical methods, based largely on phenotypic traits, to characterize organisms responsible for economic loss in food processing can be overcome by the use of molecular techniques. Moreover, insights into the evolutionary position and relatedness of organisms can be achieved by careful selection of targets that exhibit a slow rate of change in gene sequences.

The use of genetic fingerprints (RiboPrint® patterns) to characterize bacteria provides a practical means to establish the species of an unknown strain by direct genetic characterization at the rank of type.

The RiboPrinter™ Microbial Characterization System is an automated ribotyping system that generates a ribosomal DNA fingerprint from bacteria, allowing them to be genetically characterized. This system also provides the capability to store the genetic and related source data in a dy-

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dards and Accepted Practices when available. Equipment that is not covered by 3-A Sanitary Standards or Accepted Practices is evaluated according to the *USDA Guidelines for the Sanitary Design and Fabrication of Dairy Processing Equipment*. These USDA guidelines were developed to support the 3-A criteria and general principles for nonstandardized equipment. The USDA evaluations are conducted in association with our plant survey program. The service provides the purchasers of equipment a means by which they can assure themselves that the equipment will meet either 3-A or USDA requirements. Equipment evaluations are available throughout the design, fabrication and installation process. The evaluations can be conducted at USDA, at the designer's or fabricator's facilities or at the user's facilities. Users of the service are encouraged to initiate the evaluations as early in the process as possible in order to more easily correct nonconformances.

(S20) ABSTRACT NOT AVAILABLE

(S21) ABSTRACT NOT AVAILABLE

(S22) IS THE SYSTEM WORKING?

(S23) Vince Mills, Evergreen Packaging, Division of International Paper, 2400 - 6th St., S.W., Cedar Rapids, IA 52406; and Richard Smith, 3-A Sanitary Standards, 329 Huntington Lane, Elmhurst, IL 60126

The End Users of Dairy equipment have long appreciated the value of the 3-A Symbol applied to the equipment that we purchase. We know that if a piece of equipment meets the 3-A Standard for that equipment that it will most likely meet our own company standards for the sanitary design and fabrication of the equipment. It will be cleanable and inspectable which are the main criteria for sanitary design.

It makes our job easier in describing to the manufacturers, the sanitary requirements for our equipment. It also allows the manufacturers to standardize their design and offer standard pieces of equipment to us. The economics of this are obvious. One can imagine how expensive it would be if each User and each local sanitarian required a special design.

We can also buy standard dairy equipment with assurance that the equipment will satisfy the requirements of our local control authority as well as federal authorities like USDA and USPH. Without the 3-A program it might require purchasing a piece of equipment and then obtaining "approval" or to submit plans for each new piece of equipment to Washington for approval. We think using the 3-A Symbol is a much more efficient process, saving time and money for all of us.

(S24) THE SAFETY OF NOVEL FOOD BIOTECHNOLOGIES AND GENETICALLY MODIFIED ORGANISMS

Peter R. Day, Rutgers, The State University of New Jersey, New Brunswick, NJ 80903-0231; Pat Sanders, Monsanto Corp., 700 Chesterfield Parkway N., GG4J, Chesterfield, MO 63198; Steve Gendel, FDA, Center for Food Safety and Technology, 6502 S. Archer St., Summit, IL 60501; Christine Bruhn, University of California, Center for Consumer Research, Davis, CA 95616; Barbara Petersen, Novigen Sciences, Inc., 1730 Rhode Island Ave., N.W., Washington, D.C. 20036; H. Michael Wehr, National Milk Producers Federation, 1840 Wilson Blvd., Arlington, VA 22201; and Doug Powell, University of Guelph, Guelph, Ontario N1G 2W1 Canada

This symposium will address issues associated with the development of novel foods and genetic modification of foods. Basic principles of genetic modification techniques will be presented, followed by an industrial perspective on food safety issues. Critical factors relating to the assessment of safety of genetically modified foods will be considered. An objective evaluation of the issues from the consumer's perspective will be presented. One of the main arguments charged against novel foods has been their alleged effects on nutritional properties. This will be explored in depth. In the international arena, the *Codex Alimentarius* has considered the safety of bioengineered foods at length. These issues, and their impact on the maintenance of free trade in global markets, will be discussed. Finally, the effective communication of issues relating to the safety of novel foods, and ways to remove barriers to their acceptance, will be deliberated.

