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Poster Abstracts

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P001 COMPARATIVE STUDY OF *TOXOPLASMA GONDII* OOCYSTS ON RASPBERRIES AND BLUEBERRIES

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The consumption of *Toxoplasma gondii* oocysts on fresh produce may be one means of transmission for toxoplasmosis. Naturally infected cats can shed oocysts that contaminate water sources for agricultural irrigation, pesticide and fertilizer application. *T. gondii* oocysts are similar to those of *Cyclospora cayetennensis* and may be used as a model for *Cyclospora* for which no animal model exists. During the summers of 1996 and 1997 *Cyclospora* outbreaks occurred in the United States and Canada (1998 also) associated with Guatemalan raspberries. Raspberries and blueberries were inoculated individually with *T. gondii* oocysts (10-20,000). Berries inoculated with 20,000 oocysts were incubated at 4°C for 6 days, samples taken at 0, 2, 4, and 6 days post-inoculation (dpi). Diluted amounts of oocysts were used to analyze oocyst recovery and adherence to berries. Oocyst viability and recovery were analyzed using a mouse bioassay (58 mice total). Depending on the number of oocysts recovered, mice developed acute (> 500 oocysts, 5-10 dpi) or chronic infections that were determined by identifying *T. gondii* life stages in lung, liver, or brain tissue. Mice fed contaminated raspberries incubated at 4°C for 6 dpi developed acute infections, while those fed identically treated blueberries did not. Mice fed raspberries inoculated with > 10 oocysts became infected; while only mice fed blueberries inoculated with > 1,000 oocysts became infected. Electron microscopy was used to evaluate the role of berry surface topography in oocyst contamination. This study investigated differences in the contamination of raspberries and blueberries that may be important to preventing future outbreaks.

P002 DEVELOPMENT OF A STANDARD METHOD TO DETECT *GIARDIA* ON FRESH FRUIT AND VEGETABLES

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Foodborne outbreaks of Giardiasis have been documented in both developed and developing countries. Such outbreaks may occur following consumption of either local or imported surface-contaminated produce, particularly those which receive minimal treatment prior to consumption. In the United Kingdom, work has been performed on lettuce seeded with *Cryptosporidium parvum*. In this, various extraction parameters have been evaluated, which have led to the production of a workable detection method for this particular protozoan parasite from vegetables. However, there currently exists no standard method in the United Kingdom for detecting the transmissive stages [cysts] of *Giardia lamblia* in/on foods. We therefore intend to develop a standard method for eluting, concentrating and identifying cysts of *G. lamblia* from fresh fruit and vegetables.

Small numbers of cysts, seeded onto foodstuffs, are eluted by stomaching (vegetables) or rolling (fruit) in 1M glycine and concentrated by centrifugation at 4000 × g for 10 min. Further concentration and purification is performed by immunomagnetic separation (IMS). Following IMS, the magnetic bead is disassociated from the bead-cyst complex and the cysts air-dried onto microscope slides. Cysts are then visualized by fluorescence microscopy using genus-specific fluorescein isothiocyanate (FITC) labelled monoclonal antibodies and the nuclear fluorogen 4'6-diamid ino-2-phenyl indole (DAPI). In vegetable and fruit samples seeded with 100 cysts, recoveries of approximately 60% and 25% could be achieved respectively. Varying the pH of the eluant may further improve recovery.

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

P003 ISOLATION OF POTENTIAL MICROBIAL COMPETITORS OF FOODBORNE PATHOGENS FOR USE ON FRESH AND MINIMALLY-PROCESSED PRODUCE

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In many food environments, types of bacteria present are often of mixed species. Activities of one type may influence growth and activities of others that are present. The objective of this project was to evaluate the microbial ecology of fresh and minimally processed vegetables to determine if naturally occurring bacteria on produce function as competitive or antagonistic microbiota against potentially encountered pathogens. Naturally occurring bacteria were obtained from ready-to-eat salad vegetables on three occasions to allow for seasonal variation. Minimally processed vegetable samples were obtained at stages of processing that included raw through packaged product. Some portions were analyzed microbiologically within 24 h of obtainment, while other portions were stored refrigerated and analyzed after 72 h. Microbiological analysis was conducted for bacterial enumeration and in order to obtain isolates. Isolates were screened for antimicrobial activity using an agar spot method against *Staphylococcus aureus* ATCC#27664, *Escherichia coli* O157:H7 E009, *Listeria monocytogenes* LCDC, and *Salmonella montevideo*. Of the 1150 isolates screened for inhibitory

activity, 37 (3.22%) were found to have varying degrees of inhibitory activity against at least one test pathogen. Many isolates showed inhibitory activity against all four. The isolates with greatest inhibition were removed from finished lettuce pieces shreds. Of the 37 inhibitory isolates, 34 (91.9%) were gram negative. All isolates with inhibitory activity are able to multiply at both 4° and 10°C. The potential application of native microflora used as biopreservatives for ready-to-eat salad vegetables is suggested.

P004 CONSUMER HANDLING OF FRESH PRODUCE

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While fruits and vegetables are not common vehicles for foodborne disease, outbreaks associated with consumption of fresh produce have increased. This study examines consumer handling practices, which may increase the risk of foodborne disease. Consumer handling of fresh fruits and vegetables was assessed through a mail survey of two thousand randomly selected households in the United States. The objective of this survey was to quantify consumer practices relating to the purchase, transport, storage and preparation of fresh produce, with emphasis on practices that affect safety. Following a repeat mailing procedure, a response rate of 33% was obtained.

Less than one-third of respondents separate produce from meat when transporting purchases home. Over 35% indicate that they do not wash melons before preparation. Over 20% of respondents report placing meat, poultry, or fish on a refrigerator shelf above other foods. Almost half of respondents indicate they do not always wash their hands before handling fresh produce. While almost all respondents report that they always wash their food preparation surfaces after contact with meat products, 24% wash with water only. Chi-square analysis indicates that females, lower income households, people 65 years and older, and non-college graduates practice safer food handling methods than males, higher income households, people under 65 years, and college graduates.

These findings suggest that consumer education materials should emphasize safe handling practices from purchase through consumption. Educational outreach should target those specific sub-populations that have higher frequencies of unsafe produce handling practices.

P005 WITHDRAWN

P006 EVALUATION OF POSTHARVEST SURVIVAL AND GROWTH OF *SALMONELLA, ESCHERICHIA COLI,* AND *LISTERIA* ON PEACHES

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The potential for survival of human pathogenic bacteria on non-wounded and wounded skin of peaches was compared to non-pathogenic strains. The studies were conducted with pathogenic strains (*E. coli* O157:H7 four strains, *Listeria monocytogenes* LJH549, *Salmonella agona*, *Salmonella gaminarum*, *Salmonella michigan*, *Salmonella montevideo*, and *Salmonella* Typhimurium) and two non-pathogenic (*E. coli* 506rif and *Salmonella* Typhimurium LT2rif) under controlled conditions. After 24-h post-inoculation, no *E. coli* 506 or *E. coli* O157:H7 (Detection Limit = $\log_{10}$ 1.67 CFU/cm^2) were recovered from non-wounded tissue, while a limited number of cells were recovered from the pathogenic ($\log_{10}$ 1.9-2.1 CFU/cm^2) and non-pathogenic *Salmonella* ($\log_{10}$ 1.8-1.9 CFU/cm^2) inoculated peach fruit. On non-wounded fruit, most bacteria die during an initial 2-h drying phase at 20°C and approx. 60% R.H. On wounded tissue, multiplication of all the pathogenic and non-pathogenic strains was observed. Low bulk fruit tissue pH (3.3 to 3.5) was not a barrier to growth over the 24-h period at 20-22°C. When *Salmonella* (pathogenic and LT2) strains and *Listeria monocytogenes* were inoculated on non-wounded peach plus 4.5% horse serum, only *Listeria monocytogenes* showed a marked benefit in survival (0.24 log reduction compared to the initial inoculum). In commercial simulations, survival of *Salmonella* Typhimurium LT2rif was increased by at least 1 log following defuzzing (trichome removal), at the temperatures and RH conditions tested. Contact with five common pre- and postharvest fungicides used on peaches, one commercial vegetable-oil fruit coating, and one mineral oil based fruit coating did not reduce survival of the pathogens.

P007 *SALMONELLA* INACTIVATION FROM THE SURFACE OF WHOLE AND CUT PRODUCE BY GASEOUS OZONE

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Raw or minimally processed fruits and vegetables are increasingly implicated as a source of microbial pathogens. Fruits and vegetables can become contaminated with pathogenic microorganisms while growing in fields or during harvesting, processing and distribution. Surface pathogens may penetrate to interior or edible portions through cutting or juicing, or may cross-contaminate food preparation areas and handlers. The use of gaseous ozone for pathogen destruction on produce surfaces has not been well documented and may be an important method to reduce pathogen presence on fresh fruits and vegetables. The objective of this study was to demonstrate the microbiocidal effectiveness of gaseous ozone for treating raw fruits and vegetables which have been inoculated with pathogenic microorganisms including *Salmonella* spp. A cocktail of nalidixic acid-resistant *Salmonella* strains was spot inoculated onto the surface of whole strawberries, whole cantaloupe and cut cantaloupe chunks (~10g each). Samples were placed in a closed and vented chamber, which was supplied with a constant flow of gaseous ozone (125 ppm) for an exposure time up to 300 minutes. After a 210-minute exposure, there was a 2.8 log reduction in the average *Salmonella* concentration recovered from the strawberries, and a 2.5 log reduction from the cut cantaloupe when compared to untreated samples. A gaseous ozone treatment can effectively reduce the population of *Salmonella* sp. from the surface of raw produce. This process for surface decontamination has a significant potential for global impact, especially if it can not only improve microbial safety, but also increase produce shelf life.

P008 IS *SALMONELLA ENTERICA* A GOOD COLONIZER OF PLANT SURFACES?

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Many recent outbreaks of salmonellosis in the United States have been associated with contaminated produce. Our understanding of how *Salmonella enterica*, an enteric pathogen, colonizes plant surfaces is greatly lacking. A human isolate of *Salmonella enterica* serovar *thompson*, that was linked to an outbreak from cilantro, was studied for its ability to colonize cilantro plants. Comparative fitness studies of *S. thompson* with *Pseudomonas chlororaphis* and *Pantoea agglomerans*, two common epiphytic bacteria isolated from cilantro, showed that *S. thompson* exhibited lower growth rates and lower population levels on cilantro plants than the bacterial epiphytes. The study of the effect of temperature on the fitness of *S. thompson* showed that the maximum population level of *S. thompson* on cilantro leaves was 10-fold higher when the inoculated plants were incubated at 30°C than at 24°C. In addition, *S. thompson* colonized the cilantro leaves at 30°C at approximately the same rate as the indigenous microflora in the first 18 h following inoculation, showing increased competitiveness during this initial growth phase. Population studies also revealed that *S. thompson* tolerates and recovers from dry periods on plant tissue to the same extent as epiphytic bacteria. The distribution of *S. thompson* on leaves was investigated using confocal microscopy and the green fluorescent protein (GFP) as a marker. High densities and large aggregates of GFP-labeled *S. thompson* were detected in the leaf vein areas, but very few in regions away from the veins. *S. thompson* did not invade the plant tissue internally, except when lesions were present. Moreover, *S. thompson* reached 100-fold higher populations on leaves with soft rot symptoms due to the presence of *Erwinia chrysanthemi* than on healthy leaves that harbored the nonpathogenic *E. chrysanthemi* mutant. Confocal microscopy showed that *S. thompson* [dsRed] cohabitates at high densities within softrot lesions with *E. chrysanthemi* [GFP], but not with another softrot pathogen, *Pseudomonas viridiflava* [GFP].

P009 REDUCING *SALMONELLA* ON THE SURFACE OF APPLES USING WASH PRACTICES COMMONLY USED BY CONSUMERS

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The current recommendations for consumers are to wash fruits and vegetables before consumption. Our objectives were to evaluate specific practices for consumers to wash smooth surface fruits or vegetables using apples as a model system. Golden delicious apples, purchased from a local produce market, were spot inoculated on the blossom end with 50 µl of a cocktail of six serotypes of *Salmonella enterica* (total inoculum of approximately 9 log CFU/apple). The inoculum was dried for 1 h prior to treatment. Under aseptic conditions, inoculated apples were given one of a variety of treatment combinations that included wetting with 5 ml of water, rubbing for 5 or 30 s, rinsing with 200, 400, or 600 ml of water, and drying with a sterile paper towel. Residual populations of *Salmonella* were determined by rubbing the treated apple for 30 s in 20 ml of Dey-Engley neutralizing broth and plating onto bismuth sulfite agar. Rubbing for 5 or 30 s followed by a 200-ml water rinse reduced the population by 3 logs, with an insignificant difference between the 5 and 30 s rub. No further decrease in population was observed by rinsing with 400 or 600 ml of water. Drying the remaining liquid with a sterile paper towel resulted in an additional decrease of approximately 0.4 log CFU. A 3.6 log reduction was achieved by a combination of wetting and rubbing for 30 s, rinsing with 200 ml of water and drying with a paper towel.

P010 ISOLATION AND CHARACTERIZATION OF A *LACTOBACILLUS PLANTARUM* BACTERIOPHAGE FROM CUCUMBER FERMENTATION

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The objective of the study was to isolate and characterize bacteriophage active against a strain of *Lactobacillus plantarum*, MOP3-M6, which has been evaluated as a starter culture for low salt (2-4%) cucumber fermentation and as a biocontrol organism to prevent the growth of microbial pathogens in minimally processed vegetable products. A virulent *L. plantarum* bacteriophage, JL-1, was isolated from a commercial cucumber fermentation. Electron microscopy revealed that this phage belongs to the Siphoviridae family, having an icosahedral head (59 nm, diameter), a non-contractile tail (182 nm length, 11 nm width), and a complex base plate. One-step growth kinetics of JL-1 showed that the latent period was 35 min, the rise period was 40 min, and the average burst size was 22 PFU/cell. It was found that 90% of phage particles in MRS medium were adsorbed to host cells 20 min after infection. Calcium supplementation did not affect initial phage adsorption, but promoted rapid phage propagation. The phage genome was estimated to be 31 kbp in length by restriction enzyme analysis. SDS-PAGE profiles indicated that JL-1 had two major (34 and 28 kDa) and four minor structural proteins. The D values of JL-1 were estimated to be 2.7 min at 70°C and 0.2 min at 80°C. In controlled vegetable fermentation, phage infection may result in abnormal fermentation, allowing spoilage or pathogenic organisms to grow. In a biocontrol system, phage attack may give a false safety assurance. Further work is needed to evaluate the influence of *L. plantarum* phage in vegetable products.

P011 EFFECT OF GLYCINE BETAINE ON SURVIVAL OF *LACTOCOCCUS LACTIS* IN FRESH, REFRIGERATED, SPICY CUCUMBERS

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Outbreaks associated with the consumption of raw vegetables and fruits have increased in the United States during the past decade. At the same time, consumers are

looking for minimally processed foods without preservatives. We are investigating the use of bacteriocin-producing lactic acid bacteria (LAB) as biocontrol agents to inhibit the growth of pathogenic bacteria in refrigerated vegetables. A nisin-producing *Lactococcus lactis* was inoculated into FRESCU (Fresh/non-acidified Refrigerated Spicy Cucumbers) to control the natural microflora and enhance their safety. Jars were stored at 5°C for 15 days and transferred to 15°C for 10 days; *L. lactis* counts went down 4 log cycles under these conditions. Previous treatment of the strain with the osmoprotectant glycine betaine (GB) before inoculation into FRESCU improved the survival of *L. lactis* at 5°C. Bacterial counts went down 2 log cycles and at 15°C had reached 1×10^8 CFU/mL, but after 3 days of storage other LAB predominated. The addition of GB at different concentrations (1-15 mM) into M17 medium with 1% glucose containing 2% and 4% NaCl enabled cells to achieve a faster maximum specific growth rate after the prolonged lag phase (7 h), compared to the control without GB (at 20° and 30°C). The use of GB may enhance the survival and growth of *L. lactis* in FRESCU, and this could allow the use of the strain as a biocontrol agent in brined, non-acidified refrigerated vegetables.

P012 REDUCTION OF *LISTERIA MONOCYTOGENES* ON GREEN PEPPERS (*CAPSICUM ANNUUM*) BY GASEOUS AND AQUEOUS CHLORINE DIOXIDE AND WATER WASHING, AND ITS GROWTH AT REFRIGERATED TEMPERATURE

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There are increasing concerns about minimally processed and refrigerated (MPR) fruits and vegetables because of outbreaks caused by pathogens, including *Listeria monocytogenes*. The development of effective sanitation techniques to reduce pathogens is needed. Our objectives were to: (a) compare the inactivation of *L. monocytogenes* Scott A on uninjured and injured green pepper surfaces by 0.3 or 3 mg/l gaseous and aqueous chlorine dioxide (ClO_2) treatments and water washing for 10 min at 20°C, and (b) investigate the growth of the *L. monocytogenes* untreated or treated with 0.6 mg/l ClO_2 gas for 30 min at 20°C on green peppers. The bacterial viability on pepper surfaces was visualized using confocal laser scanning microscopy (CLSM). More than 6 log CFU/5g *L. monocytogenes* on uninjured surfaces and approximately 3.5 log CFU/5g on injured surfaces were inactivated by both 3 mg/l and 0.6 mg/l ClO_2 gas treatments. The 3 mg/l aqueous ClO_2 treatment achieved 3.7 and 0.4 log reductions on uninjured and injured surfaces, respectively, whereas water washing alone caused 1.4 and 0.4 log reductions, respectively. The significant difference ($P < 0.05$) between log reductions on uninjured and injured surfaces and the results from CLSM analysis suggested that injured surfaces protected bacteria from sanitation treatments. Not only could *L. monocytogenes* grow on green pepper surfaces at 7°C, bacteria surviving the 0.6 mg/l ClO_2 gas treatment also could grow. ClO_2 gas treatment was more effective in reducing *L. monocytogenes* on both uninjured and injured green pepper surfaces than either aqueous ClO_2 treatment or water washing.

P013 MOLD AND YEAST FLORA IN FRESH FRUITS

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Fresh fruits are prone to fungal contamination in the field, during harvest, transport, marketing, and with the consumer. It is important to identify fungal contaminants in fresh fruits because some molds can grow and produce mycotoxins on these commodities while certain yeasts and molds can cause infections or allergies. In this study, 223 fresh fruit samples including various types of grapes, strawberries, blueberries, raspberries, blackberries, and various citrus fruits were surface-disinfected, incubated at room temperature for up to 14 days without supplemental media, and subsequently examined for mold and yeast growth. The level of contamination varied depending on the type of fruit. About 33% of the grape samples tested were contaminated and supported mold growth; the levels of contamination (% of contaminated berries/sample) ranged from 9 to 80%. All strawberry, raspberry and blackberry samples were contaminated at levels ranging from 33 to 100% whereas 94% of the blueberry samples supported mold growth at levels between 10 and 100% of the tested berries. The most common molds isolated from these commodities were *Botrytis*, *Alternaria*, and *Cladosporium* followed by yeasts, *Rhizopus* (in strawberries), *Trichoderma*, and *Fusarium*. Eighty-four percent of the citrus fruit samples showed fungal growth at levels ranging from 25 to 100% of tested fruits. The most common fungi in citrus fruits were *Alternaria*, *Penicillium*, *Cladosporium*, and *Fusarium*. Less common were *Geotrichum*, yeasts, and *Trichoderma*. In conclusion, several fungi, including some mycotoxin producers, were present and capable of growing on fresh fruits at room temperature.

P014 IMPROVED QUALITY AND FUMONISIN LEVELS IN MEXICAN CORN

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Mexico consumes 10.5 million tons of corn (Zea mays L.), per year, which represents more than half of the total volume of foods consumed. *Fusarium moniliforme* is a contaminant mold of corn that produces fumonisin B1 (FB1). International Agency of Investigation in Cancer declares that such toxins are potentially carcinogenic in humans (class 2B). FB1 have been associated with esophagus cancer in humans.

Fumonisins have been detected in corn procesed for human consumption (1 mg FB1/kg) and in corn for direct consumption (> 100 mg FB1/kg). Only Switzerland has legislation that limits FB1 levels to 1mg/kg.

The objective was to detect physical and chemical qualities in hybrid corns and Fusarium-resistant improved yellow corn as well as fumonisin levels, to establish a possible correlation.

Random samples of hybrid corn and improved corn were analyzed to determine content of FB1 by immunoassay (RIDASCREENFAST), their content of nutrients (AOAC, 1995), and their physical characteristics according to Mexican legislation (NMX-FF-034-1995-SCFI). Relationships between physical and chemical characteristics with levels of fumonisins were correlated using PROC GLM, SAS®.

All samples of corns contained fumonisins. Levels for FB1 in improved yellow corn were 1.5 to 6.4 mg/kg and in improved white corn were 0.3 to 6.4 mg/kg. However, in control corns concentrations were 3.2, 0.7, and 0.5 respectively. Significant correlations exist between physical characteristics and FB1 content.

Results obtained show that corn had levels < 1mg/kg FB1 and were correlated with corn quality. It is necesary to legislate levels of fumonisins permitted in Mexico.

P015 SPREAD OF *LISTERIA MONOCYTOGENES* DURING PREPARATION OF FRESHLY SQUEEZED ORANGE JUICE

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Contaminated oranges may be a source of pathogens in orange juice (OJ) or to the workplace from which cross contamination may occur. The potential for cross contamination of *Listeria monocytogenes* (L m) was studied during OJ extraction. Spread of L m from inoculated oranges to working surfaces, the hands of the food preparer, or the end product was determined. The presence of L m was also studied in OJ made from uninoculated oranges but using contaminated utensils. A handwashing procedure or handwash followed by hand disinfection was studied for effectiveness in reducing L m on hands. Fresh oranges were inoculated with a rifampicin-resistant L m strain. An electric or mechanical juice extractor was used to squeeze OJ from the inoculated oranges. L m counts were made from different contact surfaces during OJ extraction on tryptic soy agar plus 0.1 g/L rifampicin. After inoculation, the oranges presented a mean L m count of 4.4 log CFU/cm^2. This contamination was spread over all utensils used in OJ squeezing. Mean L m counts on the cutting board, knives, and the hands of OJ preparers were 1.9, 0.6 and 1.5 log CFU/cm^2, and the juice contained 2.7 CFU L m/ml. L m counts per the 2 hands of the OJ preparer were 4.2 log CFU after cutting the inoculated oranges and 5.1 log CFU after squeezing OJ. No Listeriae were detected after handwash alone or followed by dipping hands in ethanol. Contact with contaminated surfaces resulted in the presence of L m in OJ made from uninoculated oranges. In addition to adherence to GMP during juice extraction, a pathogen-reduction strategy might reduce the potential for OJ contamination with bacterial pathogens.

P016 EFFECTS OF PH AND TEMPERATURE ON INACTIVATION OF *ESCHERICHIA COLI* 0157:H7 IN A MODEL APPLE CIDER SYSTEM

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Foodborne outbreaks of *E. coli* O157:H7 from unpasteurized apple cider suggest its ability to survive in acidic environments. In response, the FDA now requires a 5-log pathogenic kill for pasteurized juice or a warning label for unpasteurized juices. Members of the cider industry are requesting alternatives to heat pasteurization, including minimal or non-thermal treatments. Combinations of pH and sub-pasteurization temperatures were studied using a central composite design to determine inactivation kinetics for a 5-log reduction of *E. coli* O157:H7. D-values between 47 and 57°C were determined using a flask method with tryptic soy broth (TSB) as the heating medium. Acidity of TSB was adjusted with malic acid to the pH range 3 to 5. The interaction of temperature and pH was found to be significant ($P < 0.05$). pH had a greater effect on D-value at lower temperatures. D48.5 was approximately 51.4 min at pH 3.3 and 293.1 min at pH 4.7, a D-value ratio of 1:6, while at higher temperatures, pH had less effect, with values D55.5 = 7.5 min at pH 3.3 and D55.5 = 19.4 at pH 4.7, a D-value ratio of 1:3. As expected, the highest D-values were obtained at the lowest temperature (47°C) and highest pH (5), to result in a D-value of 650 min. This approach is also being applied to a model cider system when preservative agents are added. Information may be used to develop guidelines to produce a 5-log reduction in acidic juices with the use of preservatives at sub-pasteurization temperatures.

P017 A SURVEY OF PRODUCTION PRACTICES AND MICROBIAL CONTAMINATION IN IOWA APPLE CIDER

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Cider production practices by Iowa apple growers were investigated by means of interviews with 5 cider producers and questionnaires mailed to an additional 16 producers. Most producers sort apples before washing them, store apples under refrigeration, wash and brush apples before pressing, and do not use drop apples. Only 45% of producers surveyed use a chemical sanitizer on the apples. Most producers (73%) pasteurize their cider; the others are considering pasteurization. Microbial contamination of apples and cider at various points in the cider-making process was evaluated through in-depth visits made to 5 producers, with samples taken several times over the cider season (September to January). Standard plate counts as well as counts of coliforms and yeasts and molds were performed on apples and on cider before and after pasteurization and/or addition of preservatives. Microbial counts on apples were generally high, ranging from 10,000 to 10 million organisms per apple. Microbial loads in cider were in the following ranges of organisms per ml: 15 to >9900 for standard plate count; <1 to >2100 for coliforms; <10 to 73,000 for yeasts and molds. Typical *E. coli* colonies were seldom seen and were never greater than 10 per ml. The various types of cider could be ordered as follows, according to level of microbial contamination: nonpasteurized with no preservatives > nonpasteurized with preservatives > pasteurized cider. Appropriate good manufacturing practices, sanitation procedures, and HACCP plans were discussed with 5 producers to improve microbiological quality and safety of their cider.

P018 ELIMINATION OF *ESCHERICHIA COLI* 0157:H7 IN APPLE CIDER BY ELECTRON BEAM IRRADIATION

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FDA pathogen-reduction standards for fruit juices can be met by pasteurization, which can alter flavor and appearance. The use of electron beam irradiation as an alternative to pasteurization to inactivate *E. coli* O157:H7 in cider was studied. Two strains of *E. coli* O157:H7, both

producing a green fluorescent protein that allows easy identification, were grown in tryptic soy broth (TSB pH 7.0) for 18 h, then transferred to TSB buffered at pH 4.6 for a 4-h incubation, before inoculation into cider. This moderate acid shock allowed good survival of the organisms in the cider (pH 4.0). Irradiation at several doses between 0.0 and 2.2 kGy was conducted at the Iowa State University Linear Accelerator on cider inoculated with single cultures at 10 million cells/ml, or with a mixture of the two cultures at 100 million cells/ml. Survivors were plated onto tryptic soy agar; fluorescent colonies were counted after 24-h incubation at 37°C. The D values obtained for the individual strains and the mixture ranged between 0.30 and 0.32 kGy. These results indicated that 5-log reduction of *E. coli* O157:H7 in apple cider should be obtained with irradiation at 2.0 kGy. Sensory evaluation with a trained panel as well as instrumental analyses were conducted on cider irradiated at 2 and 4 kGy, to identify changes induced by the irradiation. The irradiated cider was lighter in color than nonirradiated cider and had an off-flavor that panelists termed "cardboard-like." Further investigation of this off-flavor is in progress.

P019 INFLUENCE OF TEMPERATURE ON INACTIVATION OF *ESCHERICHIA COLI* 0157:H7 AND *SALMONELLA* IN APPLE CIDER AND ORANGE JUICE TREATED WITH OZONE

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Inactivation of *Escherichia coli* O157:H7 and *Salmonella* in apple cider and orange juice treated with ozone was evaluated. A five-strain mixture of *E. coli* O157:H7 or *Salmonella* spp. was inoculated (7 log CFU/ml) into apple cider and orange juice. Ozone (0.9 g ozone/h) was pumped into juices held at 4 and 20°C for up to 240 min and at 50°C for up to 75 min. Samples were withdrawn, neutralized with 1.0 N NaOH, diluted in 0.1% peptone water, and surface plated onto tryptic soy (TSA), sorbitol MacConkey (SMAC), hemorrhagic coli (HC), and modified eosin methylene blue (MEMB) agars (for *E. coli* O157:H7) or TSA, bismuth sulfite (BSA), and XLT4 agars (for *Salmonella*). Recovery of both pathogens was poorer on selective media than on TSA ($P < 0.05$); thus survival was based upon confirmed recovery on TSA. For ozone treatment at 4°C, a 4.8- and 4.5-log reduction of *E. coli* O157:H7 and *Salmonella*, respectively, occurred in apple cider after 180 min and a 5.4- and 4.2-log reduction, respectively, in orange juice after 240 min. When treated at 20°C for 240 min, *E. coli* O157:H7 populations decreased by 3.0 and 2.3 log CFU/ml in apple cider and orange juice, respectively, while this treatment reduced *Salmonella* populations by 3.3 and 4.2 log CFU/ml, respectively. At 50°C, *E. coli* O157:H7 populations were undetectable (<1.0 log CFU/ml) within 45 min in apple cider and 75 min in orange juice; *Salmonella* was reduced by 4.8 log CFU/ml within 30 min in apple cider and undetectable in orange juice within 15 min. Ozone treatment of apple cider and orange juice at 50°C may provide an alternative to pasteurization for acceptable (5-log) reduction of *E. coli* O157:H7 and *Salmonella*.

P020 CHEMICAL INACTIVATION OF *ESCHERICHIA COLI* 0157:H7 AND *SALMONELLA* SPP. IN APPLE CIDER AND ORANGE JUICE

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Survival of *Escherichia coli* O157:H7 and *Salmonella* in pasteurized apple cider and orange juice treated with chemical preservatives was evaluated. A nalidixic-acid resistant four- or five-strain mixture of *E. coli* O157:H7 or *Salmonella* spp., respectively, was inoculated (7 log CFU/ml) into apple cider and orange juice containing no preservatives (control), 250 ppm dimethyl dicarbonate (DMDC), DMDC + 46 ppm sodium bisulfite (DMDC/BI), DMDC + 450 ppm sodium benzoate (DMDC/BE), and 100, 200, or 300 ppm hydrogen peroxide (H_2O_2). Inoculated juices were stored at 4°C. Samples were withdrawn at 24-h intervals, neutralized with 1.0N NaOH, serially diluted in 0.1M phosphate buffer, and surface plated onto tryptic soy agar containing 50 ppm nalidixic acid. Survival of both pathogens was better in apple cider than orange juice ($P < 0.05$), and *E. coli* O157:H7 remained viable longer than *Salmonella* ($P < 0.05$). Reductions achieved by the chemical treatments followed the order: DMDC = DMDC/BE > DMDC/BI > 300 ppm H_2O_2 > 200 ppm H_2O_2 > 100 ppm H_2O_2 ($P < 0.05$). After five days of storage, reduction of pathogens in control juices was < 1 log CFU/ml. *E. coli* O157:H7 and *Salmonella* were reduced to undetectable levels (<1 log CFU/ml) after 24 and 48 h, respectively, in orange juice treated with DMDC or DMDC/BE. In apple cider treated with DMDC or DMDC/BE, *E. coli* O157:H7 and *Salmonella* were undetectable within 48 and 64 h, respectively. Alternatives to pasteurization may include the addition of chemical treatments to apple cider and orange juice to provide acceptable (5-log) inactivation of *E. coli* O157:H7 and *Salmonella* spp.

P021 SURVIVAL OF *SALMONELLA* IN CALCIUM-FORTIFIED ORANGE JUICE AT REFRIGERATION TEMPERATURE

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Salmonellosis associated with orange juice has raised interest in determining the survival characteristics of *Salmonella* in juice fortified with calcium. Five strains each of *Salmonella muenchen* (Inoculum (1), serotypes isolated from animals or humans (Inoculum (2), and serotypes from produce-associated outbreaks (Inoculum (3) were separately inoculated into calcium-fortified and non-fortified orange juice which was then held at 4°C for up to 32 days. Initial populations of ca. 5 log CFU/ml were reduced to < 1 log CFU/ml in juice fortified in the laboratory with calcium lactate (CaL) or a combination of CaL and tricalcium phosphate (TCP) within 16 and 30 days, respectively. Both reductions were significantly ($P < 0.05$) greater than reductions in the control juice. Survival of cells in all Inocula in juice fortified with TCP or calcium citrate (CC) was enhanced. In juice fortified with calcium citrate malate (CCM) in the laboratory, survival of *Salmonella* in Inoculum 3 was enhanced. Populations of *S. muenchen* in Inoculum 1 declined more rapidly in

commercially fortified juice containing CaL/TCP than in the non-fortified control. Inocula 1 and 2 declined less rapidly in juice commercially fortified with TCP, while Inocula 1 and 3 declined less rapidly in commercial orange juice containing CC than in respective controls. PCR fingerprints of isolates of *Salmonella* colonies from juice stored for 32 days revealed that *S. heidelberg* predominated among serotypes in Inoculum 2, whereas *S. baildon* and *S. poona* were more prevalent in Inoculum 3.

P022 SURVIVAL DIFFERENCES OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157:H7 STRAINS IN THREE APPLE VARIETIES AT 25° AND 4°C

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The differences in survival among five different enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) strains in three apple varieties were determined at two temperatures. Jonathan (J), Golden Delicious (GD), and Red Delicious (RD) apples with respective pH values of 3.60, 3.75 and 4.30, were wounded with an artist knife (7 mm deep) and inoculated with 4 µl of EHEC strains C7929 (apple cider isolate), 301C (chicken isolate), 204P (pork isolate), 933 (beef isolate) or 43890 (human isolate) to give initial counts of 6 log CFU/g. The inoculated apples were stored at a constant temperature of 25°C or 4°C. Bacterial counts were determined on 1 g of tissue at the site of the wound tissue every week for 28 days. By day 28 at 25°C in GD, the pork isolate count (5.5 log CFU/g) was significantly lower than the counts for the beef (6.5 log CFU/g) and apple isolate (6.8 log CFU/g). By day 28 at 25°C in J, the count for the apple isolates (6.5 log CFU/g) was significantly greater than the count for the chicken (5.6 log CFU/g) and human isolates (5.8 log CFU/g). At 4°C, counts of the human isolate inoculated in J, GD, and RD were significantly lower (2 log CFU/g) than counts for the other strains throughout the 28 days. The apple isolate survived significantly better at 4°C, yielding the highest viable counts in J (4.6 log CFU/g), GD (4.8 log CFU/g), and RD (5.0 log CFU/g). Our study shows that EHEC strains responded differentially to their ability to adapt and survive in these three apple varieties at 25°C or 4°C.

P023 EFFECT OF LOW-TEMPERATURE, HIGH-PRESSURE TREATMENT ON THE SURVIVAL OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* IN UNPASTEURIZED FRUIT JUICES

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The destructive effect of high pressure (615 MPa) combined with low temperature (15°C) on various strains of *Escherichia coli* O157:H7 and various serovars of *Salmonella* in grapefruit, orange, apple and carrot juices was investigated. Commercial freshly squeezed juices were used, and the treatment times were 1 and 2 min. After pressurization, the samples were immediately cooled, diluted, and plated for enumeration of injured and non-injured survivors. The 3-strain cocktail of *E. coli* O157:H7 (SEA13B88, ATCC 43895 & 932) was found to be most sensitive in grapefruit juice (8.34 log reduction) and least sensitive in apple juice (0.41 log reductions) when pressurized at 615 MPa for 2 min at 15°C. Correspondingly, no injured survivor was detected in grapefruit and carrot juices under similar treatment conditions. No *Salmonella* serovar was detected in a 2-min pressure treatment (615 MPa, 15°C) of grapefruit and orange fruit juices. Except for Enteritidis, all four serovars tested had viability losses between 3.92 and 5.07 log reductions when pressurized in apple juice at 615 MPa for 2 min at 15°C. No injured cells were recovered from grapefruit and orange juices, while the same treatment demonstrated reduction in numbers of *Salmonella* serovars *agona* and *muenchen* in apple juices and, to a lesser extent with Typhimurium, *agona* and *muenchen* in carrot juice. This study demonstrated that low-temperature, high-pressure treatment has the potential to inactivate *E. coli* O157:H7 strains and different *Salmonella* serovars in different fruit juices.

P024 VALIDATION OF THERMAL PASTEURIZATION TREATMENTS FOR COMMERCIAL APPLE CIDERS USING *ESCHERICHIA COLI* O157:H7

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The thermotolerance of a six-strain cocktail of *E. coli* O157:H7 and acid-adapted *E. coli* O157:H7 was compared in apple cider adjusted to varying pH and Brix. Heat treatments were based on pasteurization conditions used by Wisconsin cider makers. The strains employed were strains linked to outbreaks (ATCC 43894 and 43895, C7927 and USDA-FSIS-380-94), or strains engineered to contain the gene for Green Fluorescent Protein (pGFP ATCC43894 and pGFP43889). Survival of *Salmonella* (CDC0778, CDC F2833 and CDC HO662) and *Listeria monocytogenes* (H0222, F8027, and F8369) was also evaluated. Inoculated cider of pH 3.3 or 4.1 and 11 or 14 Brix was heated under conditions ranging from 60°C for 14 s to 71.7°C for 3 s. A 5-log reduction of *E. coli* O157:H7 and acid-adapted *E. coli* was obtained at 68.1°C for 14 seconds. Lower temperatures, or less time at 68.1°C, did not ensure a 5-log reduction in *E. coli* O157:H7. *Salmonella* spp. were more sensitive to heat pasteurization than were the *E. coli* strains. *L. monocytogenes* survived 68.1°C for 14 s, but survivors died in cider within 24 h at 4°C. Laboratory results were validated with a surrogate *E. coli* using a bench-top plate heat-exchange pasteurizer. Results were further validated using both fresh and previously frozen commercial ciders. Consumer acceptance of cider pasteurized for 68.1°C for 14 s (Wisconsin recommendations) and at 71.1°C for 6 s (New York recommendations) was not significantly different. Hence we conclude that 68.1°C for 14 s is a validated treatment for ensuring safety of apple cider.

P025 INACTIVATION OF *LISTERIA MONOCYTOGENES* IN CINNAMON-ADDED APPLE JUICE

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Listeria monocytogenes is ubiquitous and can contaminate many fresh food and beverages. We have observed the killing effect of cinnamon on *Escherichia colt* O157:H7

in apple juice and ground beef. In this work, the lethal effect of cinnamon on *L. monocytogenes* in apple juice was investigated.

Cinnamon (0, 0.1, 0.2, and 0.3 %, w/v) was added to commercial pasteurized apple juice, both normal (pH 3.7) and adjusted (pH 5.0). *Listeria monocytogenes* Scott A 49594 was inoculated at 4 log CFU/mL. Samples were stored at 5° and 20°C. Counts on tryptic soy agar (TSA), modified Oxford medium (MOX), and thin agar layer (TAL) were determined at 1 h and 1, 3, and 7 days. The thin agar layer method (MOX overlaid with TSA) was used for recovery of injured cells. The experiment was performed twice.

Listeria monocytogenes counts of untreated apple juice at pH 3.7 were nondetectable in 3 days at 5°C, and in 1 day at 20°C. In untreated samples adjusted to pH 5.0, initial counts (about 4 log CFU/mL) declined to 2 log CFU/mL at 5°C but increased slightly (to 5 log CFU/mL) at 20°C until day 7. The TAL method was as effective as TSA for recovery of injured *L. monocytogenes* cells. At both pH values, all doses of cinnamon completely inactivated *L. monocytogenes* at 1 h after inoculation.

In conclusion, *L. monocytogenes* in apple juice is highly sensitive to cinnamon regardless of pH value. This confirms the killing effect of cinnamon itself.

P026 TRANSMISSION AND INTERNALIZATION OF *ESCHERICHIA COLI* O157:H7 FROM CONTAMINATED COW MANURE INTO LETTUCE TISSUE AS MONITORED BY LASER SCANNING CONFOCAL MICROSCOPY

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In recent years, *Escherichia coli* O157:H7 has been isolated with increasing frequency from raw vegetables, most notably lettuce. There are several potential mechanisms by which lettuce may become contaminated with foodborne pathogens, including the transfer from contaminated soil into the edible portions of the plant. We determined the migration of *E. coli* O157:H7 into and within the lettuce plant, using marker bacteria. *E. coli* O157:H7 was transformed using pGFP, encoding the green fluorescent protein, therefore allowing direct observation of the lettuce tissue and visualization of the target organism under UV illumination. Cow manure was inoculated with an overnight culture of *E. coli* O157:H7 (pGFP). Planting mixtures (soil/manure) containing 10^8, 10^6, and 10^4 CFU/g were placed into vegetable flats and planted with seeds of iceberg lettuce. Eight seedlings per treatment were harvested on days 3, 6, and 9 post-planting, surface disinfected using 0.1% $HgCl_2$, macerated, and placed onto the surface of a TSA plate. Samples were also examined using fluorescence and laser scanning confocal microscopy (LSCM) to determine the spatial location of the pathogen. Throughout the sampling period, seedlings collected from the 10^8 and 10^6 CFU/g mixtures contained cells of *E. coli* O157:H7. Fluorescence microscopy revealed aggregates of cells on root tissue and at junction zones between plant cells of edible tissues. LSCM revealed cells internalized up to depths of 10mm below the tissue surface. These data indicate that transmission of *E. coli* O157:H7 does indeed occur between manure-contaminated soil and lettuce plants. Moreover, the organism becomes internalized and is thus afforded protection from sanitizing treatment.

P027 EVALUATION OF VARIOUS HOUSEHOLD SANITIZERS FOR ELIMINATING *ESCHERICHIA COLI* ON LETTUCE

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The effectiveness of various household sanitizers like apple cider vinegar, household bleach, hydrogen peroxide, lemon juice, and white vinegar were studied for their effectiveness in reducing *E. coli* and aerobic plate counts on lettuce. Experiments were done both at 4°C and at room temperature, under static and agitated conditions, and at different time intervals (0-10 min). White vinegar (35%, 1.86% acetic acid) was the most effective in reducing the *E. coli* levels (maximum of 5 log). White vinegar was equally effective both at 4°C and at room temperature, under static and agitated conditions, and with 5 and 10 minute exposures. White vinegar was followed by apple cider vinegar (5%, 0.26% acetic acid) and lemon juice (13%, 0.64% citric acid) both at room temperature and under agitated conditions for 10 min, bleach (4%, 180 ppm available chlorine) at 4°C under agitated condition for 10 min, distilled water at room temperature under agitated condition for 10 min, and hydrogen peroxide (0.4% of 3% solution, 0.012% hydrogen peroxide) at room temperature under agitated condition for 10 min. In reducing aerobic plate counts, white vinegar was the most effective at room temperature for 10 min under both static and agitated conditions, followed by bleach at 4°C under agitated condition for 10 min, lemon juice at room temperature under agitated condition for 10 min, and apple cider vinegar at room temperature under agitated condition for 10 min.

P028 EFFECTIVENESS OF WATER RINSE AS A MEANS FOR PATHOGEN RECOVERY IN LETTUCE

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Pathogen detection in fresh produce is challenging due to the sparse and non-uniform distribution of these organisms in foods. Routine microbiological testing often fails to detect the presence of particular pathogens because the target pathogens were not present in the samples taken for analysis. One way to alleviate this problem is to increase the sample size and thus increase the chance of finding the pathogens. There exists a need to develop effective sample treatment processes for the separation/concentration of pathogens from large volumes of food samples. Blending or stomaching is commonly used to separate pathogens from produce. These processes often cause damage to the food matrix and result in messy samples, which make concentration of pathogens for further testing difficult. Tap water washing has been shown to be effective in removing about 90% of the microbial load from prepared salad. The goal of this study was to determine the effectiveness of water rinse as a means for pathogen recovery from produce. Lettuce inoculated with 10^6 CFU/g of *E. coli* O157:H7 was stored at 4°C. At each time point, 25 g of the lettuce was subjected to 1 min of blending, stomaching or swirling in tap water. The recovery of

E. coli O157:H7 by water rinse was found to be similar to that obtained by blending (within 0.01 log) or stomaching (within 0.12 log). Similar results were obtained for lettuce inoculated with lower levels of the pathogen and stored at different temperature. These results suggested that rinse water is effective in separating pathogens from lettuce and that the sampling and detection of pathogen in lettuce can be simplified by focusing only on the rinse water, which can be easily concentrated to allow large numbers of sample to be analyzed.

P029 SIMULATION OF AN *ESCHERICHIA COLI* O157:H7 LETTUCE OUTBREAK IN A RESTAURANT SETTING: SURVIVAL OF *E. COLI* O157:H7 ON AND CONTAMINATION OF SHREDDED LETTUCE

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Contamination of produce by bacterial pathogens is becoming an increasingly recognized problem. In March 1999, seventy-two patrons of a Nebraska restaurant were infected with Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7, with shredded iceberg lettuce implicated as the food source. We simulated the restaurant's lettuce preparation procedure to characterize the outbreak strain in this setting and to identify critical control points for lettuce preparation. As shown previously for other EHEC strains, the Nebraska outbreak strain survived, but did not grow, in tap water or on shredded iceberg lettuce immersed in tap water for 14 days at 4°C. EHEC inoculation experiments were conducted simulating the restaurant's storage of shredded lettuce in water in the refrigerator. EHEC were detected on lettuce pieces located at a greater distance from the inoculum when immersed lettuce was stored at room temperature, as compared to 4°C, suggesting that temperature abuse may increase the chance for widespread bacterial contamination. Eighty-nine percent of the samples tested were contaminated when one piece of lettuce inoculated with 3×10^4 CFU was mixed in a bag with 350 g of fresh-cut non-immersed lettuce. Washing the mixed lettuce with water resulted in a slight decrease in contamination to 75%, while washing with 200 ppm calcium hypochlorite resulted in 70% contamination. Lettuce contamination was verified by PCR to be EHEC O157:H7 using primers specific for *eae*, the gene encoding the outer membrane intimin protein. These data indicate that rinsing fresh-cut lettuce with water alone does not significantly reduce bacterial levels on lettuce surfaces.

P030 CHANGES IN APPEARANCE AND NATURAL MICROFLORA ON ICEBERG LETTUCE TREATED IN WARM CHLORINATED WATER AND THEN STORED AT REFRIGERATION TEMPERATURE

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Mild heat treatment of iceberg lettuce has been shown to delay browning, thus extending shelf life. The objective of this study was to determine the effect of warm, chlorinated water treatment on the survival and subsequent growth of naturally occurring microorganisms on iceberg lettuce. After dipping cut lettuce leaves in water containing 20 mg/L free chlorine for 90 s at 50°C or 20°C (control), samples were stored at 5 or 15°C for up to 18 or 7 days, respectively. Populations of aerobic mesophiles, psychrotrophs, *Enterobacteriaceae*, lactic acid bacteria, and yeasts and molds were determined. Shelf life of lettuce stored at 5°C, as determined by subjective evaluation of appearance, was about 5 days longer than that of lettuce stored at 15°C. Treatment in warm water, with or without 20 mg/L chlorine, and in chlorinated water at 20°C significantly ($P < 0.05$) reduced the initial population of natural microflora. Populations of aerobic mesophiles and psychrotrophs increased, regardless of treatment, as storage time at 5°C and 15°C increased. Yeast populations increased slightly in lettuce stored at 5°C but were consistently ca. 3 logs lower than mesophilic aerobes. Populations of molds and lactic acid bacteria were less than 2 $\log_{10}$ CFU/g of lettuce throughout storage at 5 or 15°C. Results suggest that heat (50°C) treatment may have delayed browning and reduced initial populations of some groups of microorganisms naturally occurring on lettuce but enhanced growth during subsequent storage.

P031 COMPARISON OF COMMERCIAL CLEANERS FOR EFFECTIVENESS IN REMOVING *SALMONELLA* AND *ESCHERICHIA COLI* O157:H7 FROM THE SURFACE OF APPLES

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Unpasteurized apple juice or cider have been implicated in outbreaks of *Salmonella* and *Escherichia coli* O157:H7 infections. The objective of this study was to determine the efficacy of cleaners used in the apple industry in removing *Salmonella* and *E. coli* O157:H7 from the surface of apples. Unwaxed Red Delicious apples were surface inoculated with 8.11–8.58 $\log_{10}$ CFU of *Salmonella muenchen* and *E. coli* O157:H7. Five cleaners were applied to apples at concentrations and exposure times recommended by manufacturers. Populations of *Salmonella*, *E. coli* O157:H7, total aerobic mesophiles (TAM), and yeasts and molds on treated and untreated apples were determined. Compared to washing with water, treatment with cleaners removed or killed up to an additional 3.11, 2.48, and 0.73 $\log_{10}$ CFU of *Salmonella* and *E. coli* O157:H7, TAM, and yeasts/molds, respectively. However, treatment with some of the cleaners was not more effective than water in removing or killing one or both of the pathogens, TAM, or yeasts and molds. There were differences in the effectiveness of cleaners in removing pathogens, but pH (2.0–12.0), time of exposure (0.5–2 min), and concentration (1–5%) of cleaner were not correlated with reduction in population. The use of some types of cleaners commercially formulated for apples may contribute significantly in attaining the 5-$\log_{10}$ reductions of pathogens on fruit intended for the fresh produce market or unpasteurized juice production.

P032 DESTRUCTION OF *ESCHERICHIA COLI* 0157:H7 ON APPLES OF DIFFERENT VARIETIES TREATED WITH CITRIC ACID BEFORE DRYING

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Escherichia coli O157:H7 has been associated with outbreaks of illness involving fruit juices. Apples are at risk of contamination, but varieties differ in sweetness and hardness as well as other properties that may affect reduction of contamination during apple drying. The objective of this study was to determine if reduction of inoculated *E. coli* O157:H7 during drying of slices undipped or immersed in water or citric acid differed among apples of various varieties (Gala, Granny Smith, Fuji, and Red Delicious). Half-ring slices (0.6 cm thick) of peeled apples were immersed for 30 min in a three-strain composite inoculum of *E. coli* O157:H7 (initial inoculum level approximately 10^7 CFU/g). After 30 min standing under a biohazard hood, the apple slices received: (1) no treatment (control), or a 10-min immersion in (2) sterile water or (3) 1.7% citric acid solution. Slices were then dried in home-type dehydrators at 57.2°C for 4 h. Samples were analyzed every hour by plating on tryptic soy agar, sorbitol MacConkey agar (SMAC), SMAC with cefixime and tellurite supplement, and modified Levine's methylene blue agar. Immersion of inoculated apple slices in water or citric acid resulted in changes (mostly reductions) of ≤ 1.3 log CFU/g. Reductions in bacterial populations following 4 h drying were 1.0 to 4.0, 1.2 to 5.0, and 4.3 to 5.3, respectively, for inoculated slices not treated, water-treated, and citric acid-treated before drying. The results will be presented relative to differences among apple varieties and microbiological culture media.

P033 DESTRUCTION OF *ESCHERICHIA COLI* 0157:H7 DURING DRYING OF APPLE SLICES PRE-TREATED WITH ACIDIC SOLUTIONS AFTER INOCULATION

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Escherichia coli O157:H7 has been known to cause illness from unpasteurized fruit juices and dehydrated meat products. Thus, home-dried apples may be a potential medium for the survival and growth of *E. coli* O157:H7. The objective of this study was to determine whether acidifying pre-treatments after inoculation of apple slices altered the survival of *E. coli* O157:H7 during dehydration. Half-ring slices (0.6 cm thick) of peeled and cored Gala apples were immersed for 30 min in a 3-strain composite inoculum of *E. coli* O157:H7. Inoculated slices received: (1) no pre-drying treatment (control), or a 10-min immersion in solutions of (2) 2.9% ascorbic acid, (3) 1.7% citric acid, (4) 50% commercial lemon juice, or (5) 50% commercial lemon juice with preservatives. An uninoculated control was also evaluated. Slices were dehydrated for up to 6 h at 60.3°C. Samples were plated on tryptic soy agar (TSA) and sorbitol MacConkey agar (SMAC) for direct enumeration of surviving bacteria at various time intervals. Exposure to acidic treatments reduced bacterial counts by 0.6 to 1.2 log CFU/g on apple slices. After 6 h of dehydration, bacterial populations were reduced by 2.2 (SMAC) and 3.1 (TSA) log CFU/g in the control (no pre-drying treatment) samples. In contrast, after 6 h of dehydration, bacterial populations on the four acid pre-treated products were reduced by 6.7 to 7.1 (TSA) and 6.7 to 7.3 (SMAC) log CFU/g. The results suggest that common household acidulants may serve as effective pre-drying treatments to minimize the risk for *E. coli* O157:H7 infection from home-dried apple slices.

P034 THE LOCALIZATION AND PERSISTENCE OF BACTERIAL AND VIRAL CONTAMINANTS ON THE SURFACE OF INOCULATED CANTALOUPE AND THEIR RESPONSE TO DISINFECTION TREATMENTS

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The ability of bacterial and viral contaminants to attach and persist on the surfaces of cantaloupes and their responses to disinfection treatments have not been adequately explored. The pathogenic bacteria *Escherichia coli* O157:H7 and *Salmonella montevideo*, along with the bacteriophages MS2, PRD1, and Phi-X 174 and the enterovirus polio 1, were selected as challenge organisms. Disinfection treatments included chlorine solutions (50,100, 200, 300-ppm free chlorine) and a commercial vegetable rinse product, Carnebon 200®, containing aqueous stabilized chlorine dioxide (100 ppm) and tap water. Treatments were applied using immersion combined with physical agitation for two min at 45°C, followed by a rinse/neutralization step. Localization trials revealed challenge organisms exhibited a propensity for attachment to stem and blossom scars. All chlorine treatments produced significant reductions in both bacteria and viruses ($P < 0.05$). Two hundred ppm chlorine solutions produced > 3 $\log_{10}$ reduction in the inoculated bacteria population. The virus populations were all reduced >1.7 $\log_{10}$ in response to the chlorine treatments and were also reduced >1 $\log_{10}$ in response to tap water. The chlorine dioxide product produced >1 $\log_{10}$ reductions in all challenge organisms. Bacteria and viruses were recovered 10 days post-inoculation from melons during storage trials. The results of this study indicate that bacteria and viral agents readily attach to cantaloupe surfaces and survived for extended periods under normal storage conditions. While significant reductions were achieved by the treatments evaluated, none of the treatments eliminated the presence of pathogens. These findings stress the necessity of preventing contamination rather than relying on disinfection procedures.

P035 MINIMUM BACTERIOSTATIC AND BACTERICIDAL CONCENTRATIONS OF VARIOUS HOUSEHOLD SANITIZERS FOR *ESCHERICHIA COLI*

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The minimum bacteriostatic and bactericidal concentrations of various household sanitizers were determined for four different strains of *Escherichia coli* grown in tryptic soy broth. The sanitizers included apple cider vinegar, baking soda, fruit protector, household bleach, hydrogen

peroxide, lemon juice, lime juice and white vinegar. The strains included American Type Culture Collection (ATCC) 8739 (a strain used for the assay of antimicrobial preservatives), Center for Disease Control (CDC) 1932 (a nalidixic acid resistant strain), National Veterinary Services Laboratory (NVSL) A940-1-5 (an animal isolate of O157:H7) and NVSL H8302 (human isolate of O157:H7). The bacteriostatic concentrations for the strains tested ranged from 1.17 to 2.0% for household bleach; 0.33 to 0.35% for hydrogen peroxide; and 11.67 to 15% for white vinegar. The bactericidal concentrations ranged from 1.67 to 2.5% for household bleach; 0.38 to 0.4% for hydrogen peroxide; 16.67 to 20% for white vinegar; 2.67% for apple cider vinegar; and 6.67% for both lemon and lime juices. Saturated solutions of baking soda and fruit protector showed no effect on the *E. coli* strains tested in an agar well screening method. The various sanitizers tested differed significantly ($P < 0.05$) in their inhibitory action. The only significant differences ($P < 0.05$) among the *E. coli* strains tested were seen with ATCC 8739, which was more resistant to household bleach, and CDC 1932, which was more sensitive to white vinegar.

P036 THE BACTERICIDAL EFFECT OF CHLORINE DIOXIDE TREATMENT AGAINST *SALMONELLA* SPP., *ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* INOCULATED ON TOMATOES AND CARROTS

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Chlorine dioxide (ClO_2) has been demonstrated to have potent bactericidal activity in cucumber hydrocooling water and poultry chilling water. It is explored as an alternative for aqueous chlorine. This study was designed to determine the concentration and time-related killing effectiveness of aqueous ClO_2 in eliminating *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* inoculated on tomatoes and carrots. Fresh stock aqueous ClO_2 solution was prepared in a sealed brown bottle, following the instructions, and used to prepared working solutions at 10, 20, 40, 100, and 200 ppm ClO_2. Following dipping the inoculated test samples in these solutions for 1, 3, 6, and 10 min, the treated samples were blended for 2 min and the suspension subjected to bacterial enumeration on various selective media, applying spiral plating and pour plate methods. The treatment of tomatoes with 200 ppm ClO_2 caused 4.60 and 4.70 log reductions of *Salmonella* and *E. coli* O157:H7, respectively, on tomatoes. For *L. monocytogenes*, a 3-min treatment with 100 ppm ClO_2 was needed to achieve a 4.79 log reduction. The treatment of carrots with 200 ppm ClO_2 for 10 min caused a 4.98 log reduction of *Salmonella* on carrots. However, only a 1.94 log reduction of *E. coli* O157:H7 occurred on carrots with the same treatment. Treatment with 20 ppm ClO_2 for 10 min killed nearly all *L. monocytogenes* on carrots. The antilisterial components in carrot juice might have a synergistic effect with ClO_2 in killing *L. monocytogenes* on carrots.

P037 ENHANCEMENT OF THE MICROBIOLOGICAL QUALITY OF SELECTED READY-TO-EAT VEGETABLES DISINFECTED BY CHLORAMINE, CHLORINE, ETHANOL, AND OZONE

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Broccoli, celery, lettuce, mung bean sprouts, parsley, and scallions were cut into bite-sized pieces and treated with aqueous solutions of chloramine (80ppm), chlorine (200 and 2000 ppm), ethanol (10%), or ozone (2ppm). The difference (D) between the aerobic plate counts (APC) of control and treated test portions expressed in $\log_{10}$ colony forming units (CFU)/g (logD) was used to estimate the effectiveness of different sanitation regimens. The mean APC of control test portions ranged from 5.5 to 9 $\log_{10}$ CFU/g. The overall effectiveness of ozone, chloramine, chlorine 200 ppm, ethanol, and chlorine 2000 ppm were 0.2, 0.5, 1.2, 1.7, and 1.9 logD, respectively. Significant differences ($P < 0.05$) in aerobic plate counts (logD) were seen in 1, 4, 5, 5, and 5 out of 5 produce categories tested with ozone, chlorine 200 ppm, chlorine 2000 ppm, chloramine, and ethanol, respectively. Sonication, done only in experiments with chloramine and chlorine, was found to significantly ($P < 0.05$) reduce the APC only by 0.2 logD. In practical terms, ozone was the least effective, and ethanol the most effective as well as the most economical. Moreover, the results showed the limited effectiveness of the 4 sanitation agents studied against the compact and resilient nature of bacterial biofilms formed on surfaces and/or in crevices of fresh produce.

P038 ASSESSMENT OF THE ANTIBACTERIAL EFFICACY OF FRUIT AND VEGETABLE WASHES USING IN-VITRO AND IN-SITU METHODS

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A tiered research approach was used to evaluate the antibacterial efficacy of various produce washes: USP water, 200 ppm chlorine, and a commercial fruit and vegetable wash product (Professional Line Fit®; tested at 5g/l). Three test methods were used: (1) Aqueous Suspension Testing – a modification of AOAC suspension testing for Germicidal and Detergent Sanitizing Action of Disinfectants; (2) Carrier Testing – a quantified modification of the AOAC Use Dilution Test; (3) In-Situ Testing – recently developed methodology using produce inoculated with pathogenic microorganisms. The efficacy of the produce wash after a 5-min exposure was significantly greater than that of sterile USP water and similar to that achieved by treatment with 200 ppm chlorine. In suspension and surface carrier testing, the produce wash provided > 6 log reduction for all test pathogens. In on-produce testing, the produce wash was significantly better than washing with water alone at removing *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus*, and *Vibrio cholerae* on produce such as lettuce, tomatoes, broccoli, and strawberries.

P039 INACTIVATION OF PATHOGENIC BACTERIA ON LETTUCE BY HYDROGEN PEROXIDE AND MILD HEAT

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Foodborne outbreaks associated with produce have been increasing in recent years. Iceberg lettuce is the main item in vegetable salad. Several treatments have been developed to inactivate pathogenic bacteria on lettuce surfaces, but most have adverse effects on sensory quality. In this study, heat treatment and hydrogen peroxide (H_2O_2) was determined to effectively inactivate *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Enteritidis while maintaining the sensory quality of lettuce. Iceberg lettuce inoculated with one of the three bacterial species was treated with 2% H_2O_2 at 50°C for 90 s. Control lettuce leaves were treated with deionized water under the same conditions. Up to a 6-log reduction of *E. coli* O157:H7 and *S.* Enteriditis occurred with the treatment, whereas a 3-log reduction *L. monocytogenes* occurred. The sensory quality of treated lettuce, which was rinsed with running cold tap water for 3 min, then dried and stored at 4°C, was maintained for up to 15 days. Hydrogen peroxide residue was 0 to 0.5 ppm immediately after rinsing and not detectable (detectability was 0.2 ppm) after a 3-day storage. Studies with fresh-cut lettuce treated with 2% H_2O_2 at 50°C for 90 s and then rinsed with ice water (2° to 3°C) for 10 min revealed that product with acceptable appearance and texture after storage at 4°C for 15 days could be produced.

P040 COMPARISON OF PEPTONE WATER AND DEY-ENGLEY NEUTRALIZING BROTH IN RECOVERING BACTERIA FROM THE SURFACE OF FRESH PRODUCE TREATED WITH LACTIC ACID AND HYDROGEN PEROXIDE

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Reported outbreaks of foodborne illness associated with fresh produce have increased in the United States during the last decade. A new combination of GRAS chemicals was developed to inactivate pathogenic bacteria on the surface of fresh produce. A combination of lactic acid and hydrogen peroxide (H_2O_2) was an antibacterial treatment for *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Listeria monocytogenes* on surfaces of tomatoes, apples and lettuce. Immersing inoculated apples or tomatoes into 1.5% lactic acid and 1.5% H_2O_2 at 40°C for 15 min reduced pathogen populations by more than 4 log CFU/fruit. Similar reductions were observed for *E. coli* O157:H7 and *S.* Enteritidis on lettuce leaves treated with 1.5% lactic acid plus 2% H_2O_2 at 23°C for 5 min, and a 3-log CFU/leaf reduction was obtained for *L. monocytogenes*. Dey-Engley (DE) neutralizing broth and 0.1% peptone water were compared to determine their efficacy in recovering pathogens injured by the treatment solutions. Although pH values of DE broth and peptone water were substantially different after rinsing produce, there was no significant difference ($P > 0.05$) in recovering bacteria from produce rinsed with peptone water or DE neutralizing broth.

P041 EVALUATION OF VOLATILE CHEMICAL TREATMENTS FOR LETHALITY TO *SALMONELLA* ON SEEDS AND SPROUTS

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Acetic acid, allyl isothiocyanate (AIT), t-anethole, carvacrol, cinnamic aldehyde, eugenol, linalool, methyl jasmonate, and thymol were examined for their lethality against *Salmonella* on alfalfa seeds by exposing inoculated seeds to each compound (1000 mg/L [ppm] of air) for 1, 3, and 7 h at 60°C. Acetic acid, cinnamic aldehyde, and thymol caused significant reductions in *Salmonella* populations (> 3 $\log_{10}$ CFU/g) compared to the control (1.9 $\log_{10}$ CFU/g) after treatment for 7 h. Treatment of seeds with acetic acid (100 and 300 mg/L of air) and thymol or cinnamic aldehyde (600 mg/L of air) for 12 h at 50°C significantly reduced *Salmonella* populations (>1.7 $\log_{10}$ CFU/g) on seeds without affecting germination percentage. Treatment of seeds at 50°C with AIT (100 and 300 mg/L of air) and cinnamic aldehyde or thymol (200 mg/L of air) did not significantly reduce populations compared to the control. Seed germination percentage was largely unaffected by treatment with gaseous acetic acid, AIT, cinnamic aldehyde, or thymol for up to 12 h at 50°C. Acetic acid (200 and 500 mg/L of air) reduced an initial population of 7.5 $\log_{10}$ CFU/g of alfalfa sprouts by 2.3 and 5.7 $\log_{10}$ CFU/g, respectively, within 4 days at 10°C; however, both treatments caused deterioration in sensory quality. Treatment of sprouts with 1 or 2 mg of AIT/L of air for 11 days at 10°C adversely affected sensory quality but did not reduce *Salmonella* populations.

P042 INHIBITION OF *LISTERIA MONOCYTOGENES* ON TURKEY FRANKFURTERS BY CARBON DIOXIDE AND CHEMICAL ADDITIVES

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The ability of *Listeria monocytogenes* to grow in many different processing environments causes it to be very difficult to eliminate from a food production facility. Although *L. monocytogenes* can be destroyed if heated to a high enough temperature, environmental exposure of the food product after the heat treatment can allow the organism to contaminate the product. Due to the risk of post-process contamination, alternative treatments are needed to reduce *L. monocytogenes* contamination in ready-to-eat food products. Our objective was to determine the combined and individual effects of carbon dioxide with sodium lactate, sodium acetate, and sodium diacetate on the inhibition of *L. monocytogenes* on turkey frankfurters. Turkey frankfurters were inoculated with approximately 1.0×10^3 CFU/g of a 4 strain *Listeria monocytogenes* cocktail. The frankfurters were immersed for 2 min in the culture cocktail, drained for 2 minutes and packaged, or drained, sprayed with a chemical additive treatment, drained and packaged. The chemical additives used were sodium acetate (0.25% and 0.5%), sodium diacetate (0.25% and 0.5%), and sodium lactate (2.4% and

4.8%). The frankfurters were packaged with oxygen-impermeable bags in two different atmospheres (vacuum and 100% CO_2). After packaging, the frankfurters were stored at 4°C, with samples taken on days 0, 3, 7, 14, 21, 28, and 42. Results showed that all treatments reduced bacterial counts at 4°C, with the combination of chemical additives and 100% CO_2 atmosphere exhibiting an increased inhibitory effect. Increasing chemical additive concentration also increased effectiveness of the treatment.

P043 INHIBITION OF *LISTERIA MONOCYTOGENES* BY SODIUM DIACETATE AND SODIUM LACTATE ON WIENERS AND COOKED BRATWURST

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The inhibition of *Listeria monocytogenes* (LM) by sodium lactate (SL) and sodium diacetate (SD) was evaluated in cooked bratwurst and wieners supplied by commercial manufacturers. Products were surface inoculated with 10^5 CFU LM/package and vacuum-packaged in gas-impermeable pouches. Wieners were stored for 60 days at 4°C and bratwurst for 72 days at 3 and 7°C. LM was enumerated from triplicate samples for each treatment on Modified Oxford agar. Trial 1 evaluated cured/smoked (CS) bratwurst formulated with no SL or SD or with 3.4% SL+0.1% SD, and uncured/unsmoked (U/U) bratwurst formulated with 2% SL or 3.4% SL+0.1% SD. Trial 2 evaluated the inhibition of LM on eight C/S wiener formulations manufactured with 1.32 to 3.5% SL or 1% SL+0.1% SD, 1% SL+0.25% SD, or 2% SL+0.1% SD. For bratwurst, U/U products formulated with 3.4% SL+0.1% SD delayed LM growth through 4 and 12 weeks at 7 and 3°C, respectively, compared to 1 and 2 weeks for the 2% SL treatment. LM grew >1-log after 4 weeks of storage at 3 or 7°C on C/S bratwurst without SL or SD, but growth was inhibited for 12 weeks on C/S bratwurst formulated with 3.4% SL+0.1% SD. SL levels > 3% and all combinations of SL+SD prevented Listerial growth on wieners stored for 60 days at 4°C. These results verify that combinations of lactate and diacetate inhibit growth of *L. monocytogenes* on cooked meat products stored at <7°C, and that an additional margin of safety is observed for products that are cured and smoked.

P044 RADIATION RESISTANCE OF *LISTERIA MONOCYTOGENES* ISOLATED FROM FRANKFURTERS

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Listeria monocytogenes is a frequent post-process contaminant of ready-to-eat meat products, including frankfurters. Low dose ionizing radiation can eliminate *L. monocytogenes* from frankfurters. The radiation resistances of five *L. monocytogenes* strains, including three associated with recent recalls of frankfurters, were determined. Individual *L. monocytogenes* strains were surface-inoculated onto frankfurters to a density of 9 log(10) per centimeter squared surface area. The frankfurters were then vacuum packed and irradiated, using a self-contained Cs-137 irradiator, at a product temperature of 4°C. D-gamma, the ionizing radiation dose required to reduce the population of viable microorganisms by 90 percent, ranged from 0.60 to 0.64 kGy for the three *L. monocytogenes* strains isolated from frankfurters. The D-gamma of a human clinical isolate was 0.63 kGy. A Scott A petite strain (ATCC 49594), a natural variant of a human clinical isolate, had a D-gamma of 0.49 kGy. With the exception of the Scott A petite strain, the D-gamma's of the *L. monocytogenes* strains were equivalent, as determined by analysis of covariance. The D-gamma's of the *L. monocytogenes* strains isolated from frankfurters were consistent with those of other *L. monocytogenes* strains obtained in previous studies and therefore do not represent a barrier to ionizing radiation pasteurization of ready-to-eat meat products.

P045 CONTROL OF *LISTERIA MONOCYTOGENES* ON TURKEY FRANKFURTERS BY GRAS PRESERVATIVES

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GRAS chemicals applied to the surface of turkey frankfurters were evaluated for their ability to inhibit growth of *Listeria monocytogenes*. Frankfurters were treated by dipping for 1 min in a solution of one of four preservatives (sodium benzoate, sodium propionate, potassium sorbate, and sodium diacetate) at three different concentrations (15, 20 and 25% [wt/vol]). The calculated amount of preservative present in the entire frankfurter was ca. 0.03% when treated with 25% preservative solution, and sensory tests by an untrained panel confirmed the treated frankfurters were organoleptically acceptable. The frankfurters were then placed individually in sterile stomacher bags and surface inoculated with a 5-strain mixture of 10^4 CFU *L. monocytogenes* per frankfurter. Frankfurters were held at 4, 13 and 22°C and, immediately after inoculation and at 3, 7, 10 and 14 days of storage, were enumerated for *L. monocytogenes*. Results revealed that at all three concentrations of all four preservatives the initial populations of *Listeria* decreased by up to 2 log CFU. Further inactivation occurred on all treated frankfurters during storage at 4°C. Sodium diacetate and sodium benzoate at 15, 20 and 25% were highly inhibitory to *L. monocytogenes* at 4°C, whereas sodium propionate and potassium sorbate at equivalent concentrations were moderately inhibitory. At 13°C and after 14 days of storage, Listeriae populations were 3 log CFU less on frankfurters treated with 25% sodium benzoate or 25% sodium diacetate and 2 log CFU less when treated with 25% sodium propionate or 25% potassium sorbate, compared with untreated frankfurters. In all instances, the degree of growth inhibition was directly proportional to the concentration of preservative. Only frankfurters treated with 25% sodium diacetate or sodium benzoate were significantly inhibitory to *L. monocytogenes* when held at 22°C for 7 days or longer. Interestingly, the untreated frankfurters held at 22°C were spoiled within 7 days, with copious slime formation, whereas there was no evidence of slime on any treated frankfurters after 14 days of storage. The overall keeping quality of frankfurters as judged by appearance was markedly improved by the four surface treatments. Results indicate that 25% solutions of selected GRAS chemicals, especially sodium benzoate and sodium diacetate, applied to the surface of frankfurters greatly reduced the potential for substantial *Listeria* growth on frankfurters.

P046 EFFECT OF ANTIMICROBIALS IN THE FORMULATION AND POST-PACKAGING THERMAL PASTEURIZATION ON *LISTERIA MONOCYTOGENES* INOCULATED ON FRANKFURTERS AFTER PEELING

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Contamination of ready-to-eat foods, such as frankfurters, with *Listeria monocytogenes* is a major concern that needs to be addressed in order to enhance safety of these products. The objective of this study was to determine the effectiveness of combinations of antimicrobials included in the formulation of frankfurters against *L. monocytogenes* inoculated (10^3-10^4 CFU/cm^2) on their surface after peeling and before vacuum packaging. In addition, the antimicrobial effect of dipping the packaged products, prepared with or without antimicrobials, in hot (75-80°C) water (30-90 s) was evaluated. Samples were stored at 4°C for 90 days and periodically analyzed for microbial growth on tryptic soy agar plus 0.6% yeast extract (TSAYE) and PALCAM agar, and for pH. While sodium lactate (3%) permitted growth of *L. monocytogenes* at 50 days, its combination with sodium acetate (0.25%), sodium diacetate (0.25%) or glucono-D-lactone (0.25%) inhibited bacterial growth (average population changes on PALCAM -0.8 to -1.3 log CFU/cm^2) throughout storage. Dipping of packaged frankfurters in hot water enhanced the antimicrobial effect of additives on stored product by reducing populations by 0.4-0.9 log CFU/cm^2 during heating. However, dipping of frankfurters containing no additives in hot water did not inhibit pathogen growth for more than 10-20 days, unless one frankfurter was placed per bag and heat-treated for 90 s. These results indicate that inclusion of 3% sodium lactate with 0.25% sodium acetate, sodium diacetate, or GDL in cured meat formulations may control *L. monocytogenes* growth during refrigerated (4°C) storage. This protective effect may be enhanced by post-packaging thermal treatment.

P047 TREATMENTS TO CONTROL POST-PROCESSING CONTAMINATION BY *LISTERIA MONOCYTOGENES* ON SLICED PORK BOLOGNA STORED AT 4°C IN VACUUM PACKAGES

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Post-processing contamination of cured meats with *Listeria monocytogenes* has become a major food safety concern. This study evaluated aqueous solutions of organic acids (2.5 and 5% lactic or acetic acid) or salts (2.5 or 5% sodium acetate and sodium diacetate, 5 and 10% sodium lactate, 5% potassium sorbate and potassium benzoate) as antimicrobials against *L. monocytogenes* on sliced, vacuum-packaged bologna stored for 120 days (4°C). Antimicrobials were applied by immersion (1 min) of inoculated (10^2-10^3 CFU/cm^2; 10-strain composite) slices of bologna before vacuum packaging. Bacterial growth on PALCAM agar from inoculated slices without treatment exceeded 7 log CFU/cm^2 at 20 days. No increases ($P > 0.05$) in populations occurred on product treated with 2.5 or 5% acetic acid and 5% sodium diacetate from day 0 to 120, while product treated with 5% lactic acid or 5% potassium benzoate was stored for 70 to 90 days before an increase ($P < 0.05$) in bacterial populations occurred. All other treatments permitted bacterial growth at earlier days of storage. The effective treatments also inhibited total microbial (Tryptic Soy Agar with Yeast Extract) growth. When product was first immersed in antimicrobial solutions and then inoculated with *L. monocytogenes*, the inhibitory effect of 5% acetic and lactic acids was more pronounced than when the acids were applied in the reverse order, while the bacteriostatic effect of 5% potassium benzoate was gradually lost during storage. These treatments may be useful in the development of processes to control *L. monocytogenes* cross-contamination of the product during slicing and packaging.