(S25) INTERNATIONAL TRENDS IN MICROBIOLOGICAL METHODS

Russell S. Flowers, Silliker Laboratories Corp., 900 Maple Road, Homewood, IL 60430; Paul Teufel, Federal Institute for Health Protection of Consumers and Veterinary Medicine, P.O. Box 330013, 14191 Berlin, Germany; John R. Lupien, Food Policy and Nutrition Division, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, 00100 Rome, Italy; Wallace H. Andrews, Center for Food Safety and Applied Nutrition, FDA, 200 C St., S.W., Washington, D.C. 20204; and Roy Betts, Campden & Chorleywood, Food Research Association, Chipping Campden, Glouchestershire, GL55 6DL United Kingdom

Recent trends toward globalization of the food supply will likely continue and will present ongoing challenges to food safety experts. Although HACCP (Hazard Analysis Critical Control Point) will increasingly be used to prevent microbiological hazards, microbiological examination of foods will continue to be a central component of trading foods internationally, especially as a verification of HACCP reliability. Methods for which the accuracy, reproducibility, and inter- and intra-laboratory variability have been established, will be required. During this symposium, a renowned panel of experts will address many of the factors that must be considered when determining international standards for laboratory accreditation, microbiological methods, and validation programs. The symposium will conclude with a roundtable discussion.

(S26) FOODBORNE *CYCLOSPOROSIS*: WIDESPREAD OUTBREAK CAUSED BY IMPORTED RASPBERRIES

Barbara Herwaldt, Division of Parasitic Diseases, Centers for Disease Control, Mailstop F22, 4770 Bufuord Highway N.E., Atlanta, GA 30341-3724

Cyclospora cayetanensis was recently demonstrated to be a coccidian parasite. Much about this parasite, which causes protracted episodes of gastroenteritis, is unknown (e.g., host range, viability under various conditions). An outbreak of cyclosporiasis occurred in the United States and Canada in the spring and summer of 1996. A total of 1,465 cases

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Two types of water supplies are needed on berry farms. One for drip irrigation and another that is potable. The potable water is necessary for drinking; spray irrigation; spraying with solutions of fungicides, insecticides and other products; hand washing; humidifying; cleaning sorting-and packaging-room tables and other berry-contact equipment; and other hygienic purposes. The potable portion is a relatively small volume of the total water compared to that needed for irrigation, and, therefore, manageable.

The farm-implemented HACCP system will need to be designed to include water as a critical control point. This will require critical limits for location, construction and maintenance of water supplies and sewage disposal facilities. Monitoring must be done on the farms by farm personnel. Verification will need to include periodic monitoring for fecal coliform bacteria in samples of the water and on-site observations of the source, storage and distribution.

(S32) POPULATION SUBGROUPS REQUIRING SPECIAL FOOD SAFETY ATTENTION

Morris E. Potter, Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Atlanta, GA 30333; Thomas Cebula, FDA CFSAN, 200 C St., S.W., Washington, D.C. 20204; Robert Buchanan, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038; Richard Belzer, Office of Information and Regulatory Affairs, MEOB, Rm. 1202, Washington, D.C. 20503; Don Zink, Nestle, USA, Inc., 800 N. Brand Blvd., Glendale, CA 91203; and Martha R. Roberts, Florida Dept. of Agriculture & Consumer Services, The Capitol, Tallahassee, FL 32399-0810

As we move forward with food safety assurance, hazard intervention systems and strategies like HACCP, risk analysis will play a critical role in industry and regulatory food safety decisions. In this context, it has become increasingly apparent that one key element of the food safety risk analysis, albeit risk assessment, aquation will be identifying consumers requiring special food safety consideration. Issues that, ostensibly, will need to be considered include (a) the scope and extent of the population subgroups involved, (b) the pathogen/host relationship and the severity of the hazards to which these population subgroups may be exposed, (c) the nature of the "emerging" pathogen and the genetic/evolutionary pressures that may impact on the same, (d) situations where a degree of risk may be viewed as acceptable, and (e) how best to communicate potential risks to the special consumer. This symposium will serve to give an overview of those principal elements and issues that constitute the "special consumer" risk concern, and provide an opportunity to discuss and advance strategies to deal with the same.

(S33) THE BENEFITS AND PITFALLS OF HACCP FOR THE SEAFOOD INDUSTRY

Donn R. Ward, North Carolina State University, Dept. of Food Science and Technology, North Carolina State University, Raleigh, NC 27695

The seafood industry is in the process of unprecedented change. The promulgation and eventual implementation of the seafood HACCP regulation mark a fundamental change in the Food and Drug Administration's oversight of the seafood processing industry. As a consequence, there are many who anticipate great benefits to the industry and the consumer. However, with implementation of the HACCP regulation several months away, we can only speculate as to the actual benefits, while most see HACCP as a positive change, those suggest that numerous problems should be resolved before the actual benefits are realized. These too must be gauged in light of industry's actual implementation of the regulation. This paper will attempt to summarize the potential positive and negative implementations of HACCP to the seafood processing industry.