P048 COMBINATIONS OF NISIN WITH ORGANIC ACIDS OR SALTS TO CONTROL POST-PROCESSING CONTAMINATION OF *LISTERIA MONOCYTOGENES* ON SLICED, VACUUM PACKAGED PORK BOLOGNA AT 4°C

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There is a need for control of post-processing contamination with *Listeria monocytogenes* in cured meat products. Combinations of nisin with organic acids or salts were evaluated as inhibitors of *L. monocytogenes* on bologna. Inoculated (10^2-10^3 CFU/cm^2; ten-strain mixture) bologna slices were immersed in aqueous solutions of lactic acid (1, 3, 5%), acetic acid (1, 3, 5%), sodium acetate (3, 5%), sodium diacetate (3, 5%), potassium benzoate (3%), or potassium sorbate (3%), each combined with 0.5% nisin (Nisaplinâ). Additional slices were immersed in 0.5% nisin, inoculated and then immersed in the above acid or salt solutions without nisin. Vacuum-packaged samples were plated on tryptic soy agar plus 0.6% yeast extract and PALCAM agar during storage (4°C; 120 days). Bacterial counts of inoculated bologna without antimicrobials exceeded 6 log CFU/cm^2 in 20 days. At day-0, irrespective of acid or salt presence, nisin reduced counts (1.0-1.5 log CFU/cm^2), but growth (>3 log CFU/cm^2) occurred at 20 to 35 days in nisin treatments without acids or salts. However, the initial contamination (≤ 3 log CFU/cm^2) was not restored in bologna treated with combinations of nisin with 3 or 5% acetic acid, 3% or 5% sodium diacetate, or 3% potassium benzoate before day 90. Inhibition by 3% potassium sorbate or 5% sodium acetate was greater when bologna was nisin-treated before (70 days) than after (35 to 50 days) inoculation. In contrast, the 0.5% nisin/5% lactic acid combination inhibited the pathogen for 90 days when applied after inoculation. Other treatments had weak antimicrobial activity (10-35 days).

P049 FATE OF ACID-ADAPTED AND NON-ADAPTED *LISTERIA MONOCYTOGENES* ON FRESH BEEF FOLLOWING ACID AND NON-ACID DECONTAMINATION TREATMENTS

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Recent outbreaks of illness caused by meat contaminated with *Listeria monocytogenes* have emphasized the need for its control. The objective of this study was to evaluate survival/growth of acid-adapted and nonadapted

L. monocytogenes inoculated onto fresh beef subsequently treated with acid or nonacid solutions. Beef slices from top rounds were inoculated with acid-adapted or non-adapted *L. monocytogenes* (4.6-5.0 log CFU/cm²) and dipped for 30 s in: (1) water, 55°C; (2) water, 75°C; (3) 2% lactic acid, 55°C; or, (4) 2% acetic acid, 55°C. The slices were vacuum-packaged and stored at 4 or 10°C and analyzed for bacterial populations after various times. Dipping in 55°C water, 75°C water, or acids produced immediate 0.3-0.6, 1.4-2.0 and 1.4-2.6 log CFU/cm² reductions, respectively. After storage at 10°C for 28 days, populations of *L. monocytogenes* on 55°C water-treated meat increased approximately 1.3-1.6 log CFU/cm². The pathogen on acid-treated meat survived but did not grow, whereas populations on 75°C water-treated meat increased rapidly to 3.5-4.5 log CFU/cm² by day 14. During storage at 4°C, there was no growth of the pathogen in 55 and 75°C water-treated samples for at least 21 days and longer for acid-treated samples. There were no differences in survival or growth between acid-adapted and nonadapted organisms. In conclusion, dipping of meat inoculated with *L. monocytogenes* (acid-adapted or not) into acid solutions reduced and then inhibited growth of the pathogen during storage at 4 or 10°C, while dipping in water, especially hot water, allowed growth of the pathogen during storage, despite initial reductions in contamination.

P050 LACTIC ACID SENSITIZATION OF *SALMONELLA* TYPHIMURIUM DT 104 AND *LISTERIA MONOCYTOGENES* IN NON-ACID (WATER) MEAT DECONTAMINATION FLUIDS AT 10°C

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Decontamination of meat with organic acid solutions may lead to development of acid-hardened bacterial pathogens, which may create problems through plant colonization and cross-contamination of meat. Control of acid-resistant pathogens may be enhanced by more intense use of nonacid meat decontamination technologies. To investigate this hypothesis, streptomycin-resistant *Salmonella* Typhimurium DT 104 and *Listeria monocytogenes* were inoculated (10^5 CFU/ml) in spray-washings from meat sprayed with cold (10°C) or hot (85°C) water. Changes in populations and acid tolerance response (ATR) were followed during storage of the washings at 10°C for 14 days. ATR was assessed at 2 and 8 days of storage by subsequent exposure of cells to tryptic soy broth plus 0.6% yeast extract (TSBYE, pH 3.7) or water washings (pH 3.5), both acidified with lactic acid. Both pathogens grew (0.4-2.3 logs) in the washings. Exposure to lactic acid had a fast and complete killing effect (<10 CFU/ml) on inoculated *S.* Typhimurium DT 104, irrespective of previous growth in the washings for 2 or 8 days. Inoculated *L. monocytogenes* was also sensitized to lactic acid, but to a lesser extent than *S.* Typhimurium DT 104. Higher numbers of *L. monocytogenes* survivors were detected at day 8 than day 2. Broth cultures of both pathogens were more resistant to lactic acid (TSBYE, pH 3.7) than cultures from washings. These results indicate that bacterial pathogens exposed to water washings may become sensitive to organic acid solutions. This approach may be useful in preventing acid hardening of pathogens and thus in minimizing potential risks in the meat industry.

P051 BIOFILM FORMATION BY ACID-ADAPTED AND NON-ADAPTED *LISTERIA MONOCYTOGENES* IN FRESH MEAT DECONTAMINATION WASHINGS AND ITS DESTRUCTION BY SANITIZERS

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The antimicrobial effects of sodium hypochlorite (SH) (200ppm), quaternary ammonium compound (QAC) (200ppm), and peroxyacetic acid (PAA) (150ppm) on previously acid-adapted or nonadapted *Listeria monocytogenes* grown in beef water decontamination washings were evaluated. The effects of the sanitizers were studied on planktonic cells, cells attached to stainless steel coupons, and de-attached cells in suspension. Adherent cells were obtained by incubating individual 2 × 5-cm coupons in 50 mL inoculated washings (10^5 CFU/mL) and stored at 15°C for up to 14 days. Adherent cells were exposed to sanitizers for 30, 120, and 300 s, and planktonic and de-attached cells for 15, 30, and 60 s. Survivors were determined on all-purpose and selective agar media (48 h, 30°C). The natural flora exceeded 8 log CFU/mL by day 2, while *L. monocytogenes* remained 1.5 to 2 log CFU/mL lower. The pathogen formed a biofilm of approximately 5 log CFU/cm² by day 2, which was consistent throughout storage, while the natural flora displayed higher attachment (6.0 to 6.5 log CFU/cm²). There were no differences in sanitizer resistance between acid-adapted and nonadapted cultures. The natural flora biofilms increased resistance to all sanitizers during storage, especially to QAC and SH, while *L. monocytogenes* was dramatically sensitized (<1.3 log CFU/cm²) to all sanitizers at day 14. Attached pathogenic cells showed higher resistance to SH at day 2 than day 7, while the opposite occurred in QAC. PAA was the most effective sanitizer on attached cells, but the least effective on cells in suspension. These results should be considered in application of sanitizing programs in meat plants.

P052 INACTIVATION OF *LISTERIA MONOCYTOGENES* IN PACKAGED HOT DOGS AND LUNCHEON MEATS BY HIGH PRESSURE PROCESSING (HPP)

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Several models have been developed to demonstrate inactivation in packaged hot dogs and luncheon meats of *Listeria monocytogenes* (Lm), grown optimally and sub-optimally, by high pressure processing (HPP). *L. monocytogenes* strains Scott A (SA) and V7 were grown either optimally (Tryptose Soy Broth; TSB, 18-24h @ 37°C, pH 7.0) or sub-optimally (TSB, 14d @ 7°C, pH 6.0), harvested, and inoculated into beef, pork and turkey franks (~10^7/g). Hot dogs were sealed in double polybags, and high-pressure processed using an ABB High Pressure Unit (Model QFP-6). Pressure, time, and temperature of processing were variable, within the ranges of 43 to 65 kpsi, 2 to 8 min., and 30 to 50°C, respectively. All survivors (injured and non-injured) were enumerated by direct plating to Tryptose Soy Agar + Yeast Extract

(TSA+YE), and fully viable cells (non-injured, only) by direct plating to Modified Oxford (MOX) Agar. Design of experiments and analysis of data were performed using "Design Expert, Version 6" (Stat-Ease, Inc.). According to two such models generated using this approach, for Lm SA and Lm V7 grown under optimal conditions, inoculated onto hot dogs, and processed under conditions of 65 kpsi for 6 min. at 40°C, inactivation of Lm SA was predicted to be log 5.49 (+/- log 0.63), and for Lm V7, log 5.56 (+/- log 0.54), respectively. When inoculated onto various luncheon meats and HPP treated under these conditions, the hot dog models reasonably predicted inactivation of both strains of Lm on smoked turkey and turkey salami, but over-predicted inactivation in bologna, and under-predicted inactivation in ham. Apparently, HPP does not produce a significant level of sub-lethal injury in Lm under these conditions. It has been demonstrated that >5-log inactivation of Lm by HPP can be modeled in a hot dog system, and validated in certain luncheon meats. In those meats where the hot dog model is not accurate, there may be some as yet non-elucidated factors (possibly related to product formulation) that may lead to synergy or antagonism toward inactivation of Lm in conjunction with HPP.

P053 SURVIVAL OF *SALMONELLA* SPP. AND *LISTERIA MONOCYTOGENES* DURING MANUFACTURE OF ITALIAN SALAMI

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Survival of *Salmonella* spp. and *Listeria monocytogenes* during the manufacture of typical Italian salami was evaluated. Italian salami batter was formulated with pork shoulder and divided into three batches (one for each pathogen inoculation and a control batch) to monitor the salami's chemical composition at each sampling period. The batter was fermented in a smokehouse at 30°C for 24, 40, or 72 h. Following each fermentation period, portions of each batch of salami were transferred to the drying chamber (13°C, 75 % RH) until target moisture-to-protein ratios (MPRs) of 1.9:1 and 1.4:1 were achieved. Italian salami links were sampled at each fermentation and fermentation-drying combination point for residual pathogen levels and chemical composition.

The study revealed that the intrinsic antimicrobial qualities of Italian salami, including nitrite (156 ppm), salt, (3.3%), acid development by starter culture inoculation (pH <5.0), and decreased water activity (0.88), resulted in reduction of the *Salmonella* spp. population by 3.4 log CFU/g and the *L. monocytogenes* population by <1.0 log CFU/g from initial levels of ca. 7.0 log CFU/g.

To assure the microbiological safety of traditionally processed Italian salami, raw materials should be tested to assure that the incoming levels of *Salmonella* spp. and *Listeria monocytogenes* are low and that the process effectively eliminates these pathogens from the finished product. As an alternative, processors should consider incorporating a thermal process to enhance the lethality of the overall process.

P054 *SALMONELLA* SPP. RISK ASSESSMENT FOR PRODUCTION AND COOKING OF NON-INTACT PORK PRODUCTS

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A *Salmonella* risk assessment was performed for production and cooking of non-intact pork products (blade tenderized, needle injected, needle injected and spice rubbed, and restructured). Magnitude and depth of pathogen contamination (translocation from the product surface) due to blade tenderization and needle injection were evaluated. Pork loins were surface inoculated with *Salmonella* (10^5 CFU/cm^2), blade tenderized, vacuum packaged and stored at 2°C. Chops (0.5 and 1.0 in. thick) were cooked to 140, 150, 160, 170 and 180°F. Log reductions were 2.79, 3.69, 4.31, 4.95, and 4.27, respectively. The blade tenderization process transfered 1 to 7% of surface contamination to the interior of the loin. Loins were surface inoculated (ca. 10^6 CFU/cm^2) and needle injected with a salt-phosphate solution and vacuum packaged. Chops were cooked, and respective reductions of 2.06, 3.86, 3.62, 4.28, and 5.13 log CFU/g were observed. Needle injection resulted in 4 to 8% translocation of surface contamination to the center of the loin. Loins were inoculated, needle injected and rubbed with a spice blend, stored and cooked. Respective log reductions were 1.38, 2.32,1.42, 3.52 and 3.92 log CFU/g from initial levels of 10^5 CFU/g. Loins were diced, inoculated (10^6 CFU/g), mixed, and stored overnight. The inoculated meat cubes were restructured with Fibrimex and stuffed into casings. The meat logs were stored and cooked, yielding reductions of 2.84, 3.04, 3.07, 4.54, 5.39 log CFU/g. Blade tenderization and needle injection of pork do not pose significant increased risks for *Salmonella*; however, restructuring could lead to higher internal levels of pathogens, so that higher internal cooking temperatures might be needed.

P055 BIOFILM DEVELOPMENT BY *LISTERIA MONOCYTOGENES* UNDER READY-TO-EAT MEAT PROCESSING CONDITIONS AND A CONTROL STRATEGY USING COLD PLASMA TECHNOLOGY

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This research evaluated the potential for biofilm formation by *Listeria monocytogenes*. A five-strain mixture was used to develop biofilms on stainless steel (304 or 316), buna-n and silicone rubber in a low nutrient medium for two or five days at 10°C. The number of cells recovered was highest on type 304 stainless steel and higher for all surfaces at five days. The effect of pork back fat and ready-to-eat meat residue (hot dogs) on biofilm formation and cleaning and sanitizing was evaluated. Addition of pork back fat or hot dog (0.5 to 5.0 %) reduced biofilm formation up to 90%. Immersion in an alkaline detergent was more effective than application by foam. Cleaning followed by a dual peracid sanitizer was generally effective in inactivating biofilm bacteria; however, biofilms on buna-n were the most resistant. Survival of biofilms during storage was higher at 4 than at 10°C. Increasing the percent of hot dog increased the survival rate, with growth occurring at 5%. Addition of

fatty acids (palmitic and oleic) had variable effects, while sodium lactate (2 and 4%) increased survival.

An anti-fouling coating was applied to stainless steel using a novel cold plasma technology. Cold plasma, a partially ionized gas (containing charged and neutral particles) is used to modify and/or deposit specific coatings on organic and inorganic surfaces. Biofilm formation with and without the presence of hot dog residues was reduced by up to 80% and the cleaning process was unaffected. Together with proper cleaning, this technology potentially provides an effective strategy to control biofilms.

P056 ENHANCED INHIBITION OF *LISTERIA MONOCYTOGENES* AND *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS IN BEEF BOLOGNA BY COMBINATIONS OF LACTATE AND DIACETATE

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The antimicrobial activities of salts of lactic and acetic acid are well documented, but there is limited information on their activity when added together to foods. We previously reported enhanced inhibition of *Listeria monocytogenes* (LM) and *Salmonella enterica* serovar Enteritidis (SE) in sterile comminuted beef at 5° and 10°C by combinations of sodium lactate (2.5%) and sodium diacetate (0.2%). The present study was undertaken to evaluate the inhibitory effect of these salts, alone and in combination, in a ready-to-eat meat. Single strains and six strain-cocktails of each of the pathogens were tested in beef bologna during storage at 5° and 10°C for up to 60 days. The growth rate of the *Listeria* cocktail (~3 log CFU/g) was higher than that of the single strain (Scott A) in the lactate/diacetate-free product. While each of the salts delayed growth of the Listeriae at 5°C, the effect of the salt combination was listericidal for the single strain and listeriostatic for the six-strain cocktail. Enhanced inhibition by the salt combination was also observed at 10°C. *Salmonella* numbers (~3 log CFU/g) declined in the untreated bologna and in each of the treatments to undetectable levels. However, the decline was more rapid in meat with the combination of the salts during storage for 60 days at both 5° and 10°C. Each of the salts further delayed growth of the background microflora during storage at 5°C, and their combination was most effective. The antimicrobial activity of the combination of lactate (2.5%) and diacetate (0.2%) enhances the safety of RTE meats by inhibiting Listeriae, and extends the shelf life of these products.

P057 SURVIVAL AND RECOVERY OF *LISTERIA MONOCYTOGENES* ON READY-TO-EAT MEATS INOCULATED USING DESICCATED AND NUTRITIONALLY DEPLETED VECTORS

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A five-strain *Listeria monocytogenes* culture, including serotype 4b, was continually stressed under four nutritionally depleted and dry conditions. The stressing process included the use of a sand vector maintained at two different temperatures (10°C and 22°C) with exposure to either humid air (40% RH and 88% RH) or complete desiccation. Irradiated, ready-to-eat (RTE) meats, including frankfurters, chopped sliced ham, sliced roast beef, and sliced bologna were contaminated with the inoculated sand vector.

Inoculation was carried out every 2 to 3 days over a period of one and one-half months. For each inoculation period, the RTE meats were vacuum-packed and stored at 4°C for 24 h. The populations of *L. monocytogenes* were recovered from the inoculated RTE meats by surface plating on non-selective and selective media. *L. monocytogenes* was not only found to be capable of surviving for over 40 days at 22°C and 0% RH, but was able to attach to and be recovered from the RTE meats after vacuum storage.

P058 POST-PROCESS PASTEURIZATION OF PACKAGED HAM, ROAST BEEF, AND TURKEY BREAST SURFACES TO REDUCE *LISTERIA MONOCYTOGENES*

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Recent outbreaks of *Listeria monocytogenes* from ready to eat (RTE) meat products have prompted investigations into post-process pasteurization of RTE meat products. Precooked hams (6 lbs), roast beef (10 lbs), and turkey breast (10 lbs) were surface inoculated with five strains (ca. 10^9 CFU/ml) of *Listeria monocytogenes*, vacuum packaged in CNP 320 bags, and surface pasteurized at 96.1°C for 0, 2, 3, 4 min in the "Stork-RMS-Protecon Post-Process Pasteurization Chamber." Three cores (71.25 cm^2) from top of roast beef and ham and two cores (47.50 cm^2) from bottom of roast beef and ham were taken, while five cores (121.25 cm^2) were taken from top and bottom of turkey breast. All cores for each product were combined, serially diluted and plated on Modified Oxford Agar medium. Mean inoculum levels on top of ham, roast beef, and turkey breast were 5.49, 5.98, and 6.78 and on the bottom were 5.39, 5.82, and 7.10 (log_{10} CFU/cm^2) respectively. Mean reductions in *Listeria monocytogenes* on top and bottom of surface pasteurized (2, 3, and 4 min) ham were 2.94, 3.73, 4.52 and 2.62, 2.75, 4.50, respectively. Mean reductions in *Listeria monocytogenes* on top and bottom of surface pasteurized roast beef were 2.67, 3.57, 4.48 and 2.51, 2.34, 2.53, respectively. On pasteurized turkey breast, *Listeria* reductions on top and bottom surfaces were 1.99, 2.40, 1.83 and 2.40, 1.72, 2.70, respectively. The steam-based (Stork-RMS-Protecon) post-process pasteurization of packaged, ready-to-eat meat and poultry products appears to be an effective and efficacious method of significantly reducing the risks associated with *L. monocytogenes* while providing a high quality product.

P059 POST-PROCESS PASTEURIZATION OF KIELBASA (FULL AND HALF) AND SALAMI TO REDUCE SURFACE *LISTERIA MONOCYTOGENES*

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Recent outbreaks of *Listeria* in ready-to-eat meat products have prompted scientists to investigate post-packaging pasteurization methods. Precooked kielbasa (commercially packaged in a bent link formation) and hard salami

were surface inoculated with five strains of *Listeria monocytogenes*. Half kielbasa portions were obtained by cutting the full kielbasa into two equal halves (removing the bent section) and using only one half for treatment. Kielbasa (full and half) were vacuum packaged and pasteurized in the Stork-RMS-Protecon Post-Process Pasteurization unit at 96.1°C for 0, 2 and 4 min; chilled in ice water for 10 min; and surface sampled from the middle and the end of links. Mean inoculum levels for full kielbasa were 6.37 and 6.63 (in the middle and end sections, respectively), and 6.51 and 7.00 $\log_{10}$ CFU/cm^2, respectively, for half kielbasa. Mean reductions in *Listeria* for full kielbasa pasteurized for 2 min were 4.59 (middle) and 2.26 (end) and for 4 min were 4.89 (middle) and 3.26 (end) log CFU/cm^2. For half kielbasa, 2 min reductions were 4.69 (middle) and 5.55 (end) and 4 min reductions were 3.56 (middle) and 6.13 (end) $\log_{10}$ CFU/cm^2, respectively. Salami was pasteurized at 85, 90, and 95°C for 2 or 4 min. Inoculum levels for the end and middle sections of the salami links were 4.36 and 4.49 $\log_{10}$ CFU/cm^2, respectively. Approximately 0.11 $\log_{10}$ CFU/cm^2 were recovered from middle and end surfaces of salami pasteurized at 95°C for 2 and 4 min. This establishes the efficacy and effectiveness of the Stork steam-based pasteurization treatment for reducing *Listeria monocytogenes* in these product types.

P060 INHIBITION OF *LISTERIA MONOCYTOGENES* BY SODIUM DIACETATE AND POTASSIUM LACTATE IN CURED, READY-TO-EAT PROCESSED MEAT PRODUCTS AT REFRIGERATED TEMPERATURES

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Listeria monocytogenes is an important foodborne pathogen. The control of this microorganism is of paramount concern to the producers of ready-to-eat foods, including processed meat manufacturers. This study evaluated the combined effects of sodium diacetate and potassium lactate on the growth of *L. monocytogenes* in cured, ready-to-eat processed meat products. Initial trials were conducted in four products (wieners, bologna, ham, and cotto salami) inoculated with 1-100 CFU/g of a five-strain cocktail of *L. monocytogenes*. The data from these trials demonstrated that growth could be prevented for 18 weeks in samples held at 4°C by the combined addition of 0.15% sodium diacetate and 1.5-2.5% potassium lactate. Based on these results, a complete response-surface-model (RSM) design incorporating 0-0.2% sodium diacetate, 0-5.6% potassium lactate, 0.8-3.6% sodium chloride, and 42.5-83.5% finished product moisture was run in a model cured meat system. The RSM data was used in the development of a mathematical model capable of predicting the combined effect of these four factors on the growth of *L. monocytogenes* in cured, ready-to-eat processed meat products. This model will be useful to food technologists in the formulation of cured, ready-to-eat processed meat products in which *L. monocytogenes*, if present, will not grow.

P061 APPLICATION OF THE BACTERIOCINOGENIC *LACTOBACILLUS SAKE* 2A TO PREVENT GROWTH OF *LISTERIA MONOCYTOGENES* IN BRAZILIAN SAUSAGE (LINGÜIÇA FRESCAL) PACKED WITH DIFFERENT ATMOSPHERES

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Lactobacillus sake 2a is a bacteriocin-producing organism isolated from "lingüiça frescal", a Brazilian sausage. The combined effects of modified atmosphere packaging and the addition of *L. sake* 2a on inhibition of the growth of *L. monocytogenes* in "lingüiça" was evaluated. Samples were inoculated with *L. monocytogenes* and/or *L. sake* 2a, packed in air (oxygen permeable film) or atmosphere of either 100% CO_2 or 50% CO_2/50% N_2, and stored at 6°C. Microbial counts were analyzed weekly. Sensory evaluation (triangle tests with 16 subjects) was performed after 5 and 11 days of storage. After one week of storage, the presence of *L. sake* 2a had no effect on the growth of *L. monocytogenes*; however, the use of a modified atmosphere reduced counts of *L. monocytogenes* by 1.4 $\log_{10}$ CFU/g ($P < 0.05$). By two weeks of storage, *L. monocytogenes* counts in samples inoculated with *L. sake* 2a and packaged in a modified atmosphere were 3.5 $\log_{10}$ CFU/g lower than counts in samples without any treatment.

After 4 weeks of storage, counts of *L. monocytogenes* in samples inoculated with *L. sake* 2a and packed in a modified atmosphere were 6.4 $\log_{10}$ CFU/g lower than in uninoculated samples.

Differences in sensory characteristics were detected after 5 days of storage, regardless of addition of *L. sake* 2a. However, after 11 days of storage, no significant difference in sensory quality was found between the samples with or without *L. sake* 2a packaged in a modified atmosphere. Results suggest that modified atmosphere packaging and *L. sake* 2a act synergistically to inhibit *L. monocytogenes* in "lingüiça frescal".

P062 THE PRESENCE OF *CAMPYLOBACTER* AND *SALMONELLA* IN RETAIL POULTRY AND PACKAGING

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Poultry and poultry products are a source of *Campylobacter* and *Salmonella* and the ease of transfer of the organisms from poultry to other surfaces may play an important role in infections within the home. The aim of the research was to monitor over a period of time contamination levels of packaging and poultry. A total of 300 packaged poultry (whole, breast and pieces) were purchased on a monthly basis (7 months) from 3 local supermarket chains and 3 local butchers' shops (Cardiff, South Wales, UK). Poultry packaging (external and internal) and the meat itself were sampled to determine the presence of the organisms. *Campylobacter* in poultry was determined using the Exeter method and identification was made using Gram stain, and motility testing and confirmed using API. *Salmonella* samples were cultured using the

reference method BS 5763: Part 4 and confirmed using API. Presence of the pathogens on external packaging was determined by rinsing the surface in 300 ml Maximum Recovery Diluent (MRD) and swabbing with a pre-moistened sterile sponge (50 cm^2). The total packaging (external and internal) and sponge was then stomached in the MRD (1 min). Seventy-five percent of poultry sampled from local supermarkets were *Campylobacter* positive while 31% were *Salmonella* positive. Four percent of the external surfaces were positive for *Campylobacter* (absence of *Salmonella*) while total packaging yielded 44% and 15% positive samples for *Campylobacter* and *Salmonella*, respectively. Poultry from butchers' shops provided 59% and 24% *Campylobacter*- and *Salmonella*-positive chicken samples and 21% and 6% positive total packaging, respectively. Two percent of external packaging was *Campylobacter* positive. A significant difference ($P < 0.05$) was noted between *Campylobacter* positive chickens and packaging (external and internal) purchased from supermarkets as compared with local butchers' shops. The results indicate the potential for the spread of *Campylobacter*, in particular after poultry handling, and are discussed in the context of hygiene precautions for food retailers, food service, and consumers.

P063 PCR-BASED FLUORESCENT METHOD FOR RAPID DETECTION OF *CAMPYLOBACTER JEJUNI* AND *SALMONELLA* TYPHIMURIUM IN POULTRY SAMPLES

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A DNA-binding fluorescence method based on PCR products was evaluated for rapid detection of *Campylobacter jejuni* and *Salmonella* Typhimurium in poultry products. The fluorescent dye in the SYBR Green PCR Master Mix has very weak fluorescence when bound to single strand DNA but a greatly increased fluorescence signal when bound to double strand DNA. Samples of chicken wash water and ground meat were inoculated with *C. jejuni* or *S.* Typhimurium to obtain bacterial concentrations of 10^0-10^5 CFU/ml. One ml of each sample was used to get the DNA template and 5 µl of the sample template was added into 25 µl of SYBR Green PCR Master Mix and primers. The negative control was the same but 5 µl of double-deionized water was added instead of 5 µl sample template. The reaction was carried out in a thermocycler. Finally, 10 µl of each PCR product was transferred into an optical tube with 490 µl of water and the fluorescence signal was measured using a fluorometer. The PCR products were also confirmed by ethidium bromide agarose gel. The results showed that when bacterial cells increased from 10^0 to 10^5 CFU/ml, the fluorescence signal increased. However, the fluorescence signals were not significantly different from the negative control until the cell number reached 10^2 CFU/ml for *C. jejuni* and 10^3 CFU/ml for *S.* Typhimurium. The PCR-based fluorescence method could detect the target bacteria in minutes after PCR amplification, compared to hours by gel electrophoresis, and also at any time during PCR amplification.

P064 DETERMINATION OF CRITICAL CONTROL POINTS (CCPS) AT POULTRY SLAUGHTERHOUSES IN KOREA

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Foodborne diseases cause deleterious problems in public health and the major etiology is due to contamination of livestock products. This study was conducted to assess biological hazards at each processing step and to determine critical control points (CCPs) in poultry slaughterhouses by investigation of microbial contamination. Two poultry slaughterhouses were selected in Gyounggi and Choongnam provinces. Total aerobic count (TAC), coliform count (CC) and isolation rates of *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* were measured at each step of the process: (1) post-defeathering, (2) dissection of carcass, (3) post-evisceration, (4) chilling, and (5) final product. Microbial contamination of chilling water used in the process was also measured. TAC and CC were significantly increased during dissection and post-evisceration and the microbial contamination of chilling water was also problematic. Isolation rates of *Campylobacter* spp., *S. aureus*, *L. monocytogenes* and *Salmonella* spp. were 70%, 36%, 27% and 17% in poultry meat and 45%, 33%, 14% and 20% in chilling water, respectively. Of the *Campylobacter* spp. isolated, 1% of *Campylobacter* spp. were resistant to fluoroquinolone antibiotic, ciprofloxacin. Study indicates leakage of intestinal content during evisceration and cross-contamination in the chiller tank could be major factors. Therefore, these process steps should be selected as CCPs at the site of the poultry meat production.

P065 ANTIMICROBIAL EFFECT OF ELECTROLYZED WATER FOR INACTIVATING *CAMPYLOBACTER JEJUNI* DURING POULTRY WASHING

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The effectiveness of electrolyzed (EO) water for killing *Campylobacter jejuni* was evaluated. One milliliter of a six-strain mixture (approximately 10^8 CFU/ml) of *C. jejuni* was subjected to 9 ml of deionized, EO, or chlorinated water treatment. Complete inactivation of *C. jejuni* in pure culture occurred within 10 s after exposure to EO or chlorinated water, both of which contained 50 mg/L of residual chlorine. A strong bactericidal activity was also observed on the diluted EO water (containing 25 mg/L of residual chlorine) and the mean population of *C. jejuni* was reduced to less than 10 CFU/ml after a 10-s treatment. EO water was further evaluated for its effectiveness in reducing *C. jejuni* on chicken during washing. Chicken wings inoculated with *C. jejuni* were treated with 500 ml of EO or chlorinated water, both of which contained 50 mg/L of residual chlorine, for 10 or 30 min with gentle shaking (100 rpm). The EO water treatment

was equally effective as chlorinated water and both achieved reduction of *C. jejuni* by about 3 $\log_{10}$ CFU/g on chicken, whereas deionized water treatment resulted in only 1 $\log_{10}$ CFU/g reduction. No viable cells of *C. jejuni* were recovered in EO and chlorinated water after the washing treatment, whereas high populations of *C. jejuni* (4 $\log_{10}$ CFU/ml) were recovered in the wash solution after the control treatment. Our study demonstrated that EO water not only was very effective in reducing the populations of *C. jejuni* on chicken, but also could prevent cross-contamination of processing environments.

P066 MUCOSAL HUMORAL IMMUNITY TO EXPERIMENTAL *SALMONELLA* ENTERITIDIS INFECTION IN CHICKENS

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Salmonella Enteritidis (SE) has been a major cause of human Salmonellosis related to the consumption of egg and poultry products. In chickens infected with *Salmonella* Enteritidis, the IgA directed against its antigen can be found in both bile and mucosal secretions. Because the crop is the first host gastrointestinal environment encountered by SE after infection, the presence of specific IgA at this location can influence the survival and persistence of an SE infection. The presence of specific IgA antibodies in crop has not previously been reported. In this report, we show that chickens infected with SE by oral gavage produce sIgAs that specifically bind to numerous SE antigens. Five 20-wk-old leghorn hens were infected twice with SE 13a (1×10^7 CFU/ml) while another group of five birds remained uninfected as control. Bile and crop milk were collected 2 weeks after the second infection to prepare total antibodies. Mucosal immune responses against SE were tested by ELISA, using SE flagella as the solid phase antigen. SE protein profiles were prepared for SDS-PAGE and proteins were transferred onto a nitrocellulose membrane using NOVEX™ electrophoresis system. Western immunoblotting was performed using bile and crop antibodies as primary antibody, mouse anti-chicken IgA monoclonal antibody as secondary, and goat anti-mouse IgG antibody as enzyme conjugate. Positive bands were developed using Sigma Fast™ BCIP/NBT buffered substrate. Chickens infected with SE showed strong sIgA responses against flagella in both bile and crop. The O.D values of ELISA test in positive bile and crop were 1.17 and 0.38, respectively, and were significantly different from those of negative samples. The Western immunoblotting revealed that ~13.5, ~56, ~62, ~80, and ~143 kDa polypeptides were major immunogens of bile IgA while ~56, ~62, and ~80 kDa were found to be strong antigens in crop. Although all three immunogens in crop were detected strongly in bile, the ~56 kDa polypeptides showed stronger reactions in crop than in bile. These results indicate that the crop may function as another site for mucosal immunity. The presence of lymphocytes producing antibodies in crop has not yet been reported. Further study is necessary to determine (1) the source of the crop IgA; (2) the impact of different immunization regimens on this IgA; (3) the effect of crop antibody against SE.

P067 BACTERIAL SURVIVAL, MOISTURE CONTENT, AND SOLUBLE PROTEINS IN CHICKEN PATTIES PROCESSED BY AN AIR IMPINGEMENT OVEN

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The essential design of cooking processes is to ensure thermal kill of foodborne pathogens. However, the quality and yield of cooked meat products are affected by physical and chemical changes of the products during cooking. The objectives of this study were to evaluate a cooking process and determine the effect of cooking conditions on pathogen survival, moisture content, and soluble proteins in chicken patties processed by air impingement ovens. Chicken patties were obtained from a commercial processor and inoculated with 7 logs of *Salmonella* spp. and *Listeria innocua*, respectively. The inoculated patties were processed in a pilot-scale air impingement oven at an air temperature of 149-218°C to an endpoint temperature of 55-80°C. After thermal treatment, the patties were enumerated for survival of *Salmonella* and *Listeria* and analyzed for moisture change and soluble proteins. Using the negative binomial distribution, a generalized linear model was developed to analyze the effect of product temperature, wet bulb temperature, and oven air velocity on survival of *Salmonella* spp. and *Listeria innocua*. The Deviation/DF was 1.0 and 1.2 for *Salmonella* spp. and *Listeria innocua*, respectively. Standard least square regressions were used to analyze the effect of oven air temperature and product temperature on moisture content and soluble proteins in the patties. Moisture content decreased 3 to 5 times with increasing product temperature. Soluble proteins decreased 70-100% with increasing oven temperatures. The results from this study are important for evaluating cooking processes and improving product quality and yields.

P068 KINETIC PARAMETERS FOR THERMAL INACTIVATION OF *SALMONELLA* SPP. IN COMMERCIALLY FORMULATED CHICKEN PATTIES AND FRANKS

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Currently, the market for cooked meat products is increasing rapidly. In order to determine the pathogen thermal lethality in cooked meat products, the kinetic information for pathogen thermal inactivation in these products is essential. The objective of this study was to determine thermal inactivation kinetics in commercially formulated chicken patties and franks. Raw meat products were obtained from two different commercial processors. Six *Salmonella* spp., including *S. senftenberg*, *S.* Typhimurium, *S. heidelberg*, *S. mission*, *S. montevideo*, and *S. california*, were used in this study. Cultures were individually maintained and mixed just prior to the inoculation. Seven log CFU/g of *Salmonella* were uniformly blended with uncooked meat samples. Ten grams of inoculated meat samples were placed in a 152 mm long × 12.7 mm diameter × 0.7 mm thick of cupper tubes and heated in a water bath to a final temperature of 55 to 70°C for 0 - 25 min. During heat treatments, internal temperatures of the meat samples were monitored every one

second. Periodically, meat samples were taken out of the water bath, immediately cooled in an ice-water bath, and then enumerated for the bacterial survivors. Linear regressions were performed to obtain the kinetic parameters. The z values were 7.60 and 9.83°C, the activation energies were 284.1 and 218.3 kJ/mol, and the Arrhenius constants were 1.20×10^{44} and 9.47×10^{33} for chicken patties and franks, respectively. The information from this research is important for thermal process validations of commercial products.

P069 INCIDENCE OF *CLOSTRIDIUM PERFRINGENS* IN AN INTEGRATED BROILER CHICKEN OPERATION FROM BREEDER FARM TO THE FULLY-PROCESSED PRODUCT

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In a previous study, ribotypes of some strains of *Clostridium perfringens* isolated from processed chicken carcasses matched the ribotypes of strains that had been isolated from the paper pads lining trays used to transport chicks from the hatchery to the grow-out house on the farm. A subsequent study revealed that egg shell fragments, chick fluff, and paper pads beneath chicks in the hatchery were consistently contaminated with *C. perfringens*. The results reported herein are from a study of the incidence of *C. perfringens* in breeder flocks of a poultry integrator and subsequently sampled and associated hatcheries, grow-out flocks and processed carcasses. In the first trial, *C. perfringens* was recovered from the breeder farm, hatchery, previous grow-out flock, grow-out flock at 3 weeks of age, grow-out flock at 5 weeks of age, and from processed carcasses in 4, 30, 4, 0, 2, and 16% of samples, respectively. In the second trial, the incidence of *C. perfringens* in samples collected at these facilities was 30, 30, 32, 8, 4, and 8%, respectively. The genetic relatedness of strains isolated in the current study remains to be determined. Results suggest that *C. perfringens* may be transmitted within the integrated broiler chicken operation.

P070 *CLOSTRIDIUM PERFRINGENS* LEVELS IN COOKED AND UNCOOKED MEAT AND POULTRY PRODUCTS

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In January, 1999, FSIS finalized performance standards for cooking and chilling meat and poultry products in federally inspected establishments. The more restrictive chilling standards were promulgated despite no strong evidence of public health risk based on industry practices employing the original May 1988 guidelines (FSIS Directive 7110.3). FSIS baseline data indicated a "worst case" of 10^4 *C. perfringens*/g from raw meat products. The rationale for the FSIS performance standards was based on these levels and the assumption that the numbers detected in the baseline study were spores that would have survived cooking. Consequently, this regulatory change has stimulated work in our laboratory to help address why there have been so few documented outbreaks of *C. perfringens* illness from commercially processed meat and poultry products. Our research has considered the incidence of *C. perfringens* in raw and cooked products. One hundred and fifty-four raw ground turkey samples were cooked to 165°F and analyzed for *C. perfringens* levels. Counts were determined by spread plating diluted samples onto tryptose-sulfite-cycloserine agar and biochemically confirming presumptive colonies. All samples tested contained <3 spores/g. Studies are continuing with raw pork and beef. Furthermore, we have analyzed 33 production lots of RTE meat products that have deviated from the FSIS cooling guidelines for *C. perfringens* levels. To date, 420 samples have been tested, and all but two of the samples (110 and 140 CFU/g) had *C. perfringens* levels of <100 CFU/g. Our results further support historical food safety data that suggest a very low public health risk associated with *C. perfringens* in commercially processed RTE meat and poultry products.

P071 EVALUATION OF THE MICROFOSS SYSTEM FOR ENUMERATION OF TOTAL VIABLE ORGANISMS, *ESCHERICHIA COLI*, AND COLIFORMS IN GROUND BEEF

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This study was conducted to evaluate the performance of the MicroFoss™ system for enumeration of total viable organisms, *Escherichia coli*, and coliforms in ground beef. The system performance was compared to that of the USDA Bacteriological Analytical Method (BAM) reference culture methods. Over 100 ground beef samples were analyzed for each of the three test types by the MicroFoss™ system and culture methods: aerobic plate count method for total viable count and coliform count, and the most probable (MPN) method for *E. coli*. Some ground beef samples were temperature abused at room temperature (24°C + 2°C) up to 5 - 24 h to obtain microbial counts in the high-count range. The correlation coefficients for the regression lines comparing the MicroFoss™ system detection times to the results of culture methods for total viable counts, coliform counts, and Most Probable Number (MPN) method for *E. coli* were -0.95, -0.96, and -0.97 respectively. Tests comparing the reproducibility of the MicroFoss™ system, and culture methods, showed no significant differences ($P > 0.05$) in the data generated independently from the same batch of samples tested by two technicians using these methods. The plate count methods for total viable and coliform counts, and the MPN method for *E. coli*, required 10 and 20 times respectively the amount of time required to complete the tests by the MicroFoss™ system. The MicroFoss™ system provided a faster and cost-efficient method for estimation of total viable bacteria, *E. coli*, and coliforms in ground beef.

P072 GEL PEROXYGENS AS BARRIER AND TREATMENT SYSTEMS FOR BEEF CARCASSES

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Multi-intervention strategies are being utilized to treat carcasses to achieve required regulatory pathogen reductions. These strategies include steam pasteurization with & without sprays of approved chemical components

such as peracetic acid, peroxide, and chlorine dioxide. High retention food grade biocides could serve either as a barrier or treatment method to reduce the level of pathogens on beef carcasses.

A beef coupons model was developed to evaluate food grade gel peroxygen and oxidant systems both as a barrier and treatment method vs. *Listeria monocytogenes* and *Escherichia coli* O157:H7. The barrier method involved coating the surface of a 2 × 4 cm beef coupon with the test gel peroxygen, followed by an immersion or spray pathogen challenge. The treatment method involved pathogen spray inoculation, then spray application of the respective test gel peroxygens. Liquid peroxygens were tested as well.

As a barrier using an immersion application, 100 ppm of 3 gel peroxygen systems all achieved over 1 $\log_{10}$ reduction of *L. monocytogenes* [-1.17, -1.25, -1.13] and *E. coli* O157:H7 [-1, -1.3, -1.5]. As a barrier using a spray application, gel peroxygen systems at 100 ppm, using only 5 psi at ambient temperature, achieved nearly one log cycle reductions [-0.8] of both test pathogens after a 5 minute contact period between the gels and challenge pathogens. As a treatment system, gel and liquid peroxygen systems at 200 ppm, and chlorine dioxide systems at 13 ppm, all achieved log cycle reductions of both pathogens. Gel oxidant systems show potential to reduce pathogen contamination on beef carcasses.

P073 COMPARISON OF METHODS FOR THE ISOLATION OF *ESCHERICHIA COLI* O157:H7 FROM GROUND BEEF

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A study was performed for the detection of *Escherichia coli* O157:H7 in ground beef comparing VIDAS ECO immunoassay, VIDAS ICE immunoconcentration, an alternate immunoassay, and a USDA method. Ground beef was inoculated with *E. coli* O157:H7 at a low level, 25 gram portions were enriched, and assays were performed according to the protocol specified for each method. Culture confirmation for each VIDAS method weas performed using a chromogenic plating medium, EC ID. None of the test portions by the USDA protocol or the alternative immunoassay were positive for *E. coli* O157:H7. Ninety-five percent of the test portions were positive by VIDAS ECO, and 100% by VIDAS ICE method. Based on these results, the VIDAS ECO immunoassay and ICE immunoconcentration demonstrated increased sensitivity for the detection of *E. coli* O157:H7 in ground beef.

P074 *ESCHERICHIA COLI* O157:H7 RISK ASSESSMENT FOR THE PRODUCTION AND COOKING OF RESTRUCTURED BEEF STEAKS

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Distribution of *Escherichia coli* O157:H7 in restructured beef from inoculated meat pieces and destruction of *E. coli* O157:H7 in beef steaks prepared from inoculated meat was evaluated following broiling and grilling. *Longissimus dorsi* trimmings were inoculated with fluorescently marked *E. coli* O157:H7 to visualize bacterial distribution throughout restructured steaks, using confocal laser scanning microscopy. Fluorescent density was observed along the glue lines where meat pieces were enzymatically joined. *E. coli* O157:H7 levels throughout the thickness of steaks cut from restructured beef steaks were determined. Cross sections from the steaks showed bacterial contamination to be evenly distributed within the restructured steaks (ca. 10^6 CFU/g). Beef trimmings were inoculated (10^7 CFU/g) and used to prepare restructured beef chubs. Restructured steaks of three thicknesses (1.27, 2.54 and 3.81 cm) were cooked to six internal temperatures (48.8, 54.4, 60, 65.5, 71.1, or 76.6°C) by gas grill or oven broiler. *E. coli* O157:H7 recovery was calculated after plating on MacConkey Sorbitol and Phenol Red Sorbitol agars. *E. coli* O157:H7 survival decreased as endpoint temperatures for grilled and broiled steaks incrementally increased. Broiling restructured steaks to endpoint temperatures of 48.8, 54.4, 60, 65.5, 71.1, or 76.6°C resulted in reductions of 1.03, 194, 2.70, 4.32, 6.27 and 6.08 log CFU/g, respectively. Comparable cooking on a gas grill resulted in reductions of 0.87, 1.16, 1.25, 2.62, 3.73, and 4.51 log CFU/g, respectively. Restructured steaks should be cooked in a similar manner as ground beef per USDA recommendations to achieve an internal temperature of at least 71.1°C.

P075 *ESCHERICHIA COLI* O157:H7 MAINTAINS ACID TOLERANCE IN ACID-CONTAINING BUT NOT IN NON-ACID-CONTAINING FRESH MEAT DECONTAMINATION WASTE FLUIDS

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We have shown that *Escherichia coli* O157:H7 loses its acid tolerance when exposed to water meat decontamination washings at 10°C. However, it should be investigated whether the pathogen maintains acid tolerance in fluids of sublethally low pH. This study evaluated survival and acid tolerance of acid-adapted or nonadapted cultures of a rifampicin-resistant derivative of *E. coli* O157:H7 ATCC 43895. The cultures were exposed (10^5 CFU/ml) to acid-containing (2% lactic or acetic acid, or their mixtures with water washings at ratios 1:1, 1:9 or 1:99, v/v) or nonacid-containing (water) washings of meat. Washings were analyzed for bacterial changes during storage (4 or 10°C, 14 days). Acid tolerance was assessed at 2 and 7 days by exposing cells from washings at 10°C to tryptic soy broth plus 0.6% yeast extract acidified (pH 3.5) with lactic acid. Acid-adapted and nonadapted *E. coli* O157:H7 survived without growth in water washings at 4 or 10°C. In acid-containing washings, nonadapted declined faster than acid-adapted populations; declines increased as the acid concentration in the washings increased. Declines were more dramatic in lactate compared to acetate washings, especially at 10°C. Cells exposed to acidic washings (pH 3.0 to 4.9) for 2 days survived better at pH 3.5 than those exposed to water washings (pH 6.7 to 6.8), especially when washings contained acetate and the original inoculum was acid-adapted. This response was maintained for 7 days in acid-adapted, but was reversed in nonadapted, populations. These results should be considered in application of acid decontamination technologies for meat.

P076 FOOD SAFETY: CONSUMER VIEWS OF PUBLIC VERSUS PRIVATE INTERVENTIONS RELATED TO MEAT PROCESSING

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Foodborne disease outbreaks caused by *Escherichia coli* O157:H7 bacteria in ground beef have caused the meat processing industry to employ various interventions to destroy pathogens. Four focus groups with 37 residents of Manhattan, Kansas were conducted to determine: (1) relative preferences for alternative combinations of public protection (Hazard Analysis Critical Control Points (HACCP), carcass pasteurization, irradiation) and private protection (home preparation-rare, medium, and well-done hamburgers); (2) how who is at risk (children vs. adults) influences preferences; (3) whether consumers would pay a premium for the higher levels of product safety arising from the adoption of three different innovations in processing plants. Participants were aware of food safety practices, but misconceptions also were found. For private protection, most participants (58%) preferred well-done hamburgers. For public protection, 40% of participants preferred hamburgers subjected to all three innovations. Combining both protection strategies, most participants preferred well-done, steam-pasteurized or medium, irradiated hamburgers. For children, most of the participants chose well-done, steam-pasteurized or well-done, irradiated hamburgers. Participants were willing to pay a premium of 8 cents per pound more for a steam-pasteurized hamburger and a premium of 8 cents per pound more for an irradiated hamburger. Results indicated that the availability of new safety-enhancing technology provided a marginal value to participants because private protection provides an effective means of killing pathogens. Also, the need for more information was expressed.

P077 THE INCIDENCE OF *SALMONELLA* SPP. AND BIOTYPE 1 *ESCHERICHIA COLI* ON SWINE CARCASSES PROCESSED UNDER THE HACCP-BASED INSPECTION MODELS PROJECT

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In 1999, the USDA Food Safety and Inspection Service implemented an experimental inspection system, the HACCP-based Inspection Models Project (HIMP), with selected processors of market hogs, fed cattle, and young poultry, to test whether new slaughter inspection procedures, applied in conjunction with HACCP controls, can improve food safety. The present study measured the performance of the HIMP program by examining the prevalence of *Salmonella* spp. and the prevalence and quantity of Biotype 1 *Escherichia coli* on carcasses or in pig feces at a collaborating pork processing plant operating under the HIMP program in 2000. The surfaces of carcasses were swabbed on 10 separate days immediately following exsanguination and after the carcasses were washed, eviscerated and chilled overnight. Feces were collected from 60 of the 100 swabbed, post-exsanguinated pigs. *Salmonella* spp. were detected on 73.0% of 100 pre-eviscerated carcasses, in 33.3% of 60 fecal samples, and on 0.7% of 122 chilled carcasses. Biotype 1 *E. coli* were found on 100.0% of pre-eviscerated carcasses and on 28.7% of chilled carcasses. The mean concentration of Biotype 1 *E. coli* was 1700 CFU/cm^2 at the pre-evisceration stage and 1.1 CFU/cm^2 at the chilled carcass stage. The average incidence of *Salmonella* spp. and the maximum level of Biotype 1 *E. coli* on chilled carcasses reported in a 1998 pre-HIMP baseline study for two volunteer pork processing facilities were 0.8% and 8.2 CFU/cm^2, respectively. These data show that a pork processing plant operating under the HIMP program can maintain or exceed its baseline performance levels.

P078 VERO CELL ASSAY FOR DETECTION OF CYTOPLASMIC VACUOLATION BY *ARCOBACTER* SPP. ISOLATED FROM MEAT

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Arcobacter butzleri is known to be pathogenic for humans and animals and has also been found in sewage, poultry carcasses, surface water and porcine abortion specimens. Musmano et al. showed cytotoxic effects in 17 strains of *A. butzleri* isolated from river water samples. The objective of this work was to investigate the ability of *Arcobacter* spp to induce cytoplasmic vacuolation (toxvac) in Vero cells. A total of 114 strains, isolated from chicken, beef and pork meat, were grown in minimum essential medium (Gibco/BRL) and incubated at 35°C in a microaerobic atmosphere for three days. Supernatants were filtered through 0.22µm-pore filters (Milipore) and tested immediately on Vero cells. Vero cells were seeded in microtiter plates and used when they were 80% confluent. Uninoculated broth served as a negative control, and culture filtrates of *V. cholerae* 01 (Toxvac positive) served as positive controls. Cells were examined microscopically for morphological changes at 8 h. From all strains tested, 23 (20%) were toxvac producers, 42 (36.8%) produced enterotoxins, 42 (36.8%) produced both, toxvac and enterotoxins, 1 (0.9%) had cytolethal activity, and 6 (5.3%) were negative. Our results show that cytoplasmic vacuolation could be a mechanism to produce cellular death and indicate that *Arcobacter* spp. could be potentially virulent.

P079 VALIDATION AND USE OF ALKALINE PHOSPHATASE REDUCTION AS AN INDICATOR FOR MEAT COOKING EFFICIENCY

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Heat processing is an effective method for destroying microorganisms and is likely to be an important component in many HACCP plans. Epidemiological studies in both the UK and USA have implicated inadequate heating as a risk factor in outbreaks of foodborne disease. To ensure safe food production, retrospective monitoring

of adequacy of heating food products is often required by making visual, microbiological or chemical estimations. Visual assessment can be subjective and may be inaccurate; microbiological analysis is time-consuming, but chemical analysis is more rapid.

A Cooked Meat Efficiency (CHEF) test (Charm Sciences Inc.) based on the correlation between the destruction profile of the enzyme alkaline phosphatase has been developed. Chicken and red meat products were analysed in vitro and in situ visually, microbiologically and chemically. CHEF tests were taken at the center of meat samples in a staged series of specific temperatures. A substantial decrease in phosphatase was observed above 50°C; however, meats heated to 75°C contained significantly less (P = <0.05). Prolonged heat exposure to meat samples at lower temperatures did not decrease levels of phosphatase. Data obtained from experiments enabled target levels and critical limits to be set. Standard curves will be presented to show the relationship between increase in temperature, phosphatase levels, aerobic plate counts and Enterobacteriacae counts.

A sample of 100 consumers prepared and heated a selection of home-made and convenience chicken and meat products. All samples were assessed visually, microbiologically and chemically. Use of the CHEF test enabled an immediate assessment of heating adequacy and >20% of samples passed visual assessment, but gave phosphatase levels exceeding critical limits using CHEF tests. Comparisons between CHEF test values and microbiological counts of tested food products will also be presented.

P080 ISOLATION OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* IN CATTLE MANURE AFTER A PASSIVE TREATMENT

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Cattle are the primary reservoir for *Escherichia coli* O157:H7. Cattle manure is widely used as fertilizer on fields for vegetable crops. Good agricultural practices for use of animal manure include passive treatments such as aging to reduce pathogens. The purpose of this study was to determine the presence of *E. coli* O157 in cattle manure after a passive treatment for application in agricultural fields. Manure from cattle previously diagnosed as carriers of *E. coli* O157 was sampled from a 140 m^2 treatment area. Thirty samples were collected at 30, 60 and 75 days of passive treatment from three depths: 25, 35 and 50 cm.

The method of Sanderson et al. (1995) was used to isolate *E. coli* O157. *E. coli* isolates were tested for Shiga-like toxin production on Vero tissue culture cells and for presence of 157 O-antigen. Humidity content, pH and background microflora were determinated for each sample. *E. coli* O157 was not isolated from any manure samples. pH ranged from 5.22 to 9.72, temperature from 21°C to 48°C, humidity content from 2.6% to 67.48% and the background flora ranged as follows (log CFU/g): aerobic plate count = 6.04 to 8.09, anaerobic plate count = 5.61 to 8.29, total coliforms = 1.84 to 5.51 and total molds/yeast plate count = 2.0/1.7 to 5.51/6.83. Shiga toxin producing, non-O157 *E. coli* (STEC) were isolated from 3 samples (3.33%). High levels of background microflora and high pH, as well as low humidity may have reduced the survival of *E. coli* O157 in the treated manure. However, potential sources of re-contamination were observed during and after treatment. Moreover, non-O157 STEC isolated from treated manure are a potential source of contamination of vegetables that will be grown on lands fertilized with this manure.

P081 SURVIVAL OF *ESCHERICHIA COLI* O157:H7 IN COW MANURE-AMENDED SOIL

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Raw animal manure which carries human pathogens is routinely used by farmers to fertilize crops. Our objective was to determine the survival of *E. coli* O157:H7 in manure-amended soil under different environmental conditions. A 5-strain mixture of GFP-labeled *E. coli* O157:H7 was inoculated into cow manure at ca. 10^7 CFU/g. The inoculated manure was held at 23°C for 20 to 24 h, then mixed with unautoclaved or autoclaved sandy loamy soil at a ratio of 1:100, 1:50, 1:25, and 1:10 and held at 21, 15, or 5°C. *E. coli* O157:H7 survived at least 77 days in the 1:10 formulation of manure-amended autoclaved (MAA) soil held at 5°C, and was not detected by the 63rd day in any manure-amended unautoclaved (MAU) soil. At 15 and 21°C, *E. coli* O157:H7 increased within 7 days by ca. 0.5 to 2 log CFU/g of MAA soil but declined by 3 log CFU/g of MAU soil. In MAA soil, *E. coli* O157:H7 decreased by 1 to 2 log CFU/g within 150 days in 1:25, 1:50, and 1:100 formulations and by 6 log in the 1:10 formulation at 15°C. Similar survival patterns for *E. coli* O157:H7 were observed at 21°C. Overall, *E. coli* O157:H7 survived slightly longer at 21°C than at 15°C in MAU soil, and died off more rapidly in MAU soil than in MAA soil at all three temperatures. Results indicate that *E. coli* O157:H7 can survive in manure-amended soil for more than 6 months at 15 or 21°C even under very dry conditions.

P082 SEASONAL OCCURRENCE OF *CAMPYLOBACTER* IN DAIRY CATTLE AND THEIR ENVIRONMENT

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Campylobacter spp. are a frequent cause of acute bacterial enteritis. The objective of this study was to determine the occurrence of *Campylobacter* in dairy cattle and environmental samples collected from a Dairy Cattle Experiment Station in Tennessee monthly for 12 months. To recover *Campylobacter* and *C. jejuni*, modifications of FDA enrichment/differential plating methods (BAM) were used to optimize recovery based on sample type. The occurrence of *Campylobacter* and *C. jejuni* in environmental and animal samples varied by season. The incidence of *Campylobacter* in environmental samples was greatest during fall, with 61% compared to 30%, 23% and 19% for winter, spring and summer, respectively. *Campylobacter* was more common in mixed grain (83%) and soil (42%)

than in other environmental samples during the fall months. During the winter, the majority of the isolates were recovered from bird droppings (70%) and insects (44%). The highest percentages of isolation during the spring season were in water (83%) and air (42%). Thirty-three percent (473 of 1426) of the animal samples harbored *Campylobacter* which was highest during the fall in mouth, hair, teat, and foremilk samples, with 89%, 58%, 53%, and 49% occurrence, respectively. Feces (58%) were the predominant source of *Campylobacter* during the winter months. Overall, the incidence of *Campylobacter* in animal and environmental samples was greatest during the fall and least during the summer. The frequent occurrence of *Campylobacter* in environmental samples indicates that improvements in control of *Campylobacter* in the farm environment are needed.

P083 SAMPLING OF THE DAIRY FARM ENVIRONMENT FOR *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes (LM) in the farm environment may contaminate cows and their raw products, which may cause post-processing contamination. This study determined the factors that affected the occurrence and recovery of *Listeria* spp. from the environment of dairy cattle (320 cows) and raw bulk tank milk over a twelve-month period in Tennessee. Isolation and confirmation of LM was performed according to the FDA Bacteriological Analytical Manual (AOAC) with modifications in isolation protocol to improve recovery from farm samples. LM was isolated from bedding (1%), feed bunk (2%), grain (3%), insects (1%), milking equipment (2%), and soil (1%), but not from air, bulk tank milk, silage, and chlorinated trough water (n=50 for each type of sample). *Listeria* species were isolated from all sites, except trough water. MOX was slightly more effective for detecting LM than PALCAM (57% compared to 43%). The most frequently isolated *Listeria* species was *L. seeligeri* since 40% of samples overall tested positive. These data can be used to determine the optimal sampling sites for the recovery of LM and to identify major sources of contamination where hazards might be controlled at the farm.

P084 COMPARISON OF MULTIPLEX, ELISA AND 5' NUCLEASE PCR ASSAYS FOR DETECTION OF PLASMID-BEARING VIRULENT *YERSINIA ENTEROCOLITICA* IN PIG FECES

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Swine are implicated as the principal animal reservoirs for human pathogenic plasmid-bearing cells of *Yersinia enterocolitica* (YEP+). To evaluate the utility of the PCR for detection of YEP+ in pig feces, samples were tested by multiplex PCR , PCR-ELISA and 5' nuclease PCR assays. One gram from each of 50 swine fecal samples was suspended in 9 ml of 0.1% peptone in sterile Whirl Pak bags and pummeled in a Stomacher. One ml of the suspension was diluted in 9 ml of ITC (Irgasan, ticarcillin, and KClO3) broth, vortexed, and enriched for 48 h at room temperature; then 1 ml of the culture was centrifuged and the pellet was washed with TE buffer. The DNA was extracted and subjected to the multiplex PCR employing primers from the plasmid virF gene and the chromosomal ail gene. The YEP+ were detected in 3 of 50 samples by the presence of the ail (170 bp) and virF (591 bp) amplification products by agarose gel electrophoresis. The PCR-ELISA amplifying an ail gene sequence detected 2 of the 3 multiplex PCR-positive samples. The 5' nuclease assay based on amplification of an ail gene sequence did not detect YEP+ in any of the 50 samples. The YEP+ were isolated from the PCR positive samples by the Congo red binding technique, confirming the multiplex PCR and PCR ELISA assays. These results indicate that the multiplex PCR is the most sensitive assay for detecting YEP+ in feces among the three assays evaluated.

P085 ANTIMICROBIAL SPECTRUM OF THYMOL, EUGENOL, POTASSIUM SORBATE AND SODIUM BENZOATE AT SELECTED PHS

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Several phenolic compounds present in extracts and essential oils of plants, such as thymol and eugenol, have been reported as effective natural antimicrobial agents. However, data comparing their effectiveness with that of common synthetic antimicrobials are scarce. Our objective was to evaluate and compare antimicrobial effectiveness of thymol (Th), eugenol (Eu), potassium sorbate (KS) and sodium benzoate (NaB) to inhibit the growth of *Listeria innocua*, *Escherichia coli* and *Lactobacillus reuteri* at pH 5.0, 4.5 and 4.0 as well as to inhibit growth of *Aspergillus flavus*, *A. parasiticus* and *Zygosaccharomyces rouxii* at pH 4.0, 3.5 and 3.0. Tripticase soy agar (TSA) and potato-dextrose agar (PDA) were prepared, sterilized, pH adjusted and poured on 15 × 150 Petri dishes. 50 mL of 20% aqueous KS or NaB, or 20% ethanolic Th or Eu solutions, were poured using a spiral plater. TSA plates for all antimicrobials were inoculated using a swab, making a 6-cm long radial mark with 10^6 spores or cells/mL fresh suspension, incubated at 25°C (fungi) or 35°C (bacteria), and observed for 2-5 days. Inhibitory concentrations were determined by calculating antimicrobial concentrations at growth end points. Antimicrobial inhibitory concentrations decreased along with pH. However, Th or Eu inhibitory concentrations were not pH dependent as the antimicrobial action of KS or NaB. A wide antimicrobial action of Th and Eu was observed, being capable to inhibit growth of bacteria and fungal species. Inhibitory concentrations of natural and synthetic antimicrobials were comparable, since their molecular weights are also similar. Higher antimicrobial concentrations were needed to inhibit fungi.

P086 ROPE SPOILAGE IN BREAD AND ITS CONTROL BY NATURAL ANTIMICROBIALS

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Rope, the bacterial spoilage of bread, is commonplace in South Africa and is caused mainly by strains of *Bacillus subtilis* and *B. licheniformis*. Rope spoilage is commonly controlled by using the chemical preservative calcium

propionate (CP) at ca. 0.2% based on flour mass. In situ colonization of bread crumb by a rope-inducing *Bacillus* isolate was studied by scanning electron microscopy (SEM). Furthermore, selected antimicrobials were evaluated as potential alternatives to CP, in line with modern consumer demands for naturally preserved foods. Efficacies of the natural antimicrobials lactic acid (LA), acetic acid (AA), calcium lactate (CL) and a lactate-containing fermentation cocktail (LCC), singly and in combinations, were determined by in vitro growth measurements and test bakes. SEM revealed extensive crumb colonization by bacterial cells, including the presence of confluent biofilms. In areas underlying biofilms, regular, rod-shaped depressions in the crumb matrix indicated extracellular enzymatic activity of *Bacillus* cells. In vitro, LA and AA were most effective in controlling the growth of rope-inducing *Bacillus* isolates and were also less inhibitory to yeast activity than CP or CL. In test bakes, combinations of 0.1% CP with 0.125% LA, 0.375% CL or 0.5% LCC produced acceptable rope-free shelf life. The efficacy of the 0.25% LA + 0.1% AA combination compared favorably to 0.2% CP, followed by the combinations of 0.5% LCC + 0.125% LA + 0.05% AA and 0.375% CL + 0.1% AA. In terms of cost-effectiveness and yeast activity reduction, the 0.25% LA + 0.1% AA combination was identified as a potential replacement for 0.2% CP.

P087 ANTIMYCOTIC ACTIVITY OF VANILLIN IN COMBINATION WITH SELECTED ANTIMICROBIAL AGENTS

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Debates about the use of antimicrobial agents in food products have been common for decades, encouraging the search for alternative antimicrobial agents. A wide range of natural antimicrobial sources that are being evaluated, among them vanillin, have demonstrated antimycotic activity. However, data on the effect of natural antimicrobials such as vanillin in combination with other synthetic or natural antimicrobials is scarce. The effects of selected combinations of vanillin (Vi, 100, 200, 300... up to 1300 ppm) with potassium sorbate (KS) or thymol (Th, 50, 100, 150... up to 400 ppm), sodium benzoate (NaB, 25, 50, 75... up to 200 ppm), carvacrol (Cr, 50, 100, 150... up to 300 ppm) or eugenol (Eu, 100, 200, 300... up to 600 ppm) on growth of *Aspergillus flavus* inoculated in a_w 0.99, pH 3.5 potato-dextrose agar (PDA) were evaluated. PDA prepared with each antimicrobial mixture was inoculated with 2 µl of a suspension containing 10^6 spore/m, incubated at 25°C, and observed after 5, 10, 15 and 30 days. Minimal inhibitory concentration (MIC) was defined as the minimum required to inhibit growth. Fractional inhibitory concentrations (FIC) were calculated from individual and inhibitory combinations, and the FIC index of each antimicrobial mixture was computed. MICs were 200 ppm NaB, 300 ppm Cr, 400 ppm KS or Th, 600 ppm Eu, and 1300 ppm Vi. FICs and FIC index varied with incubation time, showing synergism, additive or antagonistic effects depending on the concentrations of antimicrobials in the mixture. Synergistic combinations with FIC index <1, included Vi with KS, Th, Eu or Cr; NaB mixtures presented additive or antagonistic effects.

P088 REDUCTION OF AFLATOXINS BY KOREAN SOYBEAN PASTE AND ITS EFFECT ON CYTOTOXICITY AND REPRODUCTIVE TOXICITY: ANTIGENOTOXIC EFFECT OF THE METHANOL EXTRACT OF KOREAN SOYBEAN PASTE ON AFLATOXIN B1-INDUCED BACTERIAL REVERSE MUTATION AND CHROMOSOME ABERRATION

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We have found that the methanol extract of Korean soybean paste remarkably inhibited aflatoxin production in a culture medium in part 1 of this study (Journal of Food Protection Vol. 63, No. 9. 1295-1298). In part 2 in the series, we performed bacterial reverse mutation assays with *Salmonella* Typhimurium TA1535, TA1537, TA98, TA100, and TA102 and in vitro chromosome aberration assays with Chinese hamster lung cells, to investigate the genotoxicity of the methanol extract of Korean soybean paste (doen-jang/dwen-jahng) and its antigenotoxic activity against aflatoxin B1. The methanol extract revealed nonmutagenic potential in all the bacterial strains tested. The extract significantly reduced the numbers of revertants per plates when it was added to the assay system using *S.* Typhimurium TA100 ($P < 0.05$). The extract also exhibited significant inhibitory effects on chromosome aberration in Chinese hamster lung cells ($P < 0.05$). The findings of this work indicate that the methanol extract of Korean soybean paste could have strong potential as an antigenotoxic material.

P089 PERFORMANCE OF MYCOLOGICAL MEDIA FOR SUPPORTING COLONY FORMATION BY DESICCATED FOOD SPOILAGE YEASTS: AN INTER-LABORATORY STUDY

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Dichloran 18% glycerol (DG18) agar is being used in some laboratories as a general purpose medium for enumerating yeasts and molds in foods, although its performance in recovering yeasts from dry foods has not been evaluated. A study involving thirteen collaborators in six countries was done to compare DG18 agar with dichloran rose bengal chloramphenicol (DRBC) agar, plate count agar supplemented with chloramphenicol (PCAC), tryptone glucose yeast extract chloramphenicol (TGYC) agar, acidified potato dextrose agar (APDA), and orange serum agar (OSA) for their suitability to enumerate fourteen species of lyophilized food spoilage and industrial yeasts. The coefficient of variation for among-laboratories repeatability within yeast was 1.39% and reproducibility of counts among laboratories was 7.1%. The order of performance of media for recovering yeasts was TGYC agar > PCAC = OSA > APDA > DRBC agar > DG18 agar. In a second study done in one laboratory, TGYC agar was equal to PCAC and superior to the other four media in recovering the same fourteen lyophilized yeasts stored at -18°C, 5°C, or 25°C for up to 42 weeks. Results from both the inter-laboratory study and the storage study support a recommendation to use TGYC agar for enumerating desiccated yeasts. DG18 agar is not recommended as a general purpose medium for recovering desiccated yeasts.

P090 SIMPLATE FOR YEAST AND MOLD — COLOR INDICATOR: A NEW METHOD FOR RAPID ENUMERATION OF FUNGI IN FOOD

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SimPlate for Yeast & Mold - Color Indicator (YMCI) is a new method for the detection and quantification of the total fungal concentration of food. Quantification is achieved after incubating the YMCI medium at room temperature (22 to 25°C) in a patented SimPlate device for a minimum of 48 h. Fungi are indicated by the presence of a color change in the medium dispensed into the 84 wells of the SimPlate device. The fungal concentration is obtained by counting the number of color-changed wells in the device and referring to the SimPlate Conversion Table included with the test. The YMCI medium, a proprietary formulation developed at BioControl, is highly specific for foodborne fungi and contains a blue dye that turns a different color in the presence of actively growing organisms. YMCI was evaluated in parallel with the standard 5-day Potato Dextrose Agar (PDA) method as specified in the Bacteriological Analytical Manual (BAM). Food samples used in this study represented 20 different food matrices and, when possible, were naturally contaminated with fungi. Results of this study showed agreement ($P < 0.01$) between YMCI and the PDA method for each food matrix tested. This suggests that YMCI is a suitable alternative to PDA for routine monitoring of food for the presence of yeasts and molds.

P091 DETECTION OF ANTIFUNGAL ACTIVITY OF *LACTOBACILLUS RHAMNOSUS* AND *BACILLUS PUMILUS* USING A MILK AGAR PLATE ASSAY

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The antifungal effects of two *Lactobacillus rhamnosus* and two *Bacillus pumilus* strains, isolated from foods, which had previously exhibited strong antifungal properties, were studied in a milk environment using a Milk Agar Plate Assay. The principle of this method involved inoculating sterile skim milk with the test bacterium, adding it to 1.5% bacteriological agar containing the color-pH indicator bromcresolpurple (BCP), and allowing it to solidify so that a semisolid structure was obtained. The layer was then overlaid with soft agar (0.75%) containing BCP, and the solidified agar surface was inoculated by streaking with mold test strains. The antifungal activity was tested against 18 molds from the genera *Aspergillus*, Penicillium, and Fusarium. All 4 bacterial strains tested exhibited a certain degree of inhibition of growth and spore production of 16 of the 18 mold strains. However, the *L. rhamnosus* LBK7 and *B. pumilus* NE2 strains possessed the strongest inhibitory activities, respectively. The growth of *P. italicum* NRRL 846, *P. expansum* NRRL 2304, *F. sporotrichioides* T42, and *Fusarium* sp. DMF 0101 were completely inhibited by these strains, while with the rest of the Penicillium and Fusarium strains, only inhibition of spore production was observed. It was concluded that, overall, Penicillium and Fusarium species were more sensitive to the bacterial strains than were the *Aspergillus* species, whose growth was only delayed for the first two days of incubation, followed by amounts of mycelial growth and spore production were comparable with controls.

P092 REDUCTION OF AFLATOXINS BY KOREAN SOYBEAN PASTE AND ITS EFFECT ON CYTOTOXICITY AND REPRODUCTIVE TOXICITY: INHIBITORY EFFECT OF KOREAN SOYBEAN PASTE ON THE AFLATOXIN TOXICITY IN LAYING HENS

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We observed that an extract of Korean soybean paste reduced aflatoxin production in a culture medium in part 1 of this study (*Journal of Food Protection* Vol. 63, No. 9. 1295-1298). In part 3 in the series, a study was conducted on the effects of Korean soybean paste (doen-jang/dwen-jahng) (0.5%, 1%, and 5%) on the toxicity of 500 ppb of aflatoxin in the diet of sixty laying hens (Isa Brown) divided into five groups and treated from week 15 to week 67. The treatment of aflatoxin resulted in many deleterious effects, including severely altered cell foci and sinusoid dilatation in the livers, compared with the control. The feeding of 1% soybean paste reduced the adverse effects of aflatoxin on body weight, relative organ weights, egg production, and aflatoxin accumulation in eggs, and improved serum calcium and ALT levels, as well as the histopathological lesions of the livers. The feeding of 5% soybean paste showed the same improvements at a higher level, especially in the histopathological findings of livers. On the basis of body weight, relative organ weights, aflatoxin accumulation in eggs, serum biochemical values and enzyme activities, and histological findings of the livers, it was suggested that 5% and sometimes only 1% Korean soybean paste in the diet protected laying hens from the major deleterious effects of 500 µg of aflatoxin/kg of diet. These results suggest that Korean soybean paste has a protective effect with regard to aflatoxin toxicity.

P093 *ASPERGILLUS FLAVUS* RADIAL GROWTH RATE AND LAG TIME AS AFFECTED BY NATURAL AND SYNTHETIC ANTIMICROBIAL AGENT CONCENTRATIONS

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Determining mold growth responses under the combined effects of preservation factors (including natural antimicrobials) reveals the effectiveness, as antimycotic environments, of factor interactions. Radial growth rate (RGR) and lag time (LT) of *A. flavus* in potato-dextrose agar (PDA) formulated at selected a_w (0.99 or 0.95), pH (4.5 or 3.5) and concentrations (100, 200, 300... up to 2000 ppm) of vanillin, thymol, eugenol, carvacrol, citral, potassium sorbate (KS) or sodium benzoate (NaB) were determined. PDA was prepared with sucrose and hydrochloric acid to adjust a_w and pH, and tested antimicrobial concentrations were inoculated with 2 ml of a 10^6 spore/ml suspension, incubated at 25°C, and observed daily for 60 days. After the lag period, colony diameter was measured and

RGR determined by linear regression. RGR varied from 0.037 (a_w 0.95; pH 3.5; 500 ppm eugenol) to 0.609 mm/h (a_w 0.99; pH 4.5; 100 ppm NaB). For every antimicrobial, an ANOVA demonstrated that a_w, pH, and antimicrobial concentration, as well as their interactions, significantly ($P < 0.05$) affected *A. flavus* RGR and LT. Depending on a_w and pH, RGR decreased as antimicrobial concentration increased, or maintained approximately the same value until a critical antimicrobial concentration was reached, at which point a drastic RGR reduction was observed. Several combinations inhibited growth for 60 days, while others significantly ($P < 0.05$) delayed growth, such as 300 ppm thymol, a_w 0.99 and pH 3.5, for which growth was observed after 55 days. *A. flavus* presented important sensitivity differences to the evaluated factors. Therefore, depending on a_w and pH, a more rational selection of antimicrobials is possible.

P094 HURDLE TECHNOLOGY AND *ASPERGILLUS FLAVUS* TIME-TO-GROWTH

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The effects of incubation temperature (15 or 25°C), a_w (0.98, 0.96 or 0.94), pH (5.0, 4.0 or 3.0), vanillin (V) or sodium benzoate (NaB) concentration (0, 200, 400... up to 1000 ppm) were evaluated on *Aspergillus flavus* time-to-growth. Potato dextrose agar (PDA) was prepared in triplicate for each combination of factors, inoculated with 2 mL of a 10^6 spore/ml suspension, incubated, and observed after 100, 200, 300... up to 600 h for mold colony formation. From a total of 3888 observations, growth was observed in 1488, of which 1358 cases occurred at 25°C but only 130 occurred at 15°C. At 25°C, in 543 cases the mold grew before 600 h of incubation in PDA formulated with pH 5.0, in 464 cases at pH 4.0, and in 351 cases at pH 3.0. At 15°C, 49 growth observations occurred at pH 5.0, 44 at pH 4.0, and only 37 at pH 3.0. In 552 cases, mold growth was observed for NaB at 25°C, and in 53 cases when incubated at 15°C. V allowed mold growth in 806 cases at 25°C and in 77 cases at 15°C. For NaB incubated at 25°C, growth was observed in 222 cases when a_w was fixed at 0.98, in 159 cases at a_w 0.96, and in 171 cases at a_w 0.94. In the presence of sub-inhibitory antimicrobial concentrations, longer times-to-growth were observed at 15°C, reduced a_w and pH, indicating the efficacy of antimicrobial agents in controlling *A. flavus* spoilage in chilled, acidified, and/or sugared food products.

P095 SURVIVAL AND GROWTH OF *SALMONELLA* IN RECONSTITUTED INFANT CEREAL HYDRATED WITH WATER, MILK, OR APPLE JUICE

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Salmonella has caused foodborne illness through consumption of a variety of foods, including dry cereal and infant foods. The objective of this study was to assess survival/growth of *Salmonella* inoculated (four-strain mixture) into rice, oatmeal, and mixed rice-oatmeal-wheat infant cereal, hydrated (1/6, w/v) with apple juice, pasteurized milk (2% fat), or water. The inoculated products were stored at 4, 15, or 25°C for 0, 8 and 24 h to simulate advance preparation and abuse. Samples were analyzed by plating on tryptic soy agar with 0.6% yeast extract (TSAYE) and on XLT4 agar. There were no changes in populations of *Salmonella* in any hydrated cereal stored at 4°C for 24 h (average XLT4 agar counts 3.3 to 3.8 log CFU/g). At 15°C, XLT4 agar counts increased in cereal hydrated with water or milk from 1.8 to 2.1 log CFU/g at time 0 to 3.9 to 5.4 log CFU/g in 24 h, while at 25°C they reached 6.9 to 7.8 log CFU/g in 24 h. In cereal hydrated with apple juice, growth of *Salmonella* was 1.2 to 2.7 log CFU/g after 24 h at 25°C. Average changes at 8 h in cereal hydrated with water, milk, or apple juice were 0.8 to 1.4, 0.4 to 1.8, and -0.1 to 1.8, and 1.2 to 1.3, 0.9 to 1.6, and 0.2 to 0.4 log CFU/g, at 15 and 25°C, respectively. Bacterial populations in TSAYE followed similar trends. Thus, hydrated infant cereal should be consumed immediately after preparation or held at 4°C.

P096 EVALUATION OF LIQUID EGG WHITE PASTEURIZATION GUIDELINES FOR *SALMONELLA*

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D-values for a five-strain cocktail of *Salmonella* (*S.* Enteriditis ≤ phage type 4 & 13/, *S.* Typhimurium; TM-1, *S. blockley* and *S. heidelberg*) in liquid egg white at pH 7.8, 8.2, 8.8, and 9.3 were determined using a sealed capillary tube method. Fresh shell eggs were obtained from the University of Nebraska poultry farm. Fresh eggs were used to obtain egg whites with pH of 7.8 and 8.2. For pH values of 8.8 and 9.3, the shell eggs were held at 5°C for one and two weeks, respectively. The pH was monitored and small adjustments were made with 1N hydrochloric acid or sodium hydroxide. The egg white products were inoculated to approximately 1×10^9 CFU/ml and heated to various temperatures (54.4, 55.5, 56.7 and 57.7°C) for various time intervals in a circulating water bath. Temperatures were monitored with a calibrated thermocouple and recorded continuously on a Campbell Scientific CSI data logger. The heat resistance of *Salmonella* was greatly reduced as the pH of the egg white was increased. Using the required USDA pasteurization temperature of 56.7°C (134°F) and 3.5 min hold, for egg white without chemicals, even the highest pH tested (9.3) only reached a 4 log reduction. When the USDA regulations were written in 1969, it was based on a 9.0 pH. Eggs reach the market faster now, and the pH of the egg white is lower in fresher eggs. These results indicate that present time and temperatures may not be sufficient in liquid egg white products, especially at pH values below 9.3.

P097 NEW EASY-TO-READ, QUANTITATIVE METHOD FOR *ESCHERICHIA COLI* TESTING IN FOODS

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Accurate and rapid detection of *Escherichia coli* is of significant interest in the food industry. Enumeration of this bacterium is often a requirement by regulatory agencies for release of food products. The 3M™ Petrifilm™ Select *E. coli* Count (SEC) Plate has been developed to provide quantitative *E. coli* counts from food in 24 h with no interference from other bacteria. Samples of naturally con-

taminated foods were tested to compare the Petrifilm plate method to the TBX chromogenic agar method, which is described in ISO Standard 16649-2, Horizontal method for the enumeration of presumptive *Escherichia coli* - Colony counting technique at 44°C using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronic acid. The regression equation resulting from the analysis of 109 samples is log (TBX) = -0.04 + 0.91 log (Petrifilm SEC) with r = 0.96. Mean log difference (log Petrifilm counts minus log TBX counts) was 0.24. These results indicate that compared to ISO 16649-2, the Petrifilm SEC plate method is an acceptable new method for detecting *E. coli* in food.

P098 INHIBITORY ACTIVITY OF *BIFIDOBACTERIUM LONGUM* HY8001 AGAINST VEROCYTOTOXIN OF *ESCHERICHIA COLI* O157:H7

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Verocytotoxin (VT)-producing *Escherichia coli* (VTEC), such as *E. coli* O157:H7, are emerging foodborne pathogens worldwide. Attachment of the B subunit of VTs to its receptor, globotriaosylceramide (Gb_3), in gut epithelium is the primary step and, consequently, VTs inhibit protein synthesis in the target cell. Proinflammatory cytokines, such as TNF-α and IL-1β, upregulate Gb_3 expression so as to increase the sensitivity to VTs and enhance the ability of VT to cause disease. Currently, there is growing interest in probiotics because of increasing occurrence of antibiotic resistant bacteria, and much work has been done with regard to technological and therapeutic applications. In Korea, a neutralizing effect of culture supernatant of *Bifidobacterium longum* HY8001, Korean isolate, against the VTs from *E. coli* O157:H7 was found. Therefore, this study focused on unravelling the inhibitory effect of *B. longum* HY8001 against VTs. Mice were inoculated intragastrically with *B. longum* HY8001 culture supernatant before and after challenge with *E. coli* O157:H7. Control mice were inoculated intragastrically with *E. coli* O157:H7 only. mRNA expression of the cytokines, TNF-α and IL-1β, was decreased and expression of Gb_3 in renal tubular epithelial cells was reduced in mice treated with *B. longum* HY8001 culture supernatant. In competitive ELISA, culture supernatant of *B. longum* HY8001 primarily binds VTs to interfere with the interaction of VTs with Gb_3. These results suggest that soluble substances in *B. longum* HY8001 culture supernatant have inhibitory effects on the expression of Gb_3, or VT- Gb_3 interaction, or both. Further study will elucidate the properties of soluble substances in *B. longum* HY8001 culture supernatant.

P099 EFFECT OF GLUCOSE SUPPLEMENTATION ON GROWTH AND ACID TOLERANCE OF *ESCHERICHIA COLI* O157:H7 IN PURE AND MIXED CULTURES WITH A *PSEUDOMONAS* SPP. AT 10°C

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The natural flora may affect resistance of pathogenic bacteria, such as *Escherichia coli* O157:H7, to acid. This study evaluated growth and acid tolerance of *E. coli* O157:H7 in co-culture with a *Pseudomonas* strain of meat origin. Pure or mixed (1:1) cultures of *Pseudomonas* and a rifampicin-resistant derivative of *E. coli* O157:H7 ATCC 43895 were prepared in glucose-free tryptic soy broth plus 0.6% yeast extract (TSBYE-G), or supplemented with 1% glucose (TSBYE+G). Changes in populations of the inoculated (10^5 CFU/ml) cultures were determined during storage at 10°C for 14 days, while acid tolerance was assessed at 2 and 7 days by exposure to lactic or acetic acid (pH 3.5 or 3.7). In pure culture, *E. coli* O157:H7 exceeded 8 log CFU/ml at 14 days, irrespective of glucose supplementation. In contrast, its maximum growth was 6.1 ± 0.2 (4 days) and 7.4 ± 0.7 (14 days) CFU/ml in mixed TSBYE+G and TSBYE-G cultures, respectively, while its populations in TSBYE+G cultures declined to 5.3 ± 0.4 log CFU/ml by day 14. *Pseudomonas* grew abundantly (>8 log CFU/ml) by day 2, irrespective of glucose. At 2 days, the acid tolerance of *E. coli* O157:H7 was similar in pure or mixed cultures, with acid-adapted (TSBYE+G) being more tolerant than nonadapted (TSBYE-G) populations. At 7 days, however, acid-adapted, but not nonadapted, *E. coli* O157:H7 from mixed cultures became more sensitive than those from pure cultures. Thus, inhibition and acid weakening of *E. coli* O157:H7 may be enhanced by *Pseudomonas* in the presence of glucose in foods.

P100 INFLUENCE OF PROCESS PARAMETERS ON THE LETHALITY OF *ESCHERICHIA COLI* O157:H7 DURING PULSED ELECTRIC FIELDS PROCESSING

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Pulsed electric fields (PEF) processing is one of the promising methods for inactivation of microorganisms. Better understanding of PEF processing parameters will be helpful in designing a successful continuous PEF system. In this research, the combined effect of various PEF process parameters on the lethality of *Escherichia coli* O157:H7 was investigated. The inactivation of *E. coli* O157:H7 by PEF as a function of field strength (20-44 kV/cm), temperature (20-50°C), electrical conductivity (0.08-0.16% $CaCl_2$) with 3.25mS pulse width, 16.67mHz frequency and electrode gap (2.6 and 3.4mm) in a static treatment chamber was studied. A gellan gum gel matrix was used to hold bacterial cells in place during treatment. After treatment the samples from control and treated area were taken, dissolved using a calcium sequestrant, and enumerated. Energy input was calculated by integrating the product of voltage, current and time. Up to 4 log reduction was achieved, with the maximum reduction at 35kv/cm with 3.4mm gap at 50°C. Microbial inactivation increased with an increase in energy input to the system for all conditions except at 44kV/cm with 2.6mm gap. However, increasing electrical conductivity did not have a significant effect on microbial inactivation. Within the range of conditions of the study, temperature did not play a role in inactivation and no synergistic effect with electric field was observed. Electric field strength played a major role during inactivation. These results would aid in developing a correlation between energy input and microbial resistance to PEF processing.

P101 DETEX FOR DETECTION OF *ESCHERICHIA COLI* O157 IN RAW GROUND BEEF AND RAW GROUND POULTRY

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Escherichia coli O157:H7 is a particularly virulent serotype of enterohemorrhagic *E. coli* which causes severe disease in humans. The organism is particularly dangerous to the very young and the elderly, who may develop hemolytic uremic syndrome and other potentially lethal complications as a result of infection. The most common source of O157:H7 outbreaks is raw or undercooked beef. Since only a few cells are necessary to cause illness, detection of low levels of this organism quickly and accurately is critical for public safety. The objective of this study was to examine the efficacy of the Detex *E. coli* O157 Assay in detection of the organism in artificially-spiked raw ground beef and raw ground poultry. The Detex Assay is an antibody-based assay that utilizes a patented antibody-specific metal-plating procedure for the detection of *E. coli* O157 in enriched meat samples. Two hundred and sixty-four raw ground beef and raw ground chicken samples were spiked with either *E. coli* O157 (1-10 CFU) or non-*E. coli* O157 (100-1,000 CFU) strains and tested on Detex following enrichment in modified EC plus novobiocin. The Detex assay correctly identified 198 of 202 positive samples and 56 of 62 negative samples, resulting in a sensitivity of 98% and a specificity of 90%. In a second study, replicate samples of raw ground beef, raw ground chicken and raw ground turkey samples were assayed by using Detex and the USDA/FSIS reference method. Method agreement between the *E. coli* O157 Detex assay and the USDA/FSIS method was 96%. These results indicate that the Detex *E. coli* O157 assay is a useful new rapid method for the identification.

P102 RESUSCITATION AND GROWTH OF HEAT- AND FREEZE-INJURED *ESCHERICHIA COLI* O157:H7 IN SELECTIVE ENRICHMENT BROTHS

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Escherichia coli O157:H7 strains, ATCC 35150 and 43894, were heat-injured in beef infusion at 53°C for 40 and 50 min, respectively, and freeze-injured in the same infusion at -25°C for 30 days, as determined by plating both uninjured and injured cells on MacConkey agar with 0.60% bile salts #3 (Mac-BS) as the selective medium and on Brain Heart Infusion agar (BHIA) as the nonselective medium. Repair of injury was measured in five selective enrichment broths (buffered peptone water supplemented with vancomycin, cefsulodin, and cefixime [BPW-VCC], modified EC broth with novobiocin [mEC+n], enterohemorrhagic *E. coli* enrichment broth [EEB], double modified Tryptic soy broth [dmTSB], and BCM™ *E. coli* O157:H7 enrichment broth [BCM™-EB] versus TSB as the nonselective control) over 3 h incubation at 37°C and 42°C as determined by the differential between enumeration on Mac-BS and BHIA. In mEC+n, EEB, and dmTSB, death of both heat- and freeze-injured cells during the 3 h incubation (decrease counts on BHIA) occurred immediately with either minimal or no repair of the injured cells at both temperatures. Efficient repair of both heat- and freeze-injury was obtained with BPW-VCC and BCM™-EB. In BCM™-EB, however, repair, growth rates, and final cell concentrations for both strains exceeded BPW-VCC and were closer to the responses observed in TSB. Both BCM™-EB and BPW-VCC inhibited the growth of all tested Gram-positives and a select number of Gram-negatives. The ability of BCM™-EB to resuscitate heat- and freeze-injured *E. coli* O157:H7 warrants further testing with other types of stress and in both artificially and naturally contaminated foods.

P103 CHANGES IN THERMAL SENSITIVITY RESULTING FROM PH AND NUTRITIONAL SHIFTS OF ACID-ADAPTED AND NON-ACID-ADAPTED *LISTERIA MONOCYTOGENES* SCOTT A, A SEROTYPE 4B STRAIN

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Listeria monocytogenes cells were acid adapted (grown to stationary phase in TSB containing 1% glucose) or non-acid-adapted (grown in TSB without glucose) and heat challenged in fresh and conditioned media (filter sterilized media previously used to culture *L. monocytogenes*) at pH 4.8 and pH 7, with and without 1% glucose. Acid-adapted cells were the most thermally sensitive when challenged in pH 4.8 conditioned medium containing glucose (D60 = 0.41 min) and were most thermally resistant when challenged in fresh medium without glucose at pH 4.8 (D60 = 0.91 min). The presence of 1% glucose increased the D60 thermal sensitivity of acid-adapted cells in fresh and conditioned medium at pH 4.8 and 7, approximately 35 and 14%, respectively, compared to the corresponding challenges performed in menstrua without added glucose. Acid-adapted cells challenged in pH 4.8 fresh media with or without added glucose were more thermally resistant than cells challenged in fresh media at pH 7. Non-acid-adapted cells were most thermally resistant when challenged in fresh and conditioned pH 7 media containing 1% glucose (D60 = 1.52 and 1.28 min, respectively) and were less resistant when challenged at pH 4.8 with or without glucose or at pH 7 without glucose (D60 values ranging from 0.67 to 1.12 min). These data indicate that the presence and level of cross protection is dependent on the physiological state of the cells and nutrient availability at the time of thermal challenge. Such conditions should be considered to insure that stressed pathogens in foods are destroyed or inactivated.

P104 COMPARISON OF PREDICTIVE MODELS FOR A 4-LOG THERMAL REDUCTION OF *LISTERIA MONOCYTOGENES* WHEN GROWTH CONDITIONS DIFFERED

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Predictive models for inactivation of *Listeria monocytogenes* were developed, with one model using cells grown in tryptic soy broth (TSB) at pH 7.0 and another model using cells grown at all combinations of pH (5.0, 6.0, 7.0) and milkfat (0, 2.5, 5%). Cells were then inactivated in a 3 × 3 × 3 factorial design of temperature (55°, 60°, 65°C), pH (5.0, 6.0, 7.0) and milkfat (0, 2.5,

5.0%), with each combination done in triplicate. The 27 individual treatment conditions were modeled separately with the modified Gompertz equation and subsequently combined into a full regression model. Comparison of 4D-values at various levels for pH and milkfat combinations of the two models suggested that there were differences in thermal resistance. The most marked difference in thermal resistance was a 50 to 100% increase in 4D-values for cells grown at pH 6.0 and all milkfat levels compared to cells grown at pH 6.0 in TSB before thermal inactivation at 60°C. These differences were not noted at pH 5.0 or 7.0, which could be attributed to adaption to mildly acidic environments. This suggests that growing cells in conditions similar to those of foods could affect thermal resistance of *L. monocytogenes* cells. Therefore, predictive microbiologists should consider growth conditions of cultures used in inactivation studies because the predictive estimates could be changed by adaption of cells to sub-lethal environmental factors.

P105 THERMAL INACTIVATION STUDIES OF *LISTERIA MONOCYTOGENES* STRAINS BELONGING TO THREE DISTINCT GENOTYPIC LINEAGES

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Strains of *L. monocytogenes* have been classified into three different lineages according to their genotypic similarities. Studies carried out by Wiedmann et al. have demonstrated that most of the strains isolated from epidemic outbreaks of listeriosis belong to lineage I. These data suggest that each lineage possesses distinct characteristics. Thermal inactivation studies were performed on 21 *L. monocytogenes* strains belonging to the three different lineages to establish differences, if any, in heat sensitivity between both lineages and individual strains. Three inactivation conditions were examined: (1) 60°C, pH 6.0, 0.5M Lactate, (2) 55°C, pH 6.0, 0.5M Lactate and (3) 50°C, pH 4.0, 0.5M Lactate. In addition, cultures were grown in TSB with 1% glucose and TSB without glucose. Heat inactivation was carried out using a submerged-coil heating apparatus and the D-values calculated by linear regression. The average D values for the strains grown with glucose were 2.68, 31.49 and 0.27 min for conditions 1, 2 and 3, respectively. For the strains grown without glucose, the average D values were 3.77, 59.96 and 0.22 min, respectively. Cells grown with glucose had shorter D values than the respective cells grown without glucose for conditions 1 and 2; no differences were evident for condition 3. Lineages I and II showed higher average thermotolerance than lineage III for conditions 1 and 2. Strains from all lineages showed similar thermotolerance for condition 3. The standard deviation-to-mean ratio between strains averaged 0.29.

P106 CYCLOHEXIMIDE REPLACEMENT IN CAMPY-LINE AGAR FOR *CAMPYLOBACTER* ENUMERATION

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Recently, a new agar was developed for enumeration of *Campylobacters*. This new selective medium, Campy-Line agar (CLA), was developed to facilitate *Campylobacter* enumeration by combining selective antimicrobials and triphenyltetrazolium chloride (TTC). Growth of many microorganisms reduces the initially colorless tetrazolium salts to insoluble formazan compounds, which impart a red color to the growing colonies. Unfortunately, the manufacture of one of the key antimicrobials in the earlier CLA formulation (cycloheximide) has been curtailed and the world-wide supply is being diminished. The purpose of this study was to find replacement antimicrobials for cycloheximide which demonstrated similar selectivity without decreasing *Campylobacter* recovery. Over 150 different combinations of antimicrobials were tested against selected *Campylobacter* spp. and common poultry carcass contaminants. The trials demonstrated that the cycloheximide may be replaced by adding rifampicin (10 mg/l) and amphotericin B (25 mg/l) or by adding rifampicin (10 mg/l) and nystatin (50 mg/l). The substitution of these antibiotics was confirmed using naturally contaminated commercial, post-chill, post-drip, broiler carcasses (n=19) which were rinsed by standard methods and plated for recovery of *Campylobacter* and contaminants on the original and modified agars. The number of *Campylobacter* spp. colony forming units/ml of carcass rinse was not significantly affected by the replacement antibiotics in the modified media and no non-*Campylobacters* were observed on the plates. Recovery of *Campylobacters* on the modified media was similar to recovery on Cefex agar, however more non-*Campylobacter* contaminants (mean of 3.3 CFU/ml) were seen on the Cefex. The mean log values were not significantly different between groups ($P < 0.05$).

P107 DETEX FOR THE DETECTION OF *CAMPYLOBACTER* IN RAW AND COOKED POULTRY

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Campylobacter is considered to be the most common cause of infectious diarrhea in the United States, with estimates exceeding 2 million cases per year. Three species, *C. jejuni*, *C. coli*, and *C. lari*, account for the vast majority of clinical isolates. Risk factors for the disease include handling raw poultry and eating undercooked poultry. The objective of this study was to compare the Detex *Campylobacter* Assay to conventional culture methods for the detection of *Campylobacter* in raw and processed poultry. The assay is an antibody-based assay that utilizes a patented antibody-specific metal-plating procedure for the detection of *Campylobacter* in enriched meat samples. A total of ninety-five *Campylobacter* and non-*Campylobacter* strains were grown in *Campylobacter* Enrichment broth and tested on the Detex MC-18 apparatus using the *Campylobacter* Assay. The Detex assay identified 54 of 55 positive samples correctly (sensitivity = 98%) and 40 of 40 negative samples correctly (specificity =100%). When replicate samples of ground chicken, ground turkey and chicken carcasses were compared to the reference methods (USDA/FSIS Guidebook or Bacteriological Analytical Manual), Detex was found to be in good agreement with these methods. Thus, the Detex *Campylobacter* Assay is a useful tool for the identification of *Campylobacter* in raw and processed poultry.

P108 SURVIVAL AND THERMOTOLERANCE OF *CAMPYLOBACTER JEJUNI* IN LIQUID FOODS: EFFECTS OF TEMPERATURE AND PRESENCE OF *ESCHERICHIA COLI* AND *PSEUDOMONAS FLUORESCENS*

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To determine the survival of *Campylobacter* in liquid foods, milk and chicken broth were inoculated with *Campylobacter jejuni* (ATCC 5096) to a level of ca. 9 $\log_{10}$ CFU/ml in the presence and absence of ca. 5 $\log_{10}$ CFU/ml of *Escherichia coli* JM 109 and *Pseudomonas fluorescens* W 51 (competitive flora; CF). Viable counts were determined during storage at 4, 25, and 37°C for up to 21 days under aerobic conditions. Populations of *C. jejuni* decreased by 4 $\log_{10}$ CFU/ml in milk over 24 h at 25 and 37°C both in the presence and absence of CF and the organism was undetectable on day 14 (detection limit was 1.32 $\log_{10}$ CFU/ml). At 4°C, *C. jejuni* populations decreased by ca. 7 $\log_{10}$ CFU/ml on day 14 with and without CF. In chicken broth stored at 4, 25, and 37°C, *C. jejuni* counts decreased by 7 to 7.5 $\log_{10}$ CFU/ml on day 21 in the presence and absence of CF. At 4°C, neither *E. coli* nor *P. fluorescens* grew; however, at 25°C and 37°C, *E. coli* and *P. fluorescens* populations increased by 1.5 to 3 $\log_{10}$ CFU/ml in both milk and chicken broth over 48 h and were still detectable by day 14. Related studies examined the effect of the presence of CF on the thermotolerance of *C. jejuni*. The D-values for *C. jejuni* incubated in milk at 37°C for 24 h were 56, 40.6, and 27.6 s at 55, 58, and 62°C in the absence of CF, and 71, 46.2, and 34 s at 55, 58, and 62°C, respectively, in the presence of CF. Similar D-values were observed for *C. jejuni* in chicken broth.

P109 EFFECTIVENESS OF SELECTED CHEMICAL SANITIZERS AGAINST *CAMPYLOBACTER JEJUNI* CONTAINING BIOFILMS

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Survival of *Campylobacter jejuni* in biofilms was determined after treatment with chemical sanitizers including peracetic acid and peroctanoic acid mixture (POAA+POOA), peracetic acid, hypochlorite and quaternary ammonia. Biofilm producers were isolated from chicken house nipple drinkers. A meat plant isolate of Pseudomonas spp. was also included as a biofilm producer. Two-day-old biofilms of gram-positive rods, Y1 and W1, and *Pseudomonas* spp. grown on polyvinyl chloride plastic (PVC) coupons in R2A broth at 12°C were incubated with 10^6 CFU/ml of *C. jejuni* for 6 h to allow attachment. The coupons were rinsed and incubated in fresh media for an additional 24 h. *C. jejuni*-containing biofilms were removed by vortexing with glass beads in modified Brucella broth and then enumerated for *C. jejuni* on selective/differential media. Presence of biofilm enhanced ($P < 0.01$) attachment and survival of *C. jejuni*. Only 20 CFU/cm² of *C. jejuni* were recovered from the control without biofilms compared to 5000 to 2500 CFU/cm² in samples with preexisting biofilms. Type of biofilm affected ($P < 0.01$) the effectiveness of sanitizers against *C. jejuni*. Sodium hypochlorite was the most effective sanitizer for inactivation of *C. jejuni* in the biofilms, since it completely inactivated the pathogen after treatment at 50 ppm for 45 s. *C. jejuni* in biofilms was susceptible to all sanitizers tested but was not completely inactivated by treatment with quaternary ammonia or POAA+POOA at 50 and 200 ppm for 45 s.

P110 HEAT SHOCK ENHANCES ACID TOLERANCE OF *SHIGELLA FLEXNERI*

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Cross protection of stressed foodborne pathogenic bacteria against subsequent exposure to otherwise lethal environmental stresses enhances the potential for survival and growth. In a preliminary study, we observed that acid-adapted and acid-shocked cells of *Shigella flexneri* survived at lower pH than did cells not previously exposed to an acidic environment. The study reported here was done to determine the influence of heat shock on subsequent acid tolerance of *S. flexneri*. Cells grown in tryptic soy broth containing no glucose (TSB) were heated at 48°C for 15 min, then inoculated into TSB containing 0.25% glucose (TSBG) and acidified to pH 3.5, 4.0, and 4.5 with acetic, lactic, or propionic acid. Viable cells were enumerated over a 6-h period of incubation at 37°C. Populations of cells inoculated into TSBG acidified to pH 3.5 with all three acids rapidly declined, while a more gradual decline occurred at pH 4.0. The number of heat-shocked cells declined approximately 3 $\log_{10}$ CFU/ml, whereas unheated cells declined 5 $\log_{10}$ after 30 min in TSBG acidified to pH 3.5 with acetic acid. Populations remained constant at pH 4.5, regardless of acidulant used to adjust pH. Chloramphenicol prevented the development of heat-induced acid tolerance, indicating that synthesis of heat-shock proteins is correlated with development of acid resistance. Results suggest that exposure of *S. flexneri* cells to a mild heat treatment renders them more tolerant of acidic environments and may enhance survival and ability to grow in acidic foods.

P111 EFFECT OF ORGANIC ACIDS AND TEMPERATURE ON SURVIVAL OF *SHIGELLA FLEXNERI* IN BROTH

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Bacterial survival at low pH depends not only on the pH value, but also on the type of acid and on storage temperature. *Shigella*, a major foodborne pathogen, is acid tolerant. We studied survival of *Shigella flexneri* strain 5348 in brain heart infusion broth (BHI) supplemented with organic acids. Media containing 0.04 M acetic, citric, lactic, malic, or tartaric acid were adjusted to pH 4 with HCl or NaOH. The control medium was BHI adjusted to pH 4 with HCl. Stationary phase cells were inoculated into media to give initial populations of 6 to 7 $\log_{10}$ CFU/ml and incubated at 4, 19, 28, and 37°C. A two-phase linear inactivation model was applied to plate count data to calculate the time for a 4-$\log_{10}$ decrease in population (T4D). In all cases, survival increased with decreasing temperature. All acids inhibited *S. flexneri* to some extent: lactic > acetic > citric, malic, tartaric acid. At 28°C, the T4D values for the control medium and media

containing acetic, citric, lactic, malic, and tartaric acids were 232, 145, 153, 120, 155, and 166 h, respectively. *S. flexneri* was undetectable (<1.32 $\log_{10}$ CFU/ml) after 96 h at 28°C in BHI acidified to pH 4 with acetic or lactic acids, while the population remained unchanged in BHI acidified with HCl. Results suggest that organic acids may aid in inactivation of *Shigella* in foods.

P112 RESPONSE OF FOOD SPOILAGE *BACILLUS* SPP. TO THREE ACID-BASED SANITIZERS

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Three commercial sanitizers containing peroxyacetic/peroxyoctanoic acids (PPA), peroxyacetic acid/hydrogen peroxide (PAH) or nitric acid/iodine (NAI) were evaluated in vitro at recommended and double recommended concentrations for 1 or 10 min exposure times. Efficacy tests were performed on 8- or 24h-old *Bacillus cereus* DL5 and *B. subtilis* EL39 cells either in planktonic form or attached to stainless steel surfaces, or in planktonic form but grown in the presence of attachment surfaces (surface influenced planktonic (SIP)). Cells were grown in Tryptone Soya Broth (TSB) or 1/10 strength TSB (low nutrient medium (LNM)). Attached cells grown in LNM were less susceptible to all sanitizers than those grown in TSB. Furthermore, attached cells of both isolates were less susceptible to all sanitizers than planktonic or SIP cells, with 24h-old cells being more resistant than 8h-old cells. By contrast, planktonic and SIP cells of both isolates in LNM were in most cases more susceptible than those grown in TSB. Furthermore, 24h-old planktonic and SIP cells of both isolates were, in most cases, more susceptible than 8h-old. In most cases, sanitizer efficacy in decreasing order against both isolates was PAH>PPA>NAI, with *B. subtilis* EL39 marginally more resistant than *B. cereus* DL5. None of the sanitizers tested met proposed efficacy guidelines for planktonic (99.999% kill) or attached cells (99.9% kill), even when exposure times of 10 minutes and double the recommended concentrations were used.

P113 PRESENCE OF TOXIGENIC *BACILLUS* IN CUP DRINKS FROM AUTOMATIC VENDING MACHINES ON THE STREET

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In order to investigate the microbiological safety of cup drinks sold in automatic vending machines on the street in Seoul and its vicinity, the microbes in the cup drinks were analyzed and characterized. Fifty predominant strains isolated were Gram positive strains and 87% of them were *Bacillus* spp. as identified by the BioLog ID System. The strains having hemolytic activities and opaque halos on the *Bacillus* selective MYP medium were isolated and identified. Among 210 strains, 20 strains had the hemolysin BL gene (hbl) by PCR analysis, indicating that they seemed to be diarrheal strains. Half of them were identified as *Bacillus cereus* group by biochemical and physiological analysis and by the BioLog ID System. Emetic strains were also confirmed by the MTT assay. They showed growth even at 15°C and in 7% NaCl medium, so they could grow inside the machine on the street. Therefore, street food such as cup drinks from a vending machine need to be controlled from a safety aspect.

P114 MONTE CARLO SIMULATION OF THE INFLUENCE OF SPORE INOCULUM SIZE ON *CLOSTRIDIUM BOTULINUM* GERMINATION AND GROWTH

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Many models developed for *Clostridium botulinum* have used relatively high inoculum sizes (>1,000 spores/ml), while the contamination level in real food systems is generally very low (<1 spore/ml). When results obtained from high inocula are directly applied to a system with significantly lower inoculum size, independence between the spores is assumed. We have showed previously that inoculum size of *C. botulinum* spores influences time-to-detection, but this approach considered only the mean response of a group of replicates. In our current study, we used a different approach (Monte Carlo simulation) to consider the data provided by all the replicates in each condition, and the inherent variability in those responses. Data were obtained spectrophotometrically and modeled with the Gompertz equation, then time-to-detection was calculated from the equation parameters. Doubling time in h and the lag time were calculated for each observation through a series of equations. BestFit was used to determine the best distribution. Log logistic and logistic distribution were found to fit time-to-detection very well over all conditions. Logistic distribution was the most promising lag time distribution. No distribution is universally applicable to all doubling times. Three simulation scenarios were evaluated using @Risk and Analytica. The simulation results confirmed our conclusion by the modeling approach: inoculum size affects germination time of *C. botulinum* spores. The secondary models developed in the first stage of this research are available as a user-friendly Analytica program on the web. Further biochemical research may shed light on the mechanism behind the inoculum size effect.

P115 ESTIMATION OF BACTERIAL CELL COUNTS IN FOODS USING AN OXYGEN ELECTRODE SENSOR

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Oxygen electrode sensors have been applied to estimate bacterial cell counts in foods. Bacterial respiration was monitored in the cell equipped with the newly developed electrode. The relationship between respiration of bacteria and bacterial cell counts on agar plates was examined. Using various kinds of food samples, the new method was evaluated. Samples were processed with a stomacher for one minute in saline, and injected into the 96-well sensor plate with oxygen electrodes embedded. After nutrient broth was added to each well, concentration of dissolved oxygen in each sample was monitored continuously for 24 h at a temperature of 35°C. Detection time in the oxygen electrode method was defined as the elapsed time to the point when the dissolved oxygen has been consumed by bacterial respiration to 60% of oxygen concentration at negative control. It depended on the number of conven-

tional plate count. As for samples containing 100,000 CFU/g, bacteria detection time was approximately 6 h, and it decreased linearly with the log number of standard plate count, with a slope of -2.6 [hour/CFU/g]. Correlation coefficient for the estimated cell count with reference curve and conventional plate count was 0.83. This new method detected bacteria more rapidly, in proportion to bacterial concentration in foods.

P116 RAPID DETECTION OF *LISTERIA MONOCYTOGENES* WITHOUT DNA EXTRACTION FROM FOODS USING POLYMERASE CHAIN REACTION

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This study was conducted to develop a rapid method for the detection of *Listeria monocytogenes* in foods via polymerase chain reaction (PCR). Specificity, sensitivity, optimal condition of PCR and application of hly A gene or 16S rRNA-based primer for the detection of *L. monocytogenes* from milk, beef, and vegetables using whole cells without DNA extraction was investigated. Each of the 20 *L. monocytogenes* strains gave a single 713 bp band for hly gene and 70 bp band for 16S rRNA based primer, but other *Listeria* spp. and other bacteria did not show any bands. The sensitivity was significantly improved by the further 15 cycle after 20 cycle PCR amplication. Milk (10 mL), beef (10 g), cabbage (10 g), and mushroom (10 g) were inoculated with *L. monocytogenes* at the concentrations ranging from 0 to 10^7 CFU/mL or g to determine the best sensitivity of PCR for the detection of the bacteria. PCR assay could detect 2 cells and 2.6×10^2 cells in milk and beef with 2nd PCR using hlyA primer, whereas each 5 cells in milk, cabbage and mushroom using 16S rRNA-based primer were detected, respectively.

P117 PCR DETECTION OF *LISTERIA MONOCYTOGENES* ON HOT DOG USING OLIGONUCLEOTIDE PRIMERS TARGETING THE GENES ENCODING INTERNALIN AB

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Frequent outbreaks of listeriosis in the late 1980s prompted the USDA to adopt a zero-tolerance policy for *L. monocytogenes* in cooked, ready-to-eat meats. Since that time, numbers of meat product recalls due to *Listeria* contamination have increased dramatically. To avoid recalls and control product quality, rapid, specific and sensitive detection methods for *L. monocytogenes* are needed by the meat industry. In this study, a PCR assay targeting the genes encoding internalin (inl) AB or F was developed for detecting *L. monocytogenes* in ready-to-eat meats. Four sets of primers were evaluated. One set, targeting a 902-bp region of the inlAB, was most specific. The specific PCR product was detected in 51 *L. monocytogenes* strains belonging to 4 different serogroups (1/2a, 1/2b, 1/2c, and 4b). In contrast, the PCR product was not detected in other *Listeria* species (*L. innocua*, *L. ivanoii*, *L. geeligeri*, *L. welshimeri*, and *L. grayi*) and gram positive, non *Listeria* bacteria, indicating that the primer set was highly specific for *L. monocytogenes*. The detection limit of the PCR assay was 10^5 CFU per ml of pure cell culture. However, the assay could detect as few as 10 CFU of *L. monocytogenes* in 25 g of hot dog within 6 h after samples were enriched in modified *Listeria* enrichment broth at 37°C. The total assay time including enrichment was approximately 24 h compared to 3-4 days of conventional method. These results suggest that this PCR assay could be used to rapidly detect *L. monocytogenes* from hot dog, and possibly other types of ready-to-eat meats.

P118 INACTIVATION OF HEPATITIS A VIRUS BY A DYNAMIC HIGH PRESSURE TREATMENT

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Viral foodborne outbreaks have raised great interest during the last few years. Among viruses incriminated, hepatitis A virus (HAV) remains one of the most problematic infectious agents. Transmission of HAV by foods and its implication in acute gastroenteritidis cases were well documented in the literature. In general, all foods are susceptible to contamination with HAV. Contamination occurs through contact with contaminated irrigation water or with infected human food handlers. Several strategies were proposed to inactivate HAV in food samples including heat, irradiation, UV, ozone and microwave. Using these strategies, results are variable and the sensory quality of the product is largely affected. Therefore, new approaches remain greatly needed. In this study the effectiveness of a dynamic high pressure (DHP) treatment for the inactivation of HAV was evaluated. Wastewater as well as milk and apple juice samples were inoculated with different concentrations of the cytopathic HAV strain HM-175. Samples were then subjected to a DHP treatment using a laboratory scale device, Emulsiflex C5. The inactivation efficiency was evaluated by determining the viral concentration using the plaque assay titration method. In general and at a concentration of 10^6 PFU/ml, a pressure higher than 150 MPa is sufficient for significant inactivation of HAV. The inactivation efficiency depends on the initial HAV concentration, the pressure applied, the number of passes and the nature of the sample treated.

P119 HANDWASHING PRACTICES IN UNITED KINGDOM NURSING HOMES

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Handwashing is an important control procedure in the prevention of cross-contamination within the food industry. Research investigating the handwashing practices of food handlers has mostly concentrated on self-reported practices. However, it has been demonstrated that self-report data is not a good predictor of actual food safety behavior. Very little research has been carried out examining the actual handwashing practices of food handlers in a commercial setting. This study utilizes notational analysis to observe the handwashing practices of food handlers in UK nursing homes. Five different establishments took part in the study. Five visits of one hour were made to each premise, making a total of 25 h of observation. The

actions of the same food handler in each business was recorded at each visit to determine repeatability of specific handwashing actions. The results showed a lack of and/or inadequate implementation of handwashing at important points, specifically, after handling raw meat packaging, after handling raw eggs and before handling ready to eat foods, where handwashing was only implemented on 23%, 10% and 13% of occasions, respectively. None of the food handlers washed their hands correctly at every appropriate occasion. This study highlights the problems experienced by food handlers in relation to unsuitable equipment, notably, wastebins without foot pedals and taps which must be turned on and off by hand. No significant differences ($P > 0.05$) in the handwashing practices of the food handlers were found between visits, suggesting that this behaviour is consistent over time. This research provides important data regarding current handwashing practices in institutional catering and outlines the barriers preventing best practice.

P120 ASSESSMENT AND VARIABILITY OF CLEANING PRACTICES OF UNITED KINGDOM CONSUMERS, USING OBSERVATION, ATP, AND MICROBIOLOGICAL ASSESSMENT

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The potential spread and persistence of foodborne pathogens in domestic kitchens has been recognized as an important food safety issue. Adequate cleaning in the domestic kitchen is required to remove microbial contamination and to prevent subsequent cross contamination to other utensils or foodstuffs. The use of detergent and water, alone or in combination with a disinfectant, for adequate decontamination has been subject to much debate. Consumers were observed using CCTV while preparing set menus in a model domestic kitchen. Prior to the start of preparation the kitchen was cleaned thoroughly using a validated protocol leaving known residual values of ATP and microbial contamination. Participants were provided with a range of cleaning equipment/materials and were asked to implement normal cleaning practices after preparing the food. Cleaning practices were observed and recorded. After food preparation had been completed, ATP swabs and dipslides for microbiological analysis were taken from specified locations. Data was collected after preparation of individual and repeated food preparations. Results showed that sanitizers were used by 14% of participants. Separate chopping boards for raw and cooked foods were used by only 8% of participants and of these, adequate cleaning was observed in 13% of cases, indicating an important risk potential. Overall, only 10% of ATP values and 19% of microbial counts obtained were acceptable. The variability of individual and repeated food preparation sessions will be presented in terms of observed specific cleaning practices, ATP values and microbial counts. Variations in ATP values were found between different menus prepared, reflecting the different handling practices required for different meal preparations. Findings have indicated that domestic cleaning is inconsistent and often poorly performed, suggesting a need for consumer education.

P121 KANSAS FOOD*A*SYST: SELF-ASSESSMENT TOOLS FOR DETERMINING RISKS TO FOOD SAFETY DURING PRODUCTION AND HOME PREPARATION

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Kansas Food*A*Syst is modeled after a successful wellhead protection program, Farm*A*Syst, a series of self-assessments determining risks to water quality on the farm. Food safety self-assessment information and risk charts were developed for producers, targeting eight areas: water, wastewater, solid waste, beef production, poultry production, fruit/vegetable production, packaging/transportation, and open-air markets. Consumer information and risk charts include purchasing and home food preparation. Risk levels are tied to practices and conditions. To assure usefulness and relevance of the materials, they were evaluated by small focus groups of 3-7 consumers or growers. Members of each group tested materials in three different chapters and provided feedback. The objective was to determine best format and to refine the Kansas Food*A*Syst self-assessment tools, both in presentation format and in relevancy of content. The technical content level was found to affect placement order of risk assessment charts and relevant information. Materials for use by consumers in assessing food purchase and preparation practices have few technical terms. Information for producers, who are responsible for multiple areas, includes many technical terms. The growers preferred that technical information be presented before the risk assessment chart so that terms are defined. Conversely, presenting the chart before less technical information allows the consumer to be more honest in self-assessing risk. Producers were very interested in promoting the consumer materials to their customers. All groups indicated having learned new information. Revised assessment tools will be displayed at the poster session.

P122 EFFECT OF OZONATED WATER ON THE ASSIMILABLE ORGANIC CARBON AND COLIFORM GROWTH RESPONSE VALUES AND ON PATHOGENIC BACTERIA SURVIVAL

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The assimilable organic carbon (AOX) and coliform growth response (CGR) are bioassays used to determine water quality. AOX and CGR are better indexes in determining whether water can support the growth of bacteria than biological oxygen demand (BOD). The AOX value of reconditioned wastewater increased after ozonation from 1176 to 1758 μgC-eg/L. When the ozonated wastewater was inoculated with *Salmonella* spp., the cell counts remained constant with a slight increase after 7 days, whereas the cell counts in the non-ozonated wastewater decreased after 7 days. Ozonated tap water (20 ppm) was used to wash alfalfa seeds for 20 min. There was little decrease in the total background counts from the ozonated washwater. After washing, the washwater's AOX values increased six fold while the dissolved ozone decreased to

undetectable levels. These increases are due to ozone's strong oxidizing ability to break down refractory, large molecular weight compounds into smaller ones, which are readily used as nutrient sources for microorganisms. This same phenomenon was observed when using ozone in the treatment of drinking water. The increased nutrients would now become more readily available to any pathogenic microorganisms located on alfalfa seed surface as seen with the increase in inoculated levels of *Salmonella* in the ozonated wastewater. If the washing process using ozonated water is not followed by the recommended hypochlorite treatment, pathogen growth is still possible.

P123 ADAPTATIVE ACID TOLERANCE RESPONSE IN *VIBRIO PARAHAEMOLYTICUS* AND *V. VULNIFICUS*

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V. parahaeomolyticus (Vp) and *V. vulnificus* (Vv) are naturally occurring halophilic bacteria distributed in marine and estuarine environments throughout the world. Vp and Vv can cause gastroenteritis after consumption of raw or undercooked shellfish. The objective of the study was to examine the ability to induce acid tolerance in clinical isolates of Vp and Vv after exposure to mild acid (pH 5.5). Log-phase cultures were acidified to pH 5.5 at 35°C for 60 min and challenged to pH 4.0 for another 60 min at 35°C. Other log-phase cultures were challenged to pH 4.0 for 60 min without prior exposure to pH 5.5. Viable plate counts were determined every 15 min by serial dilution in phosphate-buffered saline and plating onto tryptic soy agar plates. Log-phase cultures of Vp and Vv required acid adaptation at pH 5.5 to induce acid tolerance. Prior exposure to pH 5.5 significantly enhanced the acid tolerance of Vp and Vv. No loss of viability of the adapted Vp was observed at pH 4.0 for up to 60 min, while numbers of the nonadapted Vp reduced by 6 log at pH 4.0 within 30 min. Numbers of the adapted Vv reduced by 2 log at pH 4.0 after acid adaptation, while no survivors of the nonadapted Vv were recovered at pH 4.0 after 15 min. This study suggests that adaptative acid tolerance in Vp and Vv may increase their ability to better survive the acid environments in the human gastrointestinal tract.

P124 THERMOTOLERANCE OF COAGULASE-NEGATIVE *STAPHYLOCOCCI* AND THEIR POTENTIAL USE AS INDICATORS OF CHEESE PLANT SANITATION

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This study examined the surface and interior of hard Italian-type cheeses for the presence of coagulase-negative Staphylococci, and evaluated thermo-tolerance of isolates to determine if they were likely introduced before or after pasteurization. Samples obtained from two cooperating cheese manufacturers and a local supermarket were plated on Baird-Parker agar with added mannitol, phenol red, and potassium tellurite (BP + MPRT). Isolates were characterized for Gram stain, catalase activity, anaerobic utilization of glucose and mannitol, biochemical profiling using the API-Staph system and coagulase production. Of 40 isolates from BP + MPRT, common isolates included *Staphylococcus epidermidis* (35.0%), *S. warneri* (5.0%), *S. caprae* (2.5%), and *S. sciuri* (2.5%), but 55% of the isolates were not identified. After exposure to 62.8°F for 30 min in skim milk, the population of each isolate usually decreased by 3.0 log CFU/ml, with counts for nine isolates decreasing at least 3.5 log CFU/ml and counts for one isolate decreasing < 2.0 log CFU/g. By comparison, two non-thermoduric *S. aureus* strains decreased in numbers by 3.6 and 3.1 log CFU/ml. These results suggest that the coagulase-negative Staphylococci were post-pasteurization contaminants from equipment or employees, with the exception of the isolate which decreased < 2.0 log CFU/ml in the thermotolerance test. This organism may have originally been present in raw milk and survived pasteurization. Therefore, the coagulase-negative Staphylococci may serve as a useful indication of post-pasteurization sanitation in plants making hard Italian-type cheeses.

P125 PROTECTING THE UNITED STATES FOOD SUPPLY IN A GLOBAL ECONOMY: AN EXPERT GAP ANALYSIS

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Foodborne disease continues to be of major concern to regulators, food manufacturers, academia and consumer protection groups worldwide. Diligent application of the HACCP approach is believed by many to provide a comprehensive system for food safety management. This belief, however, is not shared uniformly by all stakeholders. Because of this dichotomy of opinion, there is a compelling need for additional dialogue and consensus on this issue. The aim of this expert survey instrument is to identify the gaps and areas of agreement in the current food safety approaches among the various stakeholders. Two hundred thirty-one survey instruments were received out of 360 sent (64.2% response rate) to food safety professionals in academia, industry, federal and state government, and consumer protection groups. The survey consisted of four parts: (1) A series of 195 statements across the food chain describing the extent to which respondents agree with each, (2) Rank order priority ratings and degree of satisfaction with 14 statements, (3) Weighted priority rankings for 10 statements and (4) Verbatim comments. The data suggests broad support for (1) the cooperative development of a comprehensive K-12 food safety education program, (2) more consistent application of HACCP among industry and government, (3) increased focus on agricultural and animal husbandry practices for pathogen control and 4) increased focus on imported food safety. There was less consistency among stakeholders on (1) the role of microbiological finished product testing, (2) increased governmental inspection programs, and (3) impact of shelf-life on the safety of refrigerated foods.

P126 DAIRY-ASSOCIATED *BACILLUS CEREUS* GROWING AS A BIOFILM HAS A DISTINCT PROTEOME

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Biofilm-forming *Bacillus cereus* strains were isolated from alkaline wash solutions used in South African dairy factories. This study aimed to observe the cell attachment

process in situ and investigate phenotypic changes in response to surface attachment for one selected, typical isolate, *B. cereus* DL5. Biofilm development by *B. cereus* DL5 was initiated using glass wool as attachment surface, and observed by brightfield microscopy. The proteomes of 2h- and 18h-old planktonic and biofilm cells were compared using high resolution two-dimensional gel electrophoresis (2D PAGE) of whole-cell proteins. The high surface-to-volume ratio of the glass wool supported microcolony formation within 2h, and complex biofilms within 18h. Attached cells were always approximately 25% smaller than planktonic cells. The 2D PAGE analysis of whole-cell protein extracts permitted separation of more than 400 protein spots per gel. The biofilm proteome revealed ten proteins that were synthesized as a result of surface attachment of which four were unique to the biofilm profile and six were up-regulated. Seven proteins of the planktonic profile were absent in the biofilm profile. These altered proteomes indicated changes that take place in the regulation of protein expression as a result of *B. cereus* DL5 cells attaching to surfaces. Differences in protein composition between cells of the two modes of growth indicated altered phenotypic properties which may provide an explanation for phenomena such as increased tolerance of biofilm cells to sanitizers.

P127 GROWTH OF *BACILLUS CEREUS* AND *PSEUDOMONAS FLUORESCENS* BINARY BIOFILMS AND RESPONSE TO A CHLORINE DIOXIDE-CONTAINING SANITIZER IN A MODEL FLOW SYSTEM

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Single and binary biofilms of a *Bacillus cereus* DL5 from alkaline dairy wash solutions, and a *Pseudomonas fluorescens* M2 from spoiled milk, were grown on stainless steel surfaces in a laboratory scale flow chamber (flow rate 8 cm/min), and exposed daily to 100 ppm chlorine dioxide for 5 min over 6 consecutive days. Surfaces were sampled daily before and after sanitizer treatment and cells dislodged and enumerated by standard methods. Higher counts of *P. fluorescens* M2 were obtained in single species biofilms compared to binary biofilms. By contrast, higher counts of *B. cereus* DL5 were obtained in binary biofilms compared to corresponding single species biofilms. Lower spore counts were obtained for *B. cereus* DL5 in binary biofilms compared to corresponding single species biofilms. In binary biofilms, *P. fluorescens* M2 counts were always higher (0.04 to 2 logCFUcm^{-2}) than *B. cereus* DL5 counts. In binary biofilms, survival of *P. fluorescens* M2 after exposure to a chlorine dioxide-containing sanitizer was apparently enhanced by the presence of *B. cereus* DL5 compared to single species biofilms. By contrast, *B. cereus* DL5 showed increased susceptibility to sanitizer treatment in the presence of *P. fluorescens* M2 compared to single species biofilms. Generally, counts of *P. fluorescens* M2 decreased, while counts of *B. cereus* DL5 increased over the 6-day test period in their respective single species biofilms. No consistent response to sanitizer treatment was observed for counts of *B. cereus* DL5 and *P. fluorescens* M2 in binary biofilms.

P128 HEAT INACTIVATION OF *LISTERIA* BIOFILM

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Recent outbreaks of *Listeria monocytogenes* have raised concern that recontamination is occurring during or after processing, possibly due to ineffective cleaning and soil redeposition during the intercycle period. Our research objective was to develop a predictive model to determine the influence of heat treatment and/or time on the survival of *Listeria monocytogenes* and *Pseudomonas* spp. biofilms, and *Listeria-Pseudomonas* mixed culture biofilms formed on stainless steel and buna-N rubber surfaces. Biofilms were produced by immersing surfaces in 10% TSB inoculated with the appropriate cultures, followed by incubation for 4 h at 25°C, rinsing with phosphate buffer, transferring to 10% TSB and incubation for 48 h at 25°C. Duplicate biofilm samples were heat treated for 1, 3, 5, 15 min at 70, 72, 75, 77°C and tested for survivors using the fraction negative enumeration method. Positive controls were vortexed with glass beads and enumerated using PCA and *Listeria* Selective agar. The experiment was repeated six times. Time was the predominant predictive factor for biofilm survival on stainless steel, while temperature and time contributed equally to predicting the survival of biofilm on buna-N rubber. Overall, *Pseudomonas* biofilm was more heat resistant than *Listeria* biofilm on stainless steel; it had a 16% probability of survival after heat treatment of 77°C for 15 min and 0.04% on buna-N rubber, while for *Listeria* biofilm, the probability of survival was 7% on stainless steel and 0.094% on buna-N. In a mixed culture biofilm, *Listeria* had a greater possibility of survival on buna-N rubber (0.4%) than on stainless steel (0.3%).

P129 MICROBIAL GROWTH IN TRANSGENIC PORK

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Meat quality may be improved by genetically engineering livestock to express genes altering carcass composition and yields. Pigs expressing insulin-like growth factor-I (IGF-I) produce more lean tissue and less carcass fat than non-transgenic pigs. Diet can also cause compositional changes in pig carcasses; when conjugated linoleic acid (CLA) is added to pig diets, subcutaneous fat is reduced, but intramuscular fat is increased. It is unknown if these factors affect microbial growth in the meat during refrigerated storage. The objective of this study was to determine the effects of the IGF-I trans gene and dietary CLA on the refrigerated shelf-life of pork loins. Loins from control and transgenic pigs fed diets with or without added CLA were cut into cubes and packaged in air or under vacuum, stored at 3±1°C, and assayed periodically for aerobic plate counts for 3 weeks. Pathogens were inoculated into ground loin samples and growth monitored during 2 weeks storage at 7°C. Neither gene nor CLA content significantly affected the shelf-life of the pork cubes. The growth of *Listeria innocua*, *E. coli* O157:H7, *Salmonella* Typhimurium, and *Yersinia enterocolitica* was lower in transgenic pork than in controls. CLA did not affect the growth of Yersinia or *E. coli* O157:H7, but the growth of *L. innocua* and *S.* Typhimurium was higher in pigs fed diets with CLA. IGF-I transgenic pork does not spoil faster

and may be less supportive of the growth of foodborne pathogens than control pork, but increased dietary CLA may enhance pathogen growth.

P130 RECOVERY OF INJURED *YERSINIA ENTEROCOLITICA* FROM SWINE PRODUCTION SITES

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Little is known of the epidemiology and ecology of fecally-shed *Yersinia enterocolitica* (Y.e.) at swine production sites. Y.e. in swine wastes may be a source of animal and human exposure to these pathogens, especially if wastes are inadequately treated and are recycled for use as barn flush water or to irrigate produce. Swine wastes also contain high concentrations of metal residuals (copper and zinc). Such metals have been previously shown to have antibacterial or injurious activities against Y.e. and other bacteria in water. Therefore, the purpose of this investigation was to determine if there were injured Y.e. in swine waste and to compare cold-enrichment broth media for the recovery of injured Y.e. Y.e. O:3 was injured by mock lagoon liquid, inoculated into Pre-Enrichment Medium (PEM) and Phosphate Buffered Saline (PBS), and cold-enriched for 2 weeks. Additionally, fresh swine lagoon waste from two farms was cold-enriched with PEM and PBS for 2 weeks. Total viable counts were determined with non-selective and selective agar media. Recovery of Y.e. injured by mock lagoon liquid was 4-5 $\log_{10}$ greater with PEM as compared with PBS. In the fresh swine lagoon samples, Y.e. was recovered in PEM from both farms, but Y.e. was recovered in PBS from only one farm. Furthermore, recovery of Y.e. from fresh swine waste was 3 $\log_{10}$ greater with PEM as compared with PBS. These results indicate that Y.e. are injured in swine waste and that PEM is superior to PBS for their recovery.

P131 MICROBIOLOGICAL AND SENSORY QUALITY OF NEW YORK STATE FLUID MILK PRODUCTS: 1990-1999

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Cornell University's Milk Quality Improvement Program (MQIP) has conducted extensive shelf-life analyses of New York State (NYS) processed fluid milk products for many years. Microbiological and sensory data from pasteurized, homogenized, reduced-fat (2%), low-fat (1%), and non-fat milk products analyzed on the initial day of receipt and at 7, 10, and 14 days post-processing were compiled and compared for the period January 1990 through December 1999. Initial day mean bacterial numbers in processed milk remained constant during the ten year period, whereas dramatic increases in the percentages of samples meeting post-processing regulatory standards (<20,000 CFU/ml) at day 7, 10 and 14 were recorded, (21%, 20%, and 11%, respectively). The corresponding average flavor scores also improved, with an increase (on a scale of 1 to 10) of approximately 1.3 for day 7, 1.7 for day 10, and 2.2 for day 14. Unacceptable flavor scores (those below 6.0) decreased by 36% in the period from 1993 to 1999. Measurement of raw milk bacterial numbers during this period suggests that improvements in processed product quality were more likely a reflection of changes in the processing and handling of pasteurized products, rather than from reduced bacterial counts in raw milk. In summary, these results demonstrate that the quality of NYS fluid milk products has improved significantly since 1990, a result, in part, of the NYS Milk Promotion Board's support of MQIP extension efforts.

P132 SURVIVAL OF *LISTERIA MONOCYTOGENES* IN REFRIGERATED, NISIN-TREATED, SKIM, 2%, AND WHOLE MILK DURING STORAGE AT 5°C

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The ability of *Listeria monocytogenes* to proliferate in milk and the antilisterial activity of nisin are well documented. Although increasing milk fat was reported to reduce the antimicrobial activities of nisin, there is little information on the influence of milk fat on the antilisterial activities of nisin in refrigerated milk. Fresh pasteurized and homogenized milk samples (0.1, 2, and 3.5% fat) were treated with nisin (0-500 IU/ml), challenged with 4 log CFU/ml of *L. monocytogenes* strain Scott A, and stored at 5°C for 12 days. Numbers of the pathogen were determined on PALCAM agar plates every 2 h during the first 10 h, and every 3 days thereafter for up to 12 days. Total aerobes in the milk were also determined. Numbers of *L. monocytogenes* in the nisin-free refrigerated milk increased by 1-2 logs after 12 days, and similar increases were observed in total aerobes. *L. monocytogenes* was most sensitive to nisin in skim milk. Cell numbers of the pathogen declined immediately after its addition to skim milk. The rate of decline was inversely proportional to the nisin concentration, and numbers were below 1 log CFU/ml at the end of the storage period. While initial decline in *L. monocytogenes* numbers was also observed in 2% and whole milk, regrowth was observed in each of the tested samples, and numbers exceeded the initial values in whole milk samples containing ≤125 IU nisin/ml. These data demonstrate that increases in milk fat in the range of 0.1-3.5% cause reduction in the antilisterial activity of nisin in pasteurized, homogenized milk.

P133 EFFECT OF RESIDUAL SANITIZERS ON CULTURED DAIRY PRODUCTS

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Over the years, there has been concern about starter culture inhibition. Plants have experienced slowdowns in fermentation due to phage or other microbial contamination, poor starter culture, antimicrobials such as antibiotic in the milk, etc., resulting in lengthening set times. This study was performed to investigate the effect residual sanitizer has on starter culture performance, mimicking poor drainage from a CIP system. This study investigated the effect peroxyacetic acid, peroxy acid/carboxylic acid and chlorine have on mesophilic, thermophilic and flavor producing bacteria. Sanitizers at adulterated levels were added into milk prior to inoculation with the respective culture. Culture was then added and fermentations were monitored for titratable acidity (TA), pH, and growth and

compared against standard curves. Results from this study indicated chlorine had no negative impact at the concentrations tested, while peroxyacetic acid (POAA) affected all cultures; the most pronounced effect was against mesophilic cultures. The peroxy acid/carboxylic acid exhibited slight inhibition against mesophilic and thermophilic bacteria. This study concludes that when using chlorine as the standard, POAA may potentially cause starter culture inhibition, while a mixed peracid/carboxylic acid system may have minimal effect depending on the starter culture. The level where an effect of residual sanitizer on starter culture was noted, would be considered adulterated. To minimize the effect of residual sanitizer on starter culture, it is recommended that equipment be allowed to thoroughly drain, and that milk be added to the vat prior to adding culture.

P134 THE EFFECT OF OSMOTIC STRESS ADAPTATION ON HEAT RESISTANCE OF *LISTERIA MONOCYTOGENES* SCOTT A IN PORK SLURRY

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The relationship between the time that *L. monocytogenes* Scott A is exposed to pork slurry and changes in its heat resistance following osmotic adaptation was evaluated. *L. monocytogenes* was grown in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE, control) or TSBYE with 5% (wt/vol) NaCl (stress-adapted) at 35°C. Mid exponential phase cells, harvested by centrifugation, were suspended in sterile pork slurry (4°C) to give ~ 3.0×10^7 CFU/ml. Inoculated pork slurry was heated at 60°C within 4, 6, or 8 min after inoculation. At 0, 2, 4, 6, 8, and 10 min of heating, samples were surface-plated onto tryptic soy agar + 0.6% yeast extract (TSAYE). *L. monocytogenes* survivors on TSAYE were enumerated after incubation at 35°C for 72 h. Cell morphology was determined by light microscopy. Osmotic adaptation of *L. monocytogenes* resulted in significant ($P < 0.05$) increase in heat resistance and extreme elongation of cells. D values for control and stress-adapted cells, exposed to pork slurry for 4 min before heating, were 0.73 and 1.02 min, respectively. After 6 min in pork slurry, control and stress-adapted cells had D values of 0.76 and 0.93 min, respectively. There was no significant ($P > 0.05$) difference between D values for control (0.74 min) and stress-adapted cells (0.73 min) that were held in pork slurry for 8 min before heating. The results of this research indicate that the heat resistance of *L. monocytogenes*, adapted to 5% NaCl, rapidly decreases as exposure time of cells to pork slurry increases.

P135 INHIBITION OF PATHOGENS ON PROCESS CHEESE SLICES AT ABUSE TEMPERATURE

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The survival of *Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus* was evaluated on process cheese slices stored at abuse temperature. Duplicate lots of 20 commercial, standard-of-identity (21 CFR 133.169) process cheese slice formulations were surface inoculated separately with 3-$\log_{10}$ CFU/g of each of the five pathogens, placed in plastic sampling bags, and stored at 27°C for 7 days. Pathogens were enumerated for triplicate slices/lot by plating on selective agar at 0, 12, 24, 36, 48, 72, 96, and 168 h. Moisture, NaCl, pH, A_w, phosphate, citrate, sorbate, fat and protein levels were determined using AOAC procedures. *Salmonella* and *E. coli* O157:H7 populations decreased 1 to 2-$\log_{10}$ CFU/g during the 7-day storage period for all formulations. Populations remained constant or decreased <1-$\log_{10}$ CFU/g for *L. monocytogenes* and *B. cereus* during the 7-day storage period. Most formulations tested inhibited growth of *S. aureus* at 24 h. However, one formulation (40% moisture, 2.4% NaCl, pH 5.9-6.0, no added sorbate) supported *S. aureus* growth (1.1-$\log_{10}$ CFU/g increase) at 24 h, but not at 12 h. A comparable formulation with sorbate added did not support staphylococcal growth throughout the testing interval. None of the formulations tested supported growth of any pathogen through 12 h storage at 27°C. Inhibition of *S. aureus* through 96 h was associated with pH <5.6 or the presence of sorbate. In addition, populations of *Salmonella*, *E. coli* O157:H7, *L. monocytogenes* and *B. cereus* levels decreased or remained constant during the testing interval.

P136 RECOVERY OF *SALMONELLA* FROM DAIRY CATTLE AND THEIR ENVIRONMENT

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Salmonella is one of the major foodborne pathogenic bacteria carried by animals throughout the world. The objective of this study was to determine month-to-month variation and seasonal effects in the occurrence of *Salmonella* in dairy farm environments and animals. Recovery of *Salmonella* from indoor air (n=296), trough water (n=278), mixed grain (n=222), soil (n=258), insects (n=162), bird droppings (n=162), and cows (n=1486) on dairy farms was determined. Isolation of *Salmonella* from samples was performed by using a combination of multiple specialized enrichment and selective plating protocols adapted from FDA BAM, based upon sample type, followed by biochemical and serological confirmation tests. Samples were collected during fall, winter, spring and summer monthly (12 months). The major sources of *Salmonella* (% positive for *Salmonella*) were mixed grain (71%), insects (52%) and water (43%) during fall, air (91%), insects (50%) and droppings (50%) in winter, air (93%) and water (88%) during spring, and mixed grain (92%), insects (90%), droppings (75%), soils (68%) and water (58%) in summer. Recovery of *Salmonella* from animals (mouth, hair, teat, fecal and foremilk) during fall, winter, spring and summer ranged from 18-41, 28-49, 12-31 and 40-81%, respectively for each season. These data provide comprehensive information on key environmental and animal sampling sites needed to initiate an on-farm management programs for control of *Salmonella* in dairy cattle.

P137 *ESCHERICHIA COLI* O157:H7 IN DAIRY COWS AND THEIR ENVIRONMENT

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Cull dairy cows and cattle are important reservoirs of *Escherichia coli* O157:H7. The purpose of this study was to evaluate the seasonal occurrence of *E. coli* O157:H7 in the dairy farm environment and in cows. Isolation of *E. coli* O157:H7 from indoor air (n=296), trough water (n=278), mixed grain (n=258), insects (n=162), bird droppings (n=162) and cows (n=1486) from dairy farms was performed by using a combination of enrichment and selective plating procedures followed by biochemical and serological confirmation tests according to the FDA Bacteriological Analytical Manual. Samples were collected monthly (12 months) during fall, winter, spring and summer. *E. coli* O157:H7 was not recovered from any of the environmental samples or from cows during the winter. *E. coli* O157:H7 was recovered only from trough water (8%) and in 1 to 2% of animal samples in spring. In summer, *E. coli* O157:H7 was recovered from all animal samples at high levels ranging from 7 to 33% and also from environmental samples including soil (32%), air (25%), insects, water and bird droppings (8%). During the fall, isolation of *E. coli* O157:H7 occurred in approximately 2 to 3% of cows and in isolated environmental samples including insects (7%), water (6%), soil and air (3%). On-farm pre-harvest food safety management strategies for prevention of *E. coli* O157:H7 should consider the significance of environmental contamination and its correlation with occurrence in cows, particularly in summer and fall.

P138 GIS AND EPIDEMIOLOGY OF *SALMONELLA* ON DAIRY FARMS

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Several studies have examined risk factors associated with transmission of *Salmonella* by dairy herds. However, the application of Geographic Information Systems (GIS) to animal populations and their environment has received little attention. Therefore, this study was designed to examine spatial relationships that exist between animals and their environment and factors affecting transmission of *Salmonella* using GIS. The experimental design included 321 dairy cows (lactating, dry cows, and maternity) and calves in a vertically integrated production system. *Salmonella* was isolated from samples collected monthly for 12 months from cows, calves, and environmental sites including pasture, barns, bedding, feed, soil, bulk milk containers, milking equipment, calf milk, air, insects, and wild birds. A total of 2436 samples were analyzed for *Salmonella* using modifications of BAM enrichment and confirmation protocols, previously validated for each sample type. GIS analysis indicated that *Salmonella* was most often isolated at sites near maternity cows. Grain, soil, air, insects and water were the most frequent sources of *Salmonella* (over 50% positive), and were strongly indicated as vectors for transmission of *Salmonella* to cows. Recovery of *Salmonella* from environmental sites varied significantly by season. Grain (70% positive) and insects (50 to 80% positive) were the major carriers of *Salmonella* in summer and fall; however, insects (50% positive) and air (81% positive) were the most significant sources of *Salmonella* during winter and spring. We can conclude that elimination of *Salmonella* from feed grains and insect control could reduce transmission of *Salmonella* in dairy cows.

P139 ASSESSMENT OF *SALMONELLA*, *LISTERIA* AND *ESCHERICHIA COLI* O157 IN BIOSOLIDS AND STREAMS ASSOCIATED WITH A DAIRY FARM

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Biosolids ponds from dairy farms are frequently used both for irrigation water and fertilizer. The objective of this study was to evaluate the occurrence of *Listeria* spp., *Salmonella* spp. and *E. coli* O157:H7 in a dairy farm animal waste operation and in a stream adjacent to the production facility over a six month period. Feed grain, silage and bulk milk at the farm had frequently tested positive for both *Salmonella* and *Listeria*. *Salmonella*, *Listeria* and *E. coli* O157:H7 were isolated from the waste separator (separator liquid, separator solids, irrigation liquid), stream water 3km upstream of the Lewisburg Experiment Station dairy farm (Tennessee), and stream water 1km downstream from the dairy farm. Pathogens were isolated and confirmed using FDA BAM protocols (enrichment). *Listeria* and *Salmonella* were isolated from 33 to 100% of the separator liquid, separator solids, and irrigation liquid samples for all six months of the study (December-May). *Listeria* was recovered from 33% of both the upstream and downstream water samples in May. *Salmonella* was recovered from all upstream water samples at levels ranging from 33% to 100% for all months except February but was recovered downstream only in December, February and May. *E. coli* O157 was not detected in any of the environmental samples evaluated. These results show that other agricultural operations were impacting the stream quality even before the stream reached the dairy farm. The extremely high level of isolation of *Listeria* and *Salmonella* from irrigation and separator liquid and separator solids indicates that there is a continual recycling of pathogens in the feed production/animal production cycle of this dairy farm.

P140 MICROBIAL SAFETY OF PASTURE VERSUS FREE-RANGE CHICKENS USING ORGANIC AND TRADITIONAL FEED

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Campylobacter and *Salmonella* are estimated to affect over 2 million persons every year. Chickens may be infected with these microorganisms yet show no signs of illness. Infections can be spread through a common water source or through fecal material. Free-range skid, pastured pen and commercial production methods allow for a different amount of exposure to chicken fecal material. Due to consumer interest in "organic" and "natural" foods,

the pathogenic outcome of using different feed for poultry was addressed by analyzing the microbiological consequences of commercial, free-range and pasture rearing methods on *Salmonella* and *Campylobacter* levels in poultry. Chickens were reared under each production method. Free-range and pasture chickens were fed either organic or traditional feed. Visceral organs from randomly selected (N=35 per group) broiler stage chickens were used for microbial analysis. ANOVA revealed a significant production method effect on *Salmonella* but not on *Campylobacter*. The data from organically fed chickens showed a significantly lower *Salmonella* count when compared to the traditionally fed chickens. There was no significant difference in *Campylobacter* incidence. These data warrant further investigation into the microbiological consequences of production methods and feed source in poultry operations.

P141 SURVIVAL OF FECAL INDICATOR BACTERIA IN BOVINE MANURE INCORPORATED INTO SOIL

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Analyses for fecal indicator bacteria in soil with added bovine manure may be useful for a) evaluating the likelihood of enteric pathogens contaminating vegetables and b) estimating the time since manure application. Silty clay loam (SCL) and loamy sand (LS) soils were mixed with fresh bovine manure at a concentration representing typical manure land-spreading rates. Manure/soil mixtures were exposed to a daily temperature regime of 10 hrs at 21°C and 14 h at 10°C. For the first 12 weeks (Part 1), manure/soil was watered weekly; for the final seven weeks (Part 2), watering occurred every 3, 7, or 14 days. At weekly intervals, presumptive *Escherichia coli* and *Enterococcus* spp. were enumerated and the identity of selected isolates was confirmed using biochemical tests. During Part 1, *E. coli* initially increased 1 - 2 log CFU/g and then decreased < 1 and ca. 2 log CFU/g in SCL and LS, respectively. Numbers of presumptive enterococci initially increased less than *E. coli*, and then decreased at a greater rate, with a significant ($P < 0.05$) difference in rates in SCL. In Part 2, further decreases in cell numbers occurred, with no significant difference between rates of decrease for *E. coli* and enterococci. Results show that both indicator groups will survive lengthy periods in mixtures of bovine manure and soil, with numbers of *E. coli* decreasing less rapidly than those of enterococci. Quantitative analyses for these indicator bacteria is more appropriate for estimating time since manure application than for estimating the likelihood of enteric pathogen survival.

P142 A RAPID METHOD FOR THE DETECTION OF *LISTERIA* IN THE DAIRY FACTORY ENVIRONMENT

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Listeria spp. are common contaminants of the dairy factory environment. The presence of *Listeria* spp. is commonly used as an indicator for the pathogen *Listeria monocytogenes*. Rapid detection of *Listeria* spp. in the dairy environment is a critical component of pathogen control. For corrective action to be effective, results should be available as soon as possible. The choice of enrichment media is critical to ensure that sublethally damaged cells are recovered and their growth not inhibited by residual cleansing agents or competitive microflora.

In this study 110 Enviroswabs™ were taken, in triplicate, from dairy factories to determine the presence of *Listeria* spp. The swabs were enriched in three different selective broths, TECRA® Buffered *Listeria* Enrichment Broth (BLEB), University of Vermont Media (UVM) and Fraser Broth (FB). Each enrichment was examined for the presence of *Listeria* spp. using the TECRA® uniQue™ *Listeria* test as well as streaking onto selective agar.

Result with 110 dairy environmental swabs showed higher recoveries of *Listeria* spp when using Fraser Broth (17 confirmed isolates) as compared with both UVM (10 confirmed isolates) and TECRA BLEB (9 confirmed isolates).

This study shows that Fraser Broth is the preferred medium when testing swabs from a dairy environment. Using the TECRA uniQue *Listeria* test, presumptive results are available in 32 h, enabling rapid action to control *Listeria* spp. in the dairy production environment.

P143 RAPID DETECTION OF MICROORGANISMS IN DAIRY PRODUCTS USING AN AUTOMATED OPTICAL SYSTEM

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BioSys, an optical instrument detecting color changes associated with microbial growth, was used to detect organisms in dairy products. Raw and pasteurized milk were evaluated for Total Viable Counts (TVC) and coliforms. Yogurt and soft cheeses were evaluated for coliforms and yeast. Liquid milk samples were added directly to pre-filled vials; fermented dairy product were diluted 1:10, followed by pH adjustment and addition of the diluted sample into the pre-filled vial. Two and five ml of sample were used with the fluid milk for TVC and coliform, respectively. Five and one ml of 1:10 dilution of fermented products was added to coliform and yeast vials, respectively. Three media, TVC, coliform and yeast, containing bromcresol purple were developed and tested. In a comparison to the plate count methodology correlation coefficients R = -0.93 and R = -0.92 were obtained for raw and pasteurized milk, respectively. The coliform method yielded correlation coefficients of R = -0.95 for raw milk. In sixty pasteurized milk samples tested, only two samples were positive by the optical method; one had 1CFU/ml by the plate method while the other had < 1CFU/ml. Coliform organisms were isolated from the detecting vials showing the higher sensitivity of BioSys, probably due to the fact that higher volume of sample (5.0 vs 1.0 ml) of sample was utilized. Presence of yeast or coliforms in fermented dairy products was always detected without any false positive or negative results. The instrument has proven useful to the dairy industry, offering labor savings, rapidity and automation.

P144 DEAD *LISTERIA MONOCYTOGENES* CELLS ARE DETECTED IN COOKED MEAT AND SMOKED FISH WITH A COMMERCIAL PCR-BASED KIT

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By using a commercial PCR-based kit (Probelia *Listeria monocytogenes*™) for the detection of *Listeria monocytogenes* in cooked meat and smoked fish we found

a high proportion of "false positive" results, i.e., results that we were unable to confirm with culture or any other alternative method. We raised the hypothesis that dead cells of *L. monocytogenes* present in the food matrixes were detected by the PCR-based method. To test this hypothesis, cooked meat and smoked fish products that were initially negative for L. *monocytogenes*, both with the reference method (ISO 11290) and the PCR-based method, were spiked with heat inactivated *L. monocytogenes* cells and then retested with both methods. A suspension of *L. monocytogenes* in PBS buffer (about $1.5 \pm 0.5\ 10^9$ CFU/ml) was heat inactivated at 70°C for 30 minutes and then serially diluted to the tenth. Complete inactivation of the cells was confirmed by culture methods. Food products and PBS buffer were spiked with the inactivated cells in the range of 10^3 to 10^9 cells per 25g or 25 ml respectively. All experiments were done in triplicates, including for unspiked matrixes used as control. While the reference method gave negative results with all spiked and unspiked food products as well as PBS buffer samples, the PCR-based method gave positive results with both the meat and fish matrixes spiked with, respectively, 10^7 to 10^9 and 10^6 to 10^9 inactivated cells/25 g but not with unspiked samples. According to the respective enrichment procedures, the lowest dead cell concentration that generated a positive PCR result corresponded to approximately 10 inactivated cells in 5 ml of sample used for the PCR assay. The same results were obtained for PBS samples. We demonstrated that dead cells of *L. monocytogenes* generated positive PCR tests in cooked meat and smoked fish products with a threshold of 10^7 and 10^6 cells/25g respectively.

P145 ASSESSMENT OF PROTEIN FINGERPRINTING METHOD FOR SPECIES VERIFICATION OF MEATS

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This study was conducted to evaluate the use of a protein fingerprinting method for species verification of meats. The protein patterns of various species of raw, cooked and processed meats were generated using protein isoelectric focusing (IEF) technique. Protein patterns of various domestic and wild animals including those of cooked and processed meat products showed series of distinct bands that are unique to each individual animal species. Tests on reproducibility of protein patterns of raw and cooked meat species showed that the IEF technique is reproducible and certain types of characteristic protein bands can be used for meat species identification. Protein patterns of different anatomical sites of pork, beef and chicken showed identical profiles for each of the three meat species. However, meat samples from organs such as heart, liver and kidney of each of the three meat species produced different protein patterns than those obtained from muscle tissues. Freezing the meat samples up to 10 months had no effect on protein patterns. Levels of meat adulteration with other meat species could be estimated by visual examination or a computerised imaging system (AlfaImager™ System, Canberra-Packard Canada Ltd., Mississauga, ON, Canada). This study showed that the protein fingerprinting method using the IEF technique could be used for the detection and estimation of levels of adulteration of meats with other meat species.

P146 VALIDATION OF CCPS IN HACCP SYSTEMS IN SMALL MEAT AND POULTRY PROCESSING PLANTS IN NEBRASKA

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Three meat and poultry processors required validation of critical control points (CCPs) in their HACCP plans to satisfy USDA requirements for HACCP implementation. In-plant data including product/room temperatures and total aerobic plate counts, coliform and generic *E. coli* counts and presence of *Salmonella* was collected to establish or modify CCPs in the processes. Measuring the product temperature as a CCP in a poultry fabrication process while processing in a non-refrigerated area was validated by collecting data from poultry carcasses and on product contact surfaces during the processing day. Over the course of the day, there were no significant increases in microbial loads on the food contact surfaces or in product temperatures. In a beef fabrication process, data were collected to allow the processor to monitor room temperature, as opposed to product temperature as the CCP. Again, the microbial loads on the food contact surfaces did not increase as long as the room temperature remained near 50°F for less than 4 h. The product temperatures also did not significantly increase during processing. Finally, data were collected to determine the critical limits for a CCP during the production of cured pork skin and fat trim. Microbial loads on the cured fat and skin trim increased significantly during chilling using their original method. A new chilling method was implemented to reduce temperatures quickly to inhibit microbial growth. All three processors presented the data to the USDA inspector and the processors were allowed to operate using the new validated parameters.

P147 DETERMINING EXPOSURE ASSESSMENT AND MODELLING RISKS ASSOCIATED WITH THE PREPARATION OF POULTRY PRODUCTS IN THE HOME IN THE UNITED KINGDOM

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Poultry and poultry products have been implicated as a major source of *Salmonella* and *Campylobacter* and laboratory studies have indicated the potential for these microorganisms to spread within kitchens during food preparation. *Campylobacter* is usually associated with sporadic cases of food poisoning whilst *Salmonella* is frequently associated with general outbreaks. Exposure assessment is concerned with estimating the likelihood of consumption and the likely number of the pathogens that consumers may be exposed to in food and is an area of great uncertainty and variability. The aim of the study was to develop and pilot an exposure assessment model to estimate the risk associated with *Salmonella* and *Campylobacter* following poultry preparation and consumption within the home, using microbiological and observational techniques. Preparation of poultry meals was observed using notational analysis within a domestic kitchen and within consumer homes when preparing

barbecues (n=50). Raw and cooked poultry, other cooked foods, utensils and surface areas that possibly contribute to pathogen cross-contamination were sampled for microbiological examination. Additional laboratory experiments provided quantitative data for transfer rates of the pathogens to the specific surface areas and foods of particular concern and data were obtained with the use of literature studies and detailed food consumption data. Event tree modelling using '@ Risk' software was used to identify key factors that determine food poisoning risks to consumers. Consumer risk was determined as a function of rates of raw food contamination, subsequent food handling practices and the frequency and amounts of contaminated food consumed. All factors were quantified and incorporated into the event tree model. The effect of altering key factors on the risk to consumers is investigated and the results used to form risk management policies. The data generated may also aid in the prioritization of messages for food hygiene interventions for the consumer.

P148 VALIDATION OF THE USE OF ANTIBIOTIC-RESISTANT STRAINS OF *ESCHERICHIA COLI* 0157:H7 AND *SALMONELLA* SPP. FOR RECOVERY OF INJURED CELLS SUBJECTED TO STRESS CONDITIONS ENCOUNTERED DURING COMPETITIVE INHIBITION

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Pathogens subjected to antimicrobial intervention treatments may not be killed, but only injured. Injured cells may not grow on selective media so it is not appropriate to evaluate the effectiveness of an intervention treatment by plating on selective media. A method to evaluate the effectiveness of competitive inhibition of pathogens by lactic acid bacteria while precluding the growth of background flora and allowing injured cells to recover was validated in this study. Antibiotic-resistant (AR) and non-resistant (NR) strains of *Escherichia coli* O157:H7 and *Salmonella* spp. were stressed with lactic acid and cell-free supernatants (CFS) from lactic acid bacteria and plated onto Trypicase Soy Agar (TSA), Trypticase Soy Agar plus 50 ug/ml Nalidixic acid (TSAN), and selective media (XLT4 and VRBA). Both stress conditions resulted in significant reductions in populations of NR pathogens. AR pathogens showed similar recovery patterns on all media evaluated, but they were less sensitive to both stress conditions compared to NR strains. VRBA did not recover injured NR *E. coli* O157:H7 cells subjected to either stress condition. XLT4 recovered injured NR *Salmonella* subjected to cell-free supernatant stress, but not the cells subjected to acid stress. Both TSA and TSAN recovered 100% of the injured AR pathogens. AR strains of pathogens subjected to competitive inhibition can be recovered on non-selective media supplemented with the antibiotic. This method recovers injured cells at levels comparable to non-selective media and much higher than on selective media. This method will ensure that the effectiveness of the inhibitory action will not be exaggerated.

P149 OCHRATOXIN A PRODUCTION BY BLACK *ASPERGILLUS* SPECIES AND SIGNIFICANCE TO THE FOOD INDUSTRY

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Ochratoxin A (OA) production by black *Aspergillus* species in Section Nigri has only recently been recognised as a significant source of ochratoxin in coffee, wine and dried fruits. The most common species is *Aspergillus niger*, but *A. carbonarius* and *A. aculeatus* are also important. These fungi occur more commonly in warm climates and, being extremely resistant to ultraviolet irradiation, survive well during sun-drying of grains, nuts, fruits, coffee and spices. Under natural conditions, these species compete, with temperature being a strong determinant for dominance. At 25°C, and high water activity (0.99 a_w) *A. aculeatus* grows faster than the other two species, but below 0.90 a_w *A. niger* is dominant. At 30°C, *A. carbonarius* is dominant over the other two species, but at lower a_w values, *A. niger* grows fastest. At 37°C, *A. niger* out-competes both other black *Aspergillus* species over the entire a_w range for growth (0.996 to 0.80 a_w). However, *A. carbonarius* germinates more rapidly than the other two species at 25, 30 and 37°C between 0.996 and 0.85 a_w, giving it a competitive edge in establishing dominance. The conditions favoring *A. carbonarius* in the field are temperature range 30-33°C and a_w 0.996 to about 0.93. In Australia, *A. carbonarius* is the most important OA producer; production of OA by strains of *A. niger* is uncommon and by *A. aculeatus* unknown. Information is needed on the optimal combinations of temperature and water activity under which OA is produced by *A. niger* and *A. carbonarius*, and on the factors inhibiting production.

P150 EVALUATION OF ELECTROCHEMILUMINESCENT ASSAYS FOR THE RAPID DETECTION OF FOODBORNE PATHOGENS ON ENVIRONMENTAL SURFACES

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Environmental monitoring and hygiene testing of surfaces for the presence of foodborne pathogens in the food industry has gained importance. Typically, detection of contaminated surfaces involves the use of adenosine triphosphate (ATP) detection or the isolation of specific pathogens using selective media. These procedures may take as long as 4 days to complete. Therefore, a more rapid method is required to determine the presence of specific pathogens on environmental surfaces. In this study, the utility of ORIGEN® technology was evaluated on environmental surfaces. For each organism tested, 50 samples of each bacterial type and concentration were tested in duplicate. Areas (100 cm^2) of stainless steel were separately inoculated with known levels of *Salmonella* Typhimurium, *Campylobacter jejuni*, or *Listeria monocytogenes* (pure

culture or mixed cultures). Surfaces were sampled either immediately after inoculation while still wet, or after 1, 2, 4, 8, or 24 h. Samples were inoculated into selective media respective to that particular organism and incubated for 24 h. Enriched samples were tested using IGEN International's PATHIGEN™ *Listeria* test, *Salmonella* test, and *Campylobacter* test. The *Listeria* and *Salmonella* tests were able to detect the equivalent of 1 CFU/100cm^2 and the *Campylobacter* test was able to detect less than 10^0 CFU/100cm^2. The PATHIGEN tests performed equivalently when compared to B.A.M. standard plating methods. Both organism type and surface status influenced the minimum detection limits, which ranged from 1 CFU/100 cm^2 to >10^5 CFU/100 cm^2. Each assay takes approximately 1.5 h after enrichment and can be run simultaneously with other PATHIGEN tests.

P151 DEVELOPMENT AND EVALUATION OF A MULTIPLEX PCR ASSAY FOR SPECIFIC DETECTION OF *CAMPYLOBACTER JEJUNI, ESCHERICHIA COLI* 0157:H7, *LISTERIA MONOCYTOGENES*, AND *SALMONELLA* IN CONTAMINATED FOOD

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Campylobacter jejuni, *Salmonella*, *Listeria monocytogenes* and *E. coli* O157:H7 constitute four of the leading pathogens for the food industry. The use of the polymerase chain reaction in detection allows for more specific and sensitive identification. Previous research utilizing 3 sets of primers, one each for *C. jejuni*, *Salmonella*, and *E. coli* was developed and evaluated on various foods such as meats, poultry products, fruits, vegetables and dairy products. In the present research, a multiplex system assay that employs four sets of primers, one set for each bacteria, has been developed. The four organisms were grown by standard microbiological methods, harvested and digested for use in the PCR assay. One reaction tube allowed for simultaneous detection of all four organisms on the agarose gel. For *C. jejuni*, the use of primer set C-1/C-4 gave a product of 159 bp. The *Salmonella* was visible as a 360 bp product using primer set S29/S30; and *E. coli* was detected using primers UidAa/UidAb and a product of 252 bp. The *Listeria* produced a different bp depending on the primer set used. For AP4/SK6, a 94 bp was seen, for FP/RP, a 450 bp, and for LL4/LL5, a product of 520 bp was seen. The PCR assay and detection of all four organisms can be performed within an 8 h day. For the food industry, this rapid and efficient detection of bacterial pathogens is both cost effective and critical to the prevention and treatment of foodborne illnesses.

P152 MICROBIAL EFFICACY AND ORGANOLEPTIC IMPACT OF X-RAY IRRADIATION ON READY-TO-EAT HOT DOGS INOCULATED WITH *LISTERIA MONOCYTOGENES*

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The goal of this research was to evaluate the hypothesis that lower starting pathogen levels on food would allow for the use of a lower irradiation dose that would reduce microbial load on the product without producing sensory side effects. Consumer wieners were vacuum packaged, separated into groups and inoculated with *Listeria monocytogenes* at 10^0, 10^2, 10^3, and 10^4 CFU/g. The test groups were then dosed with 0, 1, 2, and 3 kGy of ionizing radiation. Inoculated wieners were then sampled and analyzed using Aerobic Plate Count (APC) and Most Probable Number (MPN) pathogen assay methods at 0, 33, 67 and 100% of their 70 day coded shelf life. Non-inoculated wieners were irradiated at 0, 1.0, 1.5, 2.0, 2.5, 3.0 kGy and analyzed for Sensory and Chemical changes at 0, 50, 100 and 125% of their coded shelf life. Chemical testing consisted of Peroxide Value (PV) and 2-Thiobarbituric Acid Values (TBA) as well as CO_2 and O_2 levels in the packaging head space. A trained sensory panel tested the organoleptic properties. The hypothesis was proven. Samples inoculated at 10^3 or lower CFU/g were successfully maintained below 10^1 CFU/g throughout the shelf life of the product with an initial irradiation dose of 2 kGy. The 3 kGy samples showed detectable undesirable sensory traits at the end of the shelf life. This shows that a lower initial bioburden level can be successfully treated with a lower irradiation dose and thereby avoid detectable undesirable sensory side effects.

P153 ESTER PERACIDS: NEW ANTIMICROBIAL COMPOSITIONS

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Peroxyacetic acid is a widely used antimicrobial in applications which include food contact surface sanitizer, vegetable and meat carcass treatment, aseptic packaging, and medical instrument sterilization. In some applications, its usefulness has been limited by the strong odor of the concentrate and in some cases the use solution. Ester peracid formulations, which have a similar spectrum of antimicrobial activity, represent a low odor alternative to peroxyacetic acid-based products. Initial results indicate that ester peracid formulations containing 90-120 ppm active peracid will meet the criteria for a no-rinse sanitizer. At this level, a five log reduction of *E. coli* ATCC 11229 and *S. aureus* ATCC 6538 was achieved in 30 seconds at 20°C. At levels of 150-300 ppm peracid, the criteria for hospital level disinfection is achieved. These criteria require passing results according to the A.O.A.C. use dilution method against *S. aureus* ATCC 6538, *S. choleraesuius* ATCC 10708 and *P. aeruginosa* ATCC 15442. Factors affecting effective level include water hardness and soil load. Results against various fungi indicate that levels ranging from 100 to 400 ppm are fungicidal depending upon genus and species of organism. Fungi tested to date include *Saccharomyces cerevisia*, *Geotrichum* sp. and *Trichophyton mentagrophytes*.

T01 EVALUATION OF METHODS FOR SAMPLING RECTAL/COLONAL FECES, HIDES, AND CARCASSES TO TEST FOR PRESENCE OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP.

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The study was designed to compare sampling methods to test for the presence of *Escherichia coli* O157:H7 and *Salmonella* spp. in feces, on cattle hides and on the surface of carcasses. Rectal palpation and colonal swabs were used to test feces. Three-site sponging, hair-clipping and hide excision, rinsing and gauze swabbing were used to test hides. Three-site, thorax, and pattern-mark sponging as well as tissue excision was used to test carcasses. Irrespective of sampling methods, of the 30 lots tested, 36.6%, 13.3% and 0.0% were positive for presence of *E. coli* O157:H7 on hide, fecal and carcass samples, respectively, while corresponding percentages for *Salmonella* spp. positive samples were 70.0%, 16.6% and 6.6% respectively. No differences ($P > 0.05$) were observed between sampling methods when testing for *E. coli* O157:H7. However, when sampling for *Salmonella* spp., the hide rinsing method was most effective ($P < 0.05$), as it recovered positive samples in 63.3% of hides. This technique was more effective ($P < 0.05$) in the recovery of *Salmonella* spp. than hide excision and hair clippings, but there were no differences between sponge or gauze sampling methods. Hide washing may not be a practical sampling method due to the dangers associated with sample collection. The second most effective sampling technique was the three-site sponging method, which found 46.7% samples positive; however, it was not statistically more effective than the other sampling methods used. Trends suggested that the three-site sponge sampling method would be the safest and most effective way to detect pathogens on cattle hides.

T02 RAPID DETECTION OF *ESCHERICHIA COLI* O157:H7 IN RAW GROUND BEEF VIA PCR USING A 375 G SAMPLE COMPOSITE AND SHORT ENRICHMENT

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Many quick serve food companies require either raw material or finished product ground beef to be screened for *E. coli* O157:H7. Because trimmed beef material is often analyzed for microbiological contaminates prior to final grind, time to final results is critical for an efficient production schedule. Enrichment of the sample remains the principal technique to increase the target organism to a detectable concentration. Enrichment time, therefore, is of major concern to suppliers focused on rapid microbiological results. To determine the minimum incubation time for rapid screening, 375 g ground beef samples were inoculated with 0, 15, or 150 CFU of *E. coli* O157:H7 (ATTC 43894) then tested with the BAX® system PCR assay at 8 ±1 h, 10 ±1 h, 12 ±1 h, and 20±1 h. The assay yielded positive results for 30% of the low level samples at only 8 ± 1 h of enrichment and 100% positive results at >10 h of enrichment. All inoculated samples were detected at the higher concentrations after 8 ±1 h , 10 ± 1 h, 12 ± 1 h, and 20 ± 1 h. One sample inoculated at the low level yielded negative results after the 20 ± 1h incubation. No false positive results were noted. The PCR assay can be used with greatly reduced incubation time (12 h) to provide acceptable sensitivity. The reduced time to result in detection at ~ 15 cells/375 g (0.04 cells/g) of raw ground beef could enable suppliers to reduce hold time and potentially reduce production costs.

T03 WITHDRAWN

T04 COMBINED TREATMENTS OF 2% LACTIC ACID (80°C) AND MICROWAVES FOR THE REDUCTION OF NATURAL MICROFLORA AND *ESCHERICHIA COLI* O157:H7 ON VACUUM-PACKAGED BEEF SUBPRIMALS

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Terminal treatment is needed to prevent recontamination of meat for greater food safety for consumers. This experiment was designed to find an effective terminal treatment for the reduction of natural microflora and inoculated *Escherichia coli* O157:H7. Beef subprimals, eye of round, (NAMP 171C) samples were randomly assigned to treatment combinations of 2% lactic acid heated to 80°C, vacuum packaged, and then microwaved. The 2% lactic acid exposure times used were 0, 2, and 4 s. The microwave exposure times used were 0, 50, 60, and 70 s. Samples were stored at 4°C. Microbial analysis was performed 24 h after treatment. Samples were plated on Tryptic Soy Agar (TSA) for total aerobic count, MacConkey Sorbitol Agar (MSA) for enterics, and Thin Agar Layer MacConkey Sorbitol Agar (TAL-MSA) for resuscitation of injured enteric organisms. TAL is a method developed at Kansas State University that recovers heat, acid, and cold injured cells much more effectively than selective media alone. Natural microflora and *E. coli* O157:H7 counts were decreased by one to two log Colony Forming Units (CFU)/cm^2 compared to controls. In this study, The Thin Agar Layer MacConkey Sorbitol Agar Method (TAL-MSA) recovered significantly higher ($P < 0.05$) counts of *E. coli* O157:H7 than MSA alone. The most effective treatments for the reduction of bacteria were 2% lactic acid (80°C) dipped for 2 s, vacuum packaging, and microwave treatment of 70 s. This method is an effective terminal treatment to reduce bacteria and prevent re-contamination of beef subprimals.

T05 INHIBITION OF *LISTERIA MONOCYTOGENES* ON HOT DOGS USING ANTIMICROBIAL WHEY PROTEIN-BASED EDIBLE CASINGS

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Heat-cured whey protein isolate (WPI) films (pH 5.2) containing 1.0% (w/v) sorbic acid (SA), p-aminobenzoic acid (PABA), or SA:PABA (1:1) were heat-sealed to form tubular casings measuring 2.0 cm in diameter and 12.0 cm in length. A standard hot dog batter containing 60% beef and 40% pork was stuffed into sealed WPI casings and commercial collagen casings (control) using a hand stuffer. After cooking and smoking, the hot dogs were surface-inoculated to contain *Listeria monocytogenes* at a level of 10^3 CFU/g and vacuum-packaged individually. Hot dogs were examined for numbers of *L. monocytogenes* after 0, 4, 7, 10, 14, 21, and 28 days of storage at 4°C. Tensile strength and % elongation of casings were determined before and after cooking and after smoking using standard methods. *Listeria* populations on hot dogs remained relatively unchanged after 21 days of storage using casings containing 1.0% SA, PABA or SA:PABA (1:1). In contrast, numbers of *L. monocytogenes* on hot dogs prepared with collagen casings increased >3 logs after 21 days. Tensile strength of casings containing 1.0% SA, PABA, or SA:PABA (1:1) decreased from 10.0, 14.0, or 9.8 to 6.7, 7.2, or 5.3 MPa while % elongation increased from 26.6, 36.3, or 50.3% to 40.5, 45.0, or 56.2, respectively, after cooking and smoking. Since *L. monocytogenes* is most often recognized as a post-processing contaminant, such antimicrobial WPI casings may provide a viable alternative for preventing growth of *Listeria* on hot dogs during long term refrigerated storage.

T06 EFFECTS OF DRIED PRUNE PUREES ON SUPPRESSION OF GROWTH OF FOODBORNE PATHOGENS IN GROUND BEEF

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Salmonella Typhimurium, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, and *Staphylococcus aureus* have been identified as agents of foodborne diseases. One approach for the control and prevention of foodborne pathogens is using naturally occurring food ingredients. Commercial prunes and prune extracts (Prunus domestica cv. French) contain phenolics, such as hydroxycinnamates, neochlorogenic acid and chlorogenic acid, which can inhibit the oxidation of low-density lipoprotein. Studies at Kansas State University have provided information on the killing effects of phenolic antioxidants on a large group of pathogens. The effect of prunes on foodborne pathogens has not been reported.

The objective of this study was to evaluate the efficacy of concentration and various times of contact of prune mixtures for controlling *S.* Typhimurium, *L. monocytogenes*, *E. coli* O157:H7, *Y. enterocolitica*, and *S. aureus* in ground beef.

The prune mixtures tested were prune puree (3% w/w) and fresh plum juice concentrate (3% v/w) obtained from the California Prune Board. The cocktail solution of *S.* Typhimurium, *L. monocytogenes*, *E. coli* O157:H7, *Y. enterocolitica*, and *S. aureus* (1ml) was added to 100 g of 20% fat ground beef with prune extracts for an initial inoculation of 4 log CFU/g. The prune/beef mixtures were stored at 4°C. Microbial analysis was performed on 4 days (0, 1, 3, and 5) on 25 g of sample.

The ground beef with prune puree or plum juice resulted in a 1 log to 2 log suppression of total count, *S.* Typhimurium, *L. monocytogenes*, *E. coli* O157:H7, *Y. enterocolitica*, and *S. aureus*.

T07 APPLICATION OF POTASSIUM SORBATE AND OTHER ANTIMICROBIAL INGREDIENTS TO CONTROL *LISTERIA MONOCYTOGENES* IN READY-TO-EAT MEAT AND POULTRY PRODUCTS

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Potassium sorbate has broad application as an antimicrobial food ingredient; however, in meat processing, it has been limited to use as a dip or spray to retard mold growth on dry sausages. The objective of this research was to determine the ability of potassium sorbate (PS), with or without sodium lactate/sodium diacetate (SL/SD), to control *Listeria monocytogenes* growth in selected ready-to-eat (RTE) meat and poultry products. Initial studies were performed using cooked sausage links. Sausage links were formulated without antimicrobial additives (control) and with 2.0% SL and 0.1% SD (SL/SD treatment). Individual dip solutions were prepared to achieve target concentrations of 0.05, 0.1, and 0.2% of PS in sausage links (PS treatments). For sausage links containing all three antimicrobials, target levels were 2.0% SL, 0.1% SD, and 0.1% PS (SL/SD/PS treatment). A five-strain *L. monocytogenes* mixture was used to inoculate test samples. Experimental storage temperatures were 4 and 10°C (abuse temperature). All levels of sorbate inhibited the growth of *L. monocytogenes* to some degree, with an increasing concentration of PS being more inhibitory. The SL/SD/PS combination was the most effective treatment. The highest level of PS (0.2%) was almost as effective in inhibiting *L. monocytogenes* as the SL/SD combination. Evaluation of PS will be expanded to determine if this antimicrobial significantly retards *L. monocytogenes* growth in other RTE meat and poultry products. Data from this research supports the use of sorbate in RTE meat and poultry products to enhance food safety.

T08 SEROTYPE TRACKING OF *SALMONELLA* THROUGH INTEGRATED BROILER CHICKEN OPERATIONS

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The widespread presence of *Salmonella* in all phases of broiler chicken production and processing is well documented. However, little information is available to show the identity and movement of specific serotypes of *Salmonella* through the different phases of an integrated operation. In this study, *Salmonella* were recovered from the breeder farm, hatchery, previous grow-out flock, at 5 weeks of grow out, and after processing 6, 98, 24, 60 and 7% of the time, respectively, in the first trial; and 7, 98, 26, 22, and 36% of the time, respectively, in the second trial. Seven different serotypes were identified in the first trial and 12 different serotpes were identified in the second trial. In both trials there was a poor correlation

between the serotypes found from the breeder farms and those found in the hatchery, indicating that not enough samples were examined from the farm or that there was an endemic population of *Salmonella* in the hatchery. A frequent association between the serotypes found in the hatchery and those found on the final processed carcasses was observed. This study confirms that development of successful intervention strategies for broiler production operations must have a component which emphasizes disinfection in the hatchery.

T09 MICROBIOLOGICAL RISK ASSESSMENT ON RAW PORK CARCASSES IN ONTARIO ABATTOIRS

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The prevalence and levels of bacteria of public health concern were estimated in a baseline study assessing the microbiological risk associated with hog carcasses processed in Ontario's provincially regulated abattoirs. The study was designed to account for experimental variations contributed by production volume (Strata), geographical location (GL) and Season. Within Stratum, abattoirs were randomly selected weekly using probabilities proportional to slaughter volume. A carcass swabbing technique using a sponge on 100 cm^2 area of the belly, ham and jowls of randomly selected carcasses was employed for specimen collection. Swabs immersed in 25 ml sterile buffered peptone water were submitted for microbiological analysis. Samples from 1557 carcasses were analyzed for indicator organisms: aerobic plate count (APC), total coliforms (TC) and *Escherichia coli* (Ec); and for pathogens: *Listeria monocytogenes* (Lm), *Salmonella* spp. (S), *Campylobacter* spp. (C) and VTEC. The bacterial contamination rates were 100.0%, 61.3%, 39.5%, 10.7%, 4.8%, 26.7% and 2.1% for APC, TC, Ec, Lm, S, C and VTEC, respectively. Strata, GL and Season affected ($P < .05$) TC, Ec, Lm and S. Season and GL significantly influenced ($P < .01$) C and VTEC values. Strata and GL had a significant impact on APC ($P < .001$). Means (log_{10} CFU/cm^2) for APC (4.69, 4.59, 4.21), TC (1.64, 1.51, 1.44) and Ec (1.37, 1.33, 1.20) were linearly different ($P < .05$) among the high, medium and low volume stratas, respectively. Data will be used to develop microbiological performance standards for pork processing in Ontario.

T10 EVALUATIONS OF ACIDIFIED SODIUM CHLORITE FOR USE ON RED MEATS

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The objective of this study was to evaluate the bactericidal effect of Acidified Sodium Chlorite (ASC) on 90:10 and 50:50 beef parts/trim following storage for 24 h at 4°C. The effects of 1000 ppm ASC were evaluated using a commercial prototype application system (patent pending) at 1, 2 or 3 oz/lb for 5, 10 or 15 seconds respectively on beef trim samples either naturally contaminated or artificially inoculated with a five strain cocktail of generic *E. coli*. Samples were chosen randomly and swab tested using a 5 × 10 cm template before and after treatment.

Results indicate that 3 oz/15 s/20,000 lb produced the most significant ($P < 0.05$) reductions. On 90:10 and 50:50, the system consistently demonstrated 2.3 and 1.9 log_{10} reductions on *E. coli* respectively. On total coliform counts the system reduced populations on 90:10 and 50:50 by 2.2 and 1.9 log_{10} respectively. Overall, total aerobic plate count reductions were lower, reflecting the generally lower level of sensitivity of these organisms to ASC. For APC's, the system reduced populations on 90:10 and 50:50 by 1.2 and 1.5 log_{10} respectively. For all species evaluated, the system consistently demonstrated significant ($P < 0.05$) reductions at levels as low as 1000 ppm.

Previous work has shown that *E. coli* O157:H7 is sensitive to ASC. The findings of these studies therefore suggest that the use of ASC as an antimicrobial rinse for beef parts/trim will be effective in the control of *E. coli* O157:H7 and other background microflora.

T11 COMPARATIVE STUDIES OF THE MICROBIAL-VAC™, A NON-DESTRUCTIVE WET-VACUUM MICROBIAL COLLECTION SYSTEM ON BEEF CARCASSES

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A novel wet-vacuum system, the Microbial-Vac™ (M-Vac) allows contained application of sterile, food safe Surface Rinse Solution (SRS) and subsequent retrieval of the applied liquid and suspended microbes off meat carcasses and food-prep surfaces. Microbes suspected present in high numbers (APC) may be detected as diluted in collected liquid aliquots (ca 50ml/100cm^2) of the SRS. Low numbers of microbes may be concentrated for improved rapid or conventional detection methods by filtering total recovered SRS through the M-Vac's 0.45 µm final filter. Fecal slurry containing approximately 700 CFU wild *Escherichia coli* was applied (0.5 ml) to adjacent sites (100 cm^2) on recently slaughtered beef-carcass plates then stored post inoculation for 24 h, 3°C, 95% relative humidity. Recovery of culturable coliforms was measured on m-Endo media comparing pummeled Excision (EX) and Sponge (SP) samples and M-Vac samples concentrated on the units final filter collected from adjacent carcass surface sites. Results of 44 observations indicate the M-Vac will recover surface microbes at levels similar ($P \geq 0.05$) to Ex (log_{10} 2.39 ± .13; 2.31 ± 16 CFU) whereas the SP sampling of adjacent areas recovered significantly lower ($P \leq 0.01$) levels (log_{10} 1.54 ± .25 CFU). Control sample sites contained no detectable coliforms. The M-Vac is also highly effective for *E. coli* recovery off commercial poly-cutting boards and smooth counter tops. These observations indicate the M-Vac may provide a more efficient non-destructive sampling method for improved low-level pathogen collection and identification on meat carcasses, food-prep and processing equipment surfaces.

T12 REAL TIME DETECTION OF PATHOGENIC *VIBRIO PARAHAEMOLYTICUS* IN OYSTERS

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Uncertain distribution of pathogenic *V. parahaemolyticus* in oysters clouds risk assessment and hinders control efforts. An efficient and sensitive real time PCR method for *V. parahaemolyticus* detection in oysters was developed, optimized and evaluated. Perkin Elmer Primer

Express® software was used to design primer sets and PCR conditions. A TaqMan® probe was used for detection of a 74 Bp amplicon within the thermostable direct hemolysin gene (tdh), the primary virulence determinant of *V. parahaemolyticus*. PCR was conducted in a Smart Cycler® or in a Handheld Advanced Nucleic Acid Analyzer developed at Lawrence Livermore National Laboratories. Specificity was demonstrated using a culture collection consisting of approximately 50 pathogenic and nonpathogenic *V. parahaemolyticus* plus over 100 strains representing various other species previously tested for tdh. Method sensitivity was determined using oysters collected seasonally and inoculated with known levels of pathogenic *V. parahaemolyticus*. Real time PCR was 10 to 1,000 times more sensitive than conventional PCR for detection of pathogenic *V. parahaemolyticus* in overnight enrichments of oyster homogenate in alkaline peptone water. Real time PCR also was conducted on frozen oyster enrichments archived from past environmental studies; 20 of 20 culture-positive enrichments were also positive by PCR. However, PCR detected tdh in some culture-negative samples. The failure to detect tdh by culture was probably due to the low ratio of pathogenic to total *V. parahaemolyticus*. Elimination of DNA purification and gel electrophoresis steps used with conventional PCR reduced analytical time from 9h to 30min.

T13 A MICROBIAL SURVEY OF TOILET PAPER AND ASSOCIATED PERFORMANCE VARIABLES RELATED TO ITS ROLE IN REDUCING COMMUNICABLE DISEASE TRANSMISSION

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During the golden age of public health, there have been numerous medical, scientific and technical advances in the field of hygiene, resulting in the generally high standards enjoyed in the developed world. Of the often credited list of advances, the invention and widespread use of toilet paper (TP) has been neglected. TP is a hygienic, absorbent and disposable cleaning implement, which through proper use, becomes a barrier to fecal contamination of hands. Literature review has uncovered numerous examples indicating the importance and effectiveness of TP in reducing disease rates. Compliance regarding TP usage improved with advancements in tissue softness. While TP combined with handwashing forms primary and secondary barriers to fecal-oral transmission, we continue to explore solutions to the handwashing compliance problem. One attempt to improve the handwashing compliance problem through linkage to toilet paper packaging is described in this presentation. TP quality varies widely in weight, thickness, construction, and dry/wet strength. Results are presented from experiments performed to show microbial penetration through successive sheets or layers of TP, and while no specific conclusions can be drawn, the barrier function is apparent. The modern paper making process (including pulping, bleaching and drying) results in the production of sanitary toilet paper products having low microbial counts. A microbial survey of 280 samples from a variety of manufacturers showed the majority to be below detection limits (10 CFU/g). With few exceptions, surviving microbes were heat-resistant spore-forming bacteria and fungi. Viable yeast cells were not found in any of the tissue samples tested.

T14 EVALUATION OF THE COMBINED EFFECTS OF SELECTIVE HANDWASHING WATER TEMPERATURES AND ANTIMICROBIAL SOAPS ON MICROBIAL REDUCTION EFFICACY AND SKIN IRRITATION

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While some personal hygiene experts ignore handwashing water temperature, others recommend using water that is warm, or as hot as tolerable. Insistence on hot water usage is described as a deterrent to handwashing compliance due to skin damage. The current study examines efficacy and skin irritation potential involving water temperatures when antimicrobial soaps are used. In the two-week study, the hands of 8 participants were contaminated with *Escherichia coli* inoculated ground beef and washed at different water temperatures (29° ± 2°C or 43° ± 2°C) with one of four highly active (USDA E2 equivalency) antibacterial soaps. A cross-over study design was utilized, with half the participants starting at 29°C, and the other half at 43°C. Hygienic efficiency was evaluated using the "glove-juice" technique for hand sampling. Before washing (days 1, 3, and 5 each week), skin condition was recorded visually and with specialized instrumentation, testing for total moisture content, transepidermal water loss, and appearance. Hands were contaminated with ground beef and immediately sampled to establish *E. coli* baseline counts. Handwashing was performed at the specified temperature, and the opposing hand was sampled to determine $\log_{10}$ reductions. Subjects then washed 11 consecutive times using their specific treatment, with irritation being documented 30 minutes after final product application. Overall, the four soap products produced similar handwashing efficacy results. Although there were slight increases in $\log_{10}$ reductions, visual skin irritation, loss of skin moisture content, and transepidermal water loss at higher handwashing temperatures, results were not statistically significant.

T15 APPLICATION OF REAL TIME TEMPERATURE MONITORING FOR FOOD SAFETY AND QUALITY MANANGEMENT IN FOOD RETAIL

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A recent FDA Retail Food Audit of 900 restaurants, foodservice establishments, and grocery stores reported improper storage temperature to be the greatest "out of compliance" risk factor. In this study, remote wireless temperature sensing technology was utilized to monitor multiple sites in the refrigerated areas of 15 test grocery stores. The data corroborated the above FDA finding with additional findings of in- and out-of-compliance temperature distribution within the same refrigeration cases. This was found to be independent of the age of the refrigeration equipment. Data from the sensors enabled corrective actions to be taken and full compliance was achieved after 30 days of monitoring. In 20% of sites, refrigeration cases were kept below the FDA prescribed temperature range as a precautionary measure, resulting in excess power consumption. Multi-point temperature monitoring enabled these sites to optimize power

management. Further, the real-time temperature monitoring approach has offered an additional tool for recovering temperature-abused perishables without violating the food code. The testing details and results from these sites will be presented.

T16 A MICROBIAL SURVEY OF HOUSEHOLD CAN OPENERS, FOOD AND BEVERAGE CAN TOPS, AND CLEANING METHODOLOGY EFFECTIVENESS

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A household can opener and food/beverage can top survey was undertaken to identify potential microbiological hazards. Results indicated the potential for high levels of bacterial and fungal contamination. Characterization of microorganisms from 10 can openers indicated the most common contaminants as *Klebsiella pneumoniae* and a variety of *Staphylococcus* and spore-forming *Bacillus* species. Cans selected from households and food vendors showed similar results to randomly obtained can openers. In contrast to commercial food service surveys, no *E. coli* or *Salmonella* species were found on the cans or can openers, however, low numbers of *Bacillus cereus* (22 of 25 cans) and *Clostridium perfringens* (15 of 25 cans) were identified. The effectiveness of various can cleaning methods was evaluated using marker bacteria (*Serratia marcescens*) suspended in soil consisting of tryptone soya broth, ground beef or vacuum cleaner dust. In addition to rinsing under running water, two paper towel types and a paper napkin product were used for wiping, moist wiping and wiping after rinsing. The most effective cleaning method was rinsing and wiping combined ($P < 0.0003$), while wiping alone was not as effective as moist wiping ($P = 0.038$) or rinsing with water ($P = 0.061$). Food cans were more easily cleaned than beverage cans due to the tab area. Soil type influenced the degree of effectiveness of the cleaning methods, with mean $\log_{10}$ reduction values for each type at 3.4 and 3.1 for food and beverage cans (tab area excluded), respectively. No significant difference was observed in the effectiveness of paper products ($P = 0.433$).

T17 INHIBITORY ACTIVITY OF HONEY AGAINST FOODBORNE PATHOGENS AS INFLUENCED BY THE PRESENCE OF HYDROGEN PEROXIDE AND LEVEL OF ANTIOXIDANT POWER

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Antimicrobial activity of honey has been attributed to hydrogen peroxide, which is produced by naturally occurring glucose oxidase, and phenolic compounds, although lethality of and inhibition by these and other components against microorganisms vary greatly, depending on the floral source of nectar. This study was undertaken to compare honeys from six floral sources for their inhibitory activity against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Shigella sonnei*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*. A disc assay revealed that development of zones of inhibition of growth depends on the type and concentration of honey, as well as the test pathogen. Growth of *B. cereus* was least affected. The inhibition of growth of *S. sonnei*, *L. monocytogenes*, and *S. aureus* in 25% solutions of honeys was reduced by treating solutions with catalase, indicating that hydrogen peroxide contributes to antimicrobial activity. Darker colored honeys were generally more inhibitory than light colored honeys. Darker honeys also contained higher antioxidant power. Since antimicrobial activity of the darker colored test honeys was not eliminated by catalase treatment, non-peroxide components such as antioxidants may contribute to controlling the growth of some foodborne pathogens. The antibacterial properties of honeys containing hydrogen peroxide and characterized by a range of antioxidant power need to be validated using model food systems.

T18 SENSITIZATION OF GRAM-NEGATIVE BACTERIA FOR ANTIMICROBIAL PEPTIDES UNDER HIGH HYDROSTATIC PRESSURE: ROLE OF CELL SURFACE CHARACTERISTICS

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High hydrostatic pressure sensitizes gram-negative bacteria to a number of natural antimicrobial peptides, such as lysozyme or nisin, for which they are normally protected by their outer membrane. Sensitization takes place by transient permeabilization of the outer membrane. In view of the cationic and hydrophobic properties of these peptides we have proposed permeabilization to occur by stimulation of the self-promoted uptake pathway, a mechanism which we have called pressure-promoted uptake. However, not all bacteria in a pure bacterial population, and not all gram-negative bacteria, were sensitized for lysozyme and nisin under high pressure. We speculate that heterogeneity of the cell surface properties in bacterial populations and outer membranes differences between species lie at the basis of the variable sensitivity. We have initiated a study on the mechanism of pressure-promoted uptake using a series of 9 *Salmonella* LT2 deep rough mutants, with decreasing size of O-polysaccharide and core polysaccharide (S, Ra, Rb1, Rb2, Rb3, Rc, Rd1, Rd2 and Re) and lysozyme-derived peptides with different charge and hydrophobicity in sensitization experiments under pressure. So far only the different strains have been tested. None of the strains was sensitive to lysozyme and nisin under atmospheric pressure under our test conditions. Under pressure, strain S (with wild-type LPS) was not sensitized to peptides, and only Re was sensitized to lysozyme and only Rb3, Rd1, Rd2 and Re were sensitized to nisin. These results indicate that loss of O-polysaccharides may increase the sensitization of gram-negative bacteria to cationic lipophilic peptides under pressure, by making the outer membrane more accessible or more hydrophobic.

T19 PROTECTIVE EFFECT OF COLANIC ACID OF *ESCHERICHIA COLI* O157:H7 TO ENVIRONMENTAL STRESS

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Strains of enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 produce under stress copious amounts of exopolysaccharide (EPS) composed of colanic acid (CA). Studies were performed to evaluate the association of the

ability to produce CA with survival of EHEC under adverse environmental conditions. A CA-deficient mutant, M4020, was obtained from a CA-proficient parental strain, *E. coli* O157:H7 W6-13, by inserting a kanamycin resistance gene cassette (kanr) into wcaD and wcaE, two of the 21 genes required for CA biosynthesis. M4020 was defective in CA production as determined by the uronic acid-to-protein ratio (UA/P) of cells grown from 1 to 4 days at 25°C on minimal glucose agar (MGA), MacConkey agar (MAC), and Sorbital MacConkey agar (SMAC), and by colony morphology on MGA. Results of stress treatment revealed that M4020 was substantially less tolerant to osmotic pressure (1.5 and 2.5 M NaCl), oxidation (10 and 20 mM H_2O_2), acid (pH 4.5 and 5.5) and heat (55° and 60°C) in comparison to W6-13, indicating that CA of *E. coli* O157:H7 has a protective effect to environmental stress.

T20 BACTERICIDAL ACTIVITY OF OLEATE TOWARDS VEGETATIVE CELLS AND ENDOSPORES OF *CLOSTRIDIUM PERFRINGENS*

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Bactericidal activity of oleate towards a *Clostridium perfringens* isolate obtained from a poultry processing plant was determined. Vegetative cells or endospores of *C. perfringens* were mixed in solutions of 0 to 0.20% or 0 to 10% of oleate, respectively, for 5 min to determine the bactericidal activity of various oleate concentrations. The effect of contact time on bactericidal activity of oleate was determined by mixing vegetative cells or endospores in 0.05% or 2.0% oleate solutions, respectively, for 5, 10, 15, 20, 25, or 30 min. After mixing in oleate, viable CFU were enumerated on Reinforced Clostridia Agar. Findings indicate that significantly fewer vegetative cells were recovered from solutions of >0.025% oleate than from controls, and that no bacteria were recovered from cultures mixed in solutions of >0.125% oleate. Additionally, significantly fewer CFU were recovered from endospores mixed in solutions of >2% oleate than from controls, and no endospores were recovered from solutions of >6% oleate. Significantly fewer CFU were recovered from vegetative cells mixed in 0.050% oleate solutions for >25 min than from cells mixed for <25 min, and no bacteria were recovered from oleate solutions for 30 min. Finally, significantly fewer CFU were recovered from endospores mixed in 2% oleate for > 20 min, while no bacteria were recovered from suspensions mixed for 30 min. In conclusion, bactericidal activity of oleate significantly reduces populations of vegetative cells and endospores of *C. perfringens* in vitro; therefore, oleate solutions may reduce the number of these bacteria associated with poultry processing.

T21 VALIDATING SANITATION REGIMES IN DRINK-VENDING AND POST-MIX SYSTEMS

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Consumers spend 1bn sterling annually in more than 350,000 refreshment vending machines in use in Britain today. The quality of drinks from vending machines and post-mix dispensers is paramount to consumers and suppliers of both equipment and food ingredients. There is evidence of biofilm formation within chilled water lines and local contamination of dispense heads arising from poor hygiene practice. Machine manufacturers must identify and operators are required to implement adequate sanitation procedures. This paper reports on development and validation of effective sanitation procedures.

Commonly used dispense heads and vending systems were examined for ease of cleaning and potential contamination problems. Field studies of vending machines and post-mix dispense systems involved testing water samples and swab sampling dispense heads. Both water samples and swabs were analysed using the membrane filtration technique. Samples were analyzed for *Escherichia coli*, coliforms and *Pseudomonas* spp. as well as a heterotrophic plate count at 22 and 37°C. Isolates were identified using biochemical characterization and used in the development and evaluation of sanitation protocols.

The five environmental isolates used were *Enterobacter cloacae*, *Acinetobacter* sp., *Xanthomonas* sp., *Enterobacter* sp. and *Pseudomonas aeruginosa*. All these have been documented to cause deterioration in product quality. Components under test were contaminated with late logarithmic stage cultures in the presence of representative organic soil and cleaned using controlled sanitation protocols. Cleaning efficacy was tested using microbiological swabbing and ATP bioluminescence.

Results are discussed in relation to the hygienic operation of vending machines and the assessment of cleanliness.

T22 PROVIDING SAFE FOOD FOR THE HOMELESS AND DESTITUTE: AN EDUCATIONAL PROGRAM FOR SOUP KITCHEN WORKERS

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As demand for meals provided by soup kitchens increases, there is growing concern about the safety of the food prepared by the largely untrained kitchen staff and volunteers. Soup kitchen guests have many challenges that may include homelessness, limited access to health care, chronic diseases, weakened immune systems, and alcohol or chemical dependencies. These individuals are very vulnerable to foodborne illnesses. The risk of foodborne disease can be reduced if kitchen workers know and practice the principles of safe food preparation.

Easy-to-use, audience-appropriate, safe food preparation educational materials for soup kitchen workers were developed and tested at 14 New York sites. Materials include a 28-page food safety reference booklet and 122 overhead transparencies or slides in a notebook containing several learning activities and information on how to teach adults and how to use the food safety materials. Topics covered include: Causes of foodborne illness, safe food receiving, food storage, good personal hygiene, safe food preparation, proper cooling temperatures, prevention of cross contamination, thermometer use, safe serving, and proper kitchenware washing, rinsing, and sanitizing.

At 8 workshops, participants' knowledge-change and food safety behavior were evaluated. Results indicated that participants increased their food safety knowledge and were successful at adopting or strengthening a specific food safety practice. Most attendees expressed enthusiasm for the food safety program and said they would

use the information provided. Both the New York City and State Health Departments adopted these food safety educational materials for training programs.

T23 MICROBIOLOGICAL SURVEY OF HOT-AIR HAND DRYERS FROM VARIOUS LOCATIONS

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Numerous studies involving various hand-drying techniques have described superior hygienic efficiency of hot-air dryers when compared to cloth or paper towels. An evaluation of methods utilized in previous studies revealed several deviations from everyday use, possibly compromising final results. In these studies, the hot-air dryers being tested were new (not used), employed specially filtered air, or were run for longer periods than would normally occur. Unpublished reports revealed the potential for hot-air dryers to serve as a source of cross-contamination by acting as a reservoir and emission source for dust containing potentially pathogenic microorganisms. In the latter work, under normal conditions of use, a greater than 500% increase in bacteria on the hand was reported, including *Staphylococcus* and *Enterobacteriaciae* species. The current study, described here, attempts to answer questions regarding the potential for hot-air dryers to become reservoirs for microbial contaminants that could be deposited onto hands. Microbiological sampling was performed on the air streams and mechanical components of over 30 hot-air hand dryers situated in various public locations (i.e., fast food outlets, food processing centers, supermarkets, hospitals, retirement homes, and hotels) in several US cities. Air exposure samples collected on selective media (Petri plate method) before and during machine operation indicated total bacterial contamination levels increasing significantly, confirming earlier work (>500%). These air samples included both coagulase-positive and negative *S. aureus*, members of the *Enterobacteriaciae*, and significant numbers of mold. Swab samples of various dryer components also revealed significant microbial contamination somewhat reflective of the air-stream data.

T24 PATHOGENIC AND INDICATOR BACTERIA ASSOCIATED WITH HANDWASHING AND DRYING CONTACT SURFACES

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Microbiological samples were taken from handwashing stations in 12 food processing/service facilities. Stations in restrooms and processing areas were tested (2 locations from each), emphasizing surfaces having hand contact potential. Supermarket delis, fast-food restaurants, and meat, poultry, seafood and dairy processing plants were evaluated. Sampling methodology focused on the detection of aerobes, coliforms, *Escherichia coli*, *Escherichia coli* O157:H7, coagulase positive and negative *Staphylococcus aureus*, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Salmonella*. Faucet handles, sink surfaces, doorknobs, paper towel dispenser fronts and parts (cranks, levers, towel exits), soap dispenser buttons, and hot/warm air dryer air streams and push-start buttons were sampled. Indicator microorganisms were found on some sample surfaces, occasionally at significant levels, revealing zigzag (donor to surface to recipient) cross-contamination potential at hand hygiene stations. Air samples of hot/warm air dryers in a seafood processing facility revealed significant levels of aerobic and coliform bacteria being expelled during operation. Other than coliforms, *S. aureus*, and *E. coli*, the presence of other potential pathogens was not indicated. The data supports the proposition that for optimal hygiene and compliance with prerequisite programs or CCP's, food processing/service workers should avoid contact with any of the surfaces tested following proper handwashing. Significant benefits may be achieved by the use of "hands-free" water faucets, soap and paper towel dispensers. The use of hot/warm air dryers may result in contamination of the environment and distribution of potentially harmful microorganisms onto hands if steps are not taken to reduce reservoir potential.

T25 AN IMPROVED TRANSPORT MEDIUM FOR THE PRESERVATION AND RECOVERY OF *LISTERIA MONOCYTOGENES* IN PLANT ENVIRONMENTAL SAMPLES

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Critical to the success of a *Listeria* control program designed to minimize LM cross-contamination risk in a food processing environment is the ability to detect monitor and control *Listeria* "hot-spots". Swab and sponge tests employing various media to preserve and transport samples are routinely used in *Listeria* environmental control programs. The transport medium employed should prevent the lethal effects of oxidation, inhibit the self-destructive enzymatic reactions within the cell, and minimize the effects of residual sanitizer, as well as other environmental stresses, encountered during sampling and transport. We compared 4 widely used transport media, i.e., Neutralizing Buffer (NB), Stuart's, Amies, and Amies w/charcoal, to a modified Amies medium (A-ML) containing sodium pyruvate, sodium thiosulfate, lecithin and Tween 80, for their ability to transport LM. Cells from stationary and log phase cultures of Scott A, CA, OH, and V7 were held in the test media at -20, 5, and 23°C for 24, 48 and 72 h to assess viability. Quat neutralizing ability was also tested. Transport temperature and sanitizer sensitivity were observed to be strain related, with CA being least and Scott A most sensitive. Stuart's was least effective in maintaining viability, with Scott A showing as much as a 3-log drop after 24 h at 23°C. NB failed to maintain LM viability at -20°C. Stuart's and Amies were ineffective in neutralizing sanitizer. NB and Amies w/charcoal quat neutralizing ability decreased with increasing sanitizer concentration. A-ML was most effective in neutralizing sanitizer and in maintaining viability. In both stationary and log phase cultures, A-ML counts increased by 2-4 logs in all strains after 24 h at 23°C. Meat and dairy plant audit data supported these observations, with A-ML recovery rates ranging 2-4 times higher than those of the other media tested.

T26 COMPARISON OF A NEW ELISA-BASED METHOD AND A MOLECULAR METHOD FOR THE DETECTION OF *LISTERIA MONOCYTOGENES* IN FOOD

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The performances of a new ELISA-based method (Transia™ Plate *Listeria monocytogenes* (TPLM) and a molecular hybridization-based assay (Gene-Trak® *Listeria monocytogenes* (GTLM)) were compared for the detection of *L. monocytogenes* in meat and seafood products. The specificity study, involving a total of 73 bacterial strains (30 *L. monocytogenes*, 23 other *Listeria* strains, and 20 strains belonging to 11 other related bacterial genera), showed that both TPLM and GTLM assays detected all the *Listeria monocytogenes* strains.

The limit of detection, evaluated with serially diluted cultures of 7 *Listeria monocytogenes* strains, was in the range of 10^6 to 10^7 CFU/ml for TPLM and in the range of 10^4 to 10^5 CFU/ml for GTLM: the probe assay offered a limit of detection 100 fold lower than the immunoassay. Five food matrixes ("rillettes," raw milk, soft-cheese, courgettes and smoked trout) were artificially contaminated with 0, 1-5, 8-26 and 45-132 CFU/25g: both alternative methods showed a limit of detection in the range of 1-9 CFU/25g of sample whatever the food type.

Finally, 130 naturally contaminated samples (80 meat and 50 seafood) were tested with both alternative methods and also with the ISO 11290 method. For the overall samples, the ISO method, TPLM and GTLM found respectively 36, 46 and 40 positive samples. The biggest discrepancies were noted with the meat samples, for which TPLM, GTLM and ISO methods showed respectively 39, 32 and 29 confirmed positive samples: the use of different sample and of different enrichment protocols could explain some of these discrepancies. Some samples found positive by TPLM (n = 3) or by GTLM (n = 3) were not confirmed by any other method and by streaking onto agar plates, and could be proposed as false positive. For the overall results, TPLM and GTLM methods were not statistically different, showing that the immunoassay and the probe-assay were equivalent for *L. monocytogenes* detection in meat and seafood products.

T27 EVALUATION OF A NEXT-DAY PCR METHOD FOR DETECTION OF *LISTERIA MONOCYTOGENES* IN FOODS

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A single 24-hour enrichment followed by a simple concentration step allowed accurate next-day detection of *L. monocytogenes* in food samples by the automated BAX® Screening system. PCR-based assays show improved sensitivity compared to other rapid assays, yet the relatively slow growth of *L. monocytogenes* still does not reproducibly result in detectable cell concentrations in a single 24-hour enrichment. In this study, 20 food types representing raw and processed meats, fresh produce and vegetables, seafood, cultured and non-cultured dairy products, egg and egg products and fruit juice were analyzed to determine whether a 10-fold concentration of the 24-hour enrichment sample would result in accurate detection of the target organism. For each food, 45 samples were enriched in standard media based on USDA or FDA methods for 22-24 h at 30°C. Twenty samples were inoculated at a low level (1-5 CFU/25 g), twenty were inoculated at a higher level (5-50 CFU/25 g) and five were left uninoculated. One-milliliter samples of the enrichments were centrifuged for one minute and then resuspended in 100 ml of peptone water. This sample was then processed by the standard procedure for the PCR assay. For comparison, the food samples were also tested using the 48-hour PCR method and the USDA or FDA culture methods. The results indicated that for the majority of food types the 24-hour method yielded comparable sensitivity and specificity to the 48-hour method and the culture method. This new method will allow food processors to reduce hold time for raw material or finished product.

T28 *CAMPYLOBACTER* DETECTION IN FOOD USING AN ELISA-BASED METHOD

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As one of the most important causes of acute bacterial gastroenteritis in humans, *Campylobacter jejuni* and *Campylobacter coli* need to be quickly and easily monitored in the food chain. A new method, called Transia Plate *Campylobacter*, combining a two-step enrichment done in Bolton broth and an ELISA test, has been developed for food applications.

The ELISA test was shown highly specific to the thermophilic *Campylobacter* as it detected the overall *Campylobacter* strains tested (13 *Campylobacter jejuni* and 17 *Campylobacter coli*) and did not cross react with any other bacterial genus such as *Citrobacter*, *Salmonella*, *Staphylococcus*... (21 different genera composed of 46 strains totally).

The limit of detection of the ELISA test, evaluated with 4 *Campylobacter jejuni* and 4 *Campylobacter coli* strains, was between 10^5 and 10^6 CFU/ml.

Six food matrices (raw milk, fish filet, raw milk cheese, wrapped cabbages, pork filet and poultry) were artificially contaminated with 4 different levels of *Campylobacter* strains (respectively 0, 3, 20 and 100 cells/25g of sample) and tested simultaneously by the ISO method 10 272/1995 (Preston broth 18 h at 42°C then streaking onto CCDA and Karmali agar plates and incubation 2 to 5 days at 42°C) and the ELISA based method. The limit of detection of the Transia Plate *Campylobacter* method was found lower than 10 CFU/25 g of food and equivalent to the ISO method.

Finally, when screening naturally contaminated food samples (totally 239 samples including raw and processed meat (n=95), dairy products (n=99), seafood products (n=30) and vegetables (n=15)), the Transia Plate *Campylobacter* method offered equivalent results to the ISO method, within 48 h. Furthermore, that study confirmed the high specificity of the new ELISA, suggesting that there was no need for further confirmation step with any positive ELISA test.

T29 A COMPARISON OF THE SURVIVAL RATES OF *CAMPYLOBACTER JEJUNI* UNDER VARYING ORGANIC LOADS AND FOOD CONTACT SURFACES

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Although many cases of *Campylobacter enteritis* have been attributed to undercooking of foods, cross-contamination between raw and cooked foods via food contact surfaces and worker contact has been identified as a significant risk factor for *Campylobacter* infections. The objective of this study was to determine the survival rate of *C. jejuni* under varying organic loads and food contact surfaces. D- values at 26.7°C and 60-62% rh were determined for *C. jejuni* (five pooled strains) suspended in either a phosphate buffered saline solution (PBS) or trypticase soy broth (TSB) and then inoculated (0.1 ml) on 5cm^2 samples of Formica™ laminate, ceramic tile, stainless steel, and a cotton dishcloth. Triplicate samples per surface were sampled and survivors recovered on Campy Cefex agar plates (3 replicates). The rate of inactivation was influenced by both contact surface and suspending media. The initial lag phases were followed by a linear ($r > -0.94$) decrease in cell population that varied by rate depending on the contact surface. D-values of 12.5(D), 19.1(BC), 24.1(AB), and 29.7(A) minutes and 23.7(AB), 10.5(D), 12.7(D), and 13.9(CD) minutes were detected for organisms suspended in PBS and TSB and then spotted on the dishcloth, Formica™, stainless steel, and ceramic tile surfaces, respectively. *C. jejuni* cells suspended in a nutritionally enriched media (TSB) died at a rate of nearly double that of cells suspended in PBS. The exception was for the dishcloths. These findings indicate that both the contact surface and level of organic matter can influence the survival and persistence of *C. jejuni*.

T30 WITHDRAWN

T31 FACTORS THAT INFLUENCE THE RECOVERY OF *ESCHERICHIA COLI* O157:H7 AFTER AN ACID SHOCK

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Escherichia coli O157:H7 is a foodborne pathogen capable of tolerating very low pH (e.g., acidic foods). Acid resistance can be induced by pre-exposure to mildly acidic pH or organic acids. The recovery of survivors appears to be highly dependent on the composition of the acid shock medium (ASM). This project was undertaken to determine the effect of media composition on the recovery of *E. coli* O157:H7 after an acid shock. O157:H7 strains were grown overnight in LBG medium (pH 5) and in an anaerobic basal medium. Acid shock experiments were conducted in pH 2 media for 1 h at 37°C. The survivor number was estimated by MPN in LB broth. When 100,000,000 cells were diluted 1000-fold into HCl-acidified distilled water no survivors were detected. A similar result was observed for all strains at any growth condition. If the ASM was replaced by Casaminoacids solution (CAA,10 g/l, pH 2), the survival rate could be as high as 100%, in anaerobic and aerobic cultures. A significant variation on survival rate in CAA was observed among strains, but ATCC43895 was the most resistant. When strain ATCC43895 was grown in LBG and suspended in pH 2 medium containing either glutamate, arginine or lysine, the survival rate was higher than 10%. Anaerobic acidic or acetate-added ATCC43895 cultures were also acid resistant, but only glutamate stimulated more than 10% recovery. These results indicated that acid resistance could only be elicited if aminoacids are present and that arginine- and lysine-dependent resistance is not induced under anaerobic conditions.

T32 DEVELOPMENT OF A DIGITAL DATABASE OF LACTIC ACID BACTERIA IN EUROPE

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Lactic acid bacteria (LAB) are organisms of economic importance in diverse agricultural, food and industrial applications including dairy and meat products, brewing and wine making, and animal silage production. They also offer potential as probiotics and in vaccine delivery. A collaborative network was formed to create a comprehensive electronic database combining molecular genetic, biochemical, and conventional microbiology with historical information about diverse lactic acid bacteria genera. With high standards applied to the information collected and methods of analysis, the evolving database will have considerable scientific and economic relevance. Ultimately, the goal is to allow database access to scientists and other users through a simple electronic query. All LAB strains in the database are fingerprinted with the RiboPrinter® Microbial Characterization System (Qualicon, Inc., Wilmington, DE) as the key to data accessioning and retrieval. At present, 400 discrete ribotype patterns have been assembled from strains of *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Weissella* species. Among these, 70 discrete *Lactobacillus casei* group isolates have been characterized. By incorporating advanced database and analytical software (BioNumerics, Applied Maths, Kortrijk, Belgium) with automated ribotype analysis and other molecular, biochemical, and descriptive data, this project may serve as a model for evolution of a microbiology enterprise operating with the speed and ease of the Worldwide Web.

T33 THE RISKS OF USING DATA LOGGERS TO MONITOR AVERAGE TEMPERATURE EXPOSURES

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Data loggers are routinely used to monitor temperature exposure of products during shipment and storage. In doing so, they generate large amounts of time and temperature data. From this data, it is easy to quickly compute the average temperature over the time period studied. Unfortunately, this average is wrong. All food products have a nonlinear dependency between time and temperature given by the Arrhenius equation, $t = k \exp(E/RT)$, where t is time, k is a constant, E is the activation energy, R is the gas constant and T is temperature. The calculation of the average temperature ignores this nonlinear relationship and therefore is erroneous. The Arrhenius equation does not treat all temperatures equally but instead places greater weights on higher temperatures than lower temperatures. By properly including the Arrhenius equation into the calculations, a new average called the "effective temperature" can be found. The effective temperature is a true measure for the product's

thermal history. This talk will show how the equations for calculating the effective temperature are derived and used to calculate the effective temperature. It will be shown that the effective temperature is always greater than the average temperature for non-constant temperature histories and that this difference is more significant for high activation energy food products. Finally, it will be shown that using the average temperature instead of the effective temperature can result in falsely overpredicting the shelflife of products by as much as 5 times, a potentially dangerous result.

T34 AN EVALUATION OF SURFACE HYGIENE MONITORING TECHNIQUES FOR USE IN THE FOOD INDUSTRY

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Assessing the hygienic status of food preparation areas and the efficacy of cleaning is an important component of food safety management systems. However, no ideal method exists to determine the cleanliness of surfaces and as such there is no standard method, technique or protocol, for surface hygiene monitoring. This can lead to difficulties and errors in selecting the most appropriate method for use within different food companies.

Four newly developed protein detection systems were assessed for their ability to evaluate surface cleanliness. Their performance was compared to that of ATP bioluminescence and to 3 traditional agar-based microbiological methods. Food-grade stainless steel surfaces were inoculated with known levels of food debris and/or microorganisms and sampled using standardised techniques or the manufacturer's instructions.

The ability of the various hygiene monitoring methods to detect bioburden depended not only on the level of contamination but also on the combination of microbial and food debris that was present. All 3 traditional microbiological methods were capable of detecting lower levels of bacterial contamination present on a wet surface than ATP bioluminescence could. This could lead, in a relatively small number of occasions, to wet surfaces passing ATP but failing microbiological methods. When microorganisms were absent or at levels proportionally much lower than that of the food debris, surfaces acceptable for food production by means of the microbiological methods were deemed unclean by the ATP bioluminescence technique. The most sensitive protein detection tests were superior to ATP bioluminescence when used to detect bioburden high in soluble protein (> 1 mg/g). However, in the presence of bioburden with a low protein content (< 10 ug /g) but a high microbial count, none of the protein detection tests indicated that the surfaces were unsuitable for food production, despite agar-based microbiological methods indicating the presence of large numbers of bacteria. The implications of these findings will be discussed in relation to hygiene monitoring in the food industry.

T35 DETECTION OF HEPATITIS A VIRUS IN A COMPLEX FOOD: STRAWBERRY FROSTING MIX

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Uncooked foods containing fresh frozen strawberries may be a source of hepatitis A. Outbreaks have been reported in which frozen strawberries were epidemiologically linked to disease, and items consumed in these outbreaks included desserts containing strawberry frosting. To evaluate recovery of hepatitis A virus (HAV), spiked strawberry frosting mix was prepared by adding one microliter of HAV stool preparation to 253 grams of strawberries and combining it with a commercial frosting mix. The contaminated food was diluted with an equal volume of 1.5% beef extract and 0.05% glycine (BE/G) followed by homogenization with 1/4th volume of Freon R (trichlorotrifluoroethane). After re-extraction of the interface between upper aqueous and lower Freon fractions, the aqueous fractions were concentrated twice in succession with polyethylene glycol (8% PEG and 0.3 M NaCl). RNA was prepared from multiple aliquots of the final concentrate with a commercial kit (QIAamp Viral RNA, Qiagen Co.) and analyzed by RT-PCR. Viral titer was estimated by the Most Probable Number (MPN) method, and expressed as RT-PCR units. Recoveries of seeded wild-type HAV from strawberry frosting mix, based upon calculated MPNs of recovered viruses and MPN titers of stool preparations, were 23% of HLD-2 strain and 56% of Q#1 strain of HAV. HLD-2 HAV has been titrated in primates and the inoculum in strawberry frosting corresponds to one primate infectious dose. Therefore, the method we have developed can detect one infectious dose of HAV corresponding to about 1400 viral genome copies. This method should be useful in detection of very low levels of HAV in complex food matrices.

T36 DEVELOPMENT OF PCR PRIMERS FOR DETECTION OF PROLIFIC HISTAMINE FORMER, *MORGANELLA MORGANII*

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Morganella morganii is the most important histamine former in seafoods because of its prevalence and high histamine producing capability. Identification of the bacteria has relied on conventional culture method, despite its limitations of being labor intensive and time-consuming. In this study, we developed a molecular technique, using PCR assay, for detection of *M. morganii*. The 16S rDNA of *M. morganii* was amplified using PCR. Primers used were: forward primer, 27°F (5' AGAGTTTGATCCTGGCTCAG-3'); and reverse primer, 1492R (5'-GGTTACCTTGTTACGACTT-3'). The PCR product was separated and purified using Gel Extraction Kit (Qiagen). The purified PCR product was cloned into pCR2.1 vector. Plasmid DNAs containing the insert were purified and sequenced. The 16S rDNA sequence (1,503 bp) of *M. morganii* obtained showed 95% of identity to those of enteric bacteria, i.e., *Enterobacter* spp., *Klebsiella* spp., *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp., and *Providencia* spp. *Proteus* spp. were most closely related to *M. morganii*, when phyrogenetic tree was constructed based on 16S rDNA sequences of *M. morgnaii* and other enteric bacteria. To differentiate *M. morganii* from other enteric bacteria, various PCR primers based on variable regions of 16S rDNA sequences were tested. Unique primers found for *M. morganii* were: forward primer, 5'-CTCGCACCATCAGATGAACCCATAT-3'; and reverse primer, 5'-CAAAGCATCTCTGCTAAGTTCTCTGGATG-3'. The 16S rDNA sequences and unique PCR primers obtained from this study should be useful for developing rapid and sensitive detection methods for *M. morganii* in food using PCR or other molecular-based techniques.

T37 ISOLATION, IDENTIFICATION, AND SELECTION OF LACTIC ACID BACTERIA FROM ALFALFA SPROUTS FOR COMPETITIVE INHIBITION OF FOODBORNE PATHOGENS

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The purpose of this study was to isolate and identify lactic acid bacteria with the intention of adding selected isolates to alfalfa sprouts for the purpose of inhibiting the growth of pathogens during sprouting. Fifty-eight lactic acid bacteria isolates were isolated from alfalfa seeds and sprouts. These isolates were evaluated for inhibitory action against *Salmonella* Enteritidis, *Salmonella* Typhimurium, four strains of *Escherichia coli* O157:H7, and four strains of *Listeria monocytogenes* using agar spot tests. Plates were incubated at 37°C and zones of inhibition were measured at 24 and 48 h. Diameters > 0.5 mm were considered inhibitory. *Salmonella* growth was inhibited by 56 (96.5%) of the isolates, *E. coli* by 55 (94.8%) and *Listeria* by 41 (70.6%). The isolates were identified using the API evaluation of carbohydrate utilization profile. The isolates showing inhibitory action toward all three pathogens were identified as *Lactobacillus* sp., *Lactococcus lactis* subsp. lactis, and *Leuconostoc mesenteroides* subsp. mesenteroides v. dextranicum. Isolates identified as *Lactobacillus delbrueckii* subsp. bulgaricus also showed inhibitory action against *Salmonella* and *E. coli*. Isolates taken from one sample of alfalfa seeds and identified as *Lactococcus lactis* subsp. *lactis* showed rings of inhibition of 4.0 mm or greater for all pathogens. We have selected four of the most inhibitory isolates to evaluate the effectiveness of the inhibitory action towards the pathogens during the sprouting process.

T38 EFFICACY OF DISINFECTION METHODS AGAINST CALICIVIRUSES ON FRESH FRUITS, VEGETABLES, AND FOOD-CONTACT SURFACES

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Norwalk and Norwalk-like viruses (NLVs) are important causes of foodborne gastroenteritis in restaurant-related outbreaks. Efficacy of common disinfection methods against these viruses on food-contact surfaces and fresh produce is not known due to their non-culturability. Seven commercial disinfectants for food-contact surfaces and three sanitizers for fruits and vegetables were tested against cultivable feline calicivirus (FCV). Discs of stainless steel, strawberry and lettuce were contaminated with FCV. The disinfectants were applied at 1, 2 and 4 times the manufacturer's recommended concentration for contact times of 1 and 10 min. The action of disinfectant was stopped by dilution. Surviving FCV was titrated in Crandell feline kidney cells. A product was considered effective if it reduced the virus titer by at least 3 $\log_{10}$. None of the products was effective when used at manufacturer's recommended concentration for 10 min. Phenolic compounds, when used at 2-4 times the recommended concentration, completely inactivated FCV on contact surfaces. Quaternary ammonium compound (QAC) in combination with sodium carbonate was effective on contact surfaces at twice the recommended concentration. Rinsing of produce with water alone reduced the FCV titer by 2 $\log_{10}$. On artificially contaminated strawberry and lettuce, peroxy acid-hydrogen peroxide was the only effective formulation when used at 4 times the manufacturers' recommended concentration for 10 min. These findings suggest that FCV and perhaps NLVs are very resistant to commercial disinfectants; however, phenolic compounds at 2-4 times their recommended concentrations appear to be effective at decontaminating environmental surfaces and may help control foodborne outbreaks of calicivirus in restaurants.

T39 CONCENTRATION AND DETECTION OF VIRUSES FROM FRESH PRODUCE AND FOOD-CONTACT SURFACES

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Fresh fruits, vegetables and food-contact surfaces have been implicated as vehicles of foodborne human calicivirus outbreaks. However, methods for detection of low-level calicivirus contamination of produce and surfaces are not available. We developed simple methods for recovery of caliciviruses from strawberries, lettuce and food-contact surfaces artificially contaminated with feline calicivirus (FCV). The strawberries were contaminated by immersing them in FCV suspension at pH 3.5 for 15 min. FCV was applied directly on the lettuce surface. The virus was eluted by rinsing both produce in 0.05M glycine buffer (pH 9.5). Stainless-steel surfaces were spotted with FCV. Virus from contaminated surfaces was recovered by applying 0.05M glycine buffer (pH 6.5, 10 ml/sq. ft), scraping the surfaces with cell scrapers and aspirating the buffer. For concentration, the eluate was passed through positively charged 1-MDS filter, and FCV from the filter was eluted in 1/100th volume of 0.05M glycine buffer (pH 9.5) containing 3% beef extract. The pH of the eluate was adjusted to 7.2 and virus was titrated in CRFK cells and also by RT-PCR. FCV recovery from both produce and contaminated surfaces ranged between 60 and 100%, and as little as 100 TCID50 of FCV could be detected from contaminated produce and surfaces. Organic flocculation and filter adsorption gave similar results for virus concentration from the eluate. Using these methods, we could detect 100 PCR units of Norwalk Virus contamination from food-contact surfaces.

T40 INACTIVATION OF *CRYPTOSPORIDIUM PARVUM* IN APPLE CIDER USING ULTRAVIOLET LIGHT

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Cryptosporidium and *Cyclospora* are protozoan parasites associated with water and foodborne illness outbreaks. In order to assess the efficacy of novel processing methods for the treatment of fruit juices such as apple cider, pathogens implicated in past foodborne illness outbreaks must be used to ascertain the level of effectiveness. *Cryptosporidium parvum* and *Escherichia coli* O157:H7 are the two microbial pathogens that have been associated with foodborne illness due to the consumption of unpasteurized apple cider. Ultraviolet light (CiderSure 3500) as a non-thermal processing alternative has been shown to be effective in achieving a 5-log or greater reduction in *E. coli* O157:H7 in apple cider.

This study was designed to determine the effectiveness of ultraviolet light in the inactivation of *Cryptosporidium parvum* oocysts inoculated into unfiltered apple cider and treated with the same UV exposure that was shown to be capable of achieving a greater than 5-log reduction of *E. coli* O157:H7 in apple cider. Apple cider was inoculated with 3 levels of *Cryptosporidium parvum* oocysts and treated with UV light. To determine the level of surviving oocysts, the treated cider was used in two parallel mice feeding trials (GKO and suckling). The results of this study showed that a greater than 6-log reduction of *Cryptosporidium parvum* oocysts was achieved. UV light as a non-thermal processing alternative to reduce contamination levels of *E. coli* O157:H7 and *Cryptosporidium parvum* in apple cider is effective and a means to enhance the safety of apple cider.

T41 EFFECTS OF HYDROGEN PEROXIDE ON THE SURVIVAL OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS IN UNPASTEURIZED FRUIT JUICES

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Cryptosporidium parvum has historically been associated with waterborne outbreaks of diarrheal illness. More recently, foodborne cryptosporidiosis has been associated with unpasteurized apple cider. Infectious oocysts are shed in the feces of common ruminants like cattle and deer in/near orchards. The addition of hydrogen peroxide (H_2O_2) to fruit juice to inhibit survival of *C. parvum* without using pasteurization was analyzed in this study. H_2O_2 was added on a wt/wt basis to apple cider, orange juice, and grape juices. Concentrations ranging from 3%-0.025% H_2O_2 were evaluated. Oocyst viability was analyzed with a cell culture infectivity assay, using a human colon cell line (HCT-8) that is most similar to human oral infectivity. Cell monolayers were infected with 10^6 treated oocysts. Parasitic life stages were visualized through immunohistochemistry and 100 microscope fields counted per monolayer. As little as 0.025% hydrogen peroxide completely inhibited the survival of *C. parvum* oocysts in apple cider after 24 h; however, incubation with the same concentration of hydrogen peroxide for two h resulted in only 68% reduction of viable organisms. Similar results were observed for all juices. Addition of 0.10% H_2O_2 to water caused a 100% decrease in viability after 2 h. Sensory analysis was performed on treated juice samples to determine palatable levels of H_2O_2. The effects of catalase on H_2O_2 decomposition were evaluated over 24 h. Further analysis will better evaluate if H_2O_2 is inhibiting excystation directly or through degredative products and quinone formation. Addition of low concentrations of H_2O_2 offers a valuable alternative to pasteurization.

T42 INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* IN APPLE CIDER AND ORANGE JUICE