(S34) EXPERIENCES IN IMPLEMENTATION OF HACCP IN A SEAFOOD PROCESSING PLANT

Michael Mondragon, Tyson Seafood Group, Pier 91, Bldg. 392, Box C 119, Seattle, WA 98119

During the last decade, the Tyson Seafood Company has had to deal with three different sets of HACCP-type requirements. The presentation is to discuss our experience on the implementation and transition of these programs. (1) Prelude to implementation of HACCP for the Tyson Seafood offshore facilities (1985 to 1991): we experienced the State of Arkansas Plan of Operations (AKPOP), Quality Assurance Programs, and the commonalities between NMFS/USDC HACCP and AK POP; (2) Differentiating between NMFS and FDA HACCP programs (1991 to 1993): we experienced the implementation of NMFS/USDC program and found the pros and cons of the program. The pros include (i) increase in awareness level for at sea personnel and (ii) putting into perspective sanitation requirements. The cons include (i) cost of certifying personnel, (ii) quality and economic fraud issues, (iii) some proprietary quality info, (iv) over burdened HACCP program — cumbersome, and (v) cost and space for personnel needed to maintain records; (3) Implementing FDA HACCP program (1994 to present): we have been separating the quality issue from safety, conducting hazard analysis, and managing multiple plans. The company has been establishing management commitment awareness at the offshore facilities level and conducting training programs.

(S35) ABSTRACT NOT AVAILABLE

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(S54) MOLECULAR TYPING SYSTEMS FOR *CAMPYLOBACTER*

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Campylobacter jejuni is one of the most common foodborne causes of bacterial gastroenteritis in humans with an estimated 2 million cases or more each year in the United States. In order to study the epidemiology and pathogenesis of *Campylobacter* infection, numerous typing systems have been developed that range in complexity and ability to differentiate strains. The most common method used for typing *Campylobacter* is serotyping and includes the O (Penner) and HL (Lior) systems. Few laboratories, however, have the ability to perform serotyping and the availability of typing sera is almost non-existent. Several molecular-typing systems have been developed for *Campylobacter* and offers the advantage of standardization, relatively low reagent cost, and is less labor intensive than current serotyping methods. We have developed a molecular-typing system, called flagellin gene typing, based on polymorphisms in the flagellin gene, flaA, of *C. jejuni* and *E. coli* and have identified over 100 types to date. This system has already been shown to correlate with serotyping methods in an analysis of epidemiologically-related strains, yet can distinguish among strains within a particular serotype. In an effort to standardize the typing system, a multicenter evaluation at the University of Pennsylvania, USDA and Minnesota Dept. of Health has shown good correlation of typing results. With further refinements, flagellin gene typing should become a useful epidemiologic tool for studying *Campylobacter*.

(S55) FATTY ACID ANALYSIS AND RANDOMLY POLYMORPHIC DNA FOR EPIDEMIOLOGICAL TYPING IN FOOD MICROBIOLOGY

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Analysis of bacterial fatty acid composition has been used traditionally to identify microorganisms at the genus and species level. The development of an automated system for fatty analysis, the microbial identification system (MIS), has brought this technique to be applied in many fields of microbiology. Beyond identification, fatty acid profiles can be used for epidemiological typing of microorganisms. Thus, the MIS has been employed successfully as a typing tool for dairy *Bacillus* spp. Fatty acid profiles from over 500 *Bacillus cereus* isolated from milk processing lines were analyzed to determine sources of *B. cereus* contaminating and spoiling pasteurized milk. Results from these studies demonstrated the presence of plant- specific *B. cereus*. They also indicated that these plant-specific strains may originate from certain dairy farms. Fatty acid analysis has thus proven to be a suitable tool for epidemiological typing of microorganisms. However, in many cases it is preferred to rely on genetic information for microbial typing. Randomly amplified polymorphic DNA (RAPD) is a rapid and sensitive nucleic acid-based typing technique that uses the enteric bacterial genome as a template for generating a DNA profile. This technique has been reliably used for typing of *B. cereus*. However, for its application in extensive epidemiological investigations, more sophisticated systems for data capturing and analysis need to be developed.

(S56) AUTOMATED RIBOTYPING FOR CHARACTERIZATION AND IDENTIFICATION OF PATHOGENS AND FOOD SPOILAGE ORGANISMS

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Until recently, food microbiologists have been frustrated by the lack of appropriate tools to facilitate identification and characterization of pathogenic and food spoilage organisms. The difficulty associated with using classical methods, based largely on phenotypic traits, to characterize organisms responsible for economic loss in food processing can be overcome by the use of molecular techniques. Moreover, insights into the evolutionary position and relatedness of organisms can be achieved by careful selection of targets that exhibit a slow rate of change in gene sequences.

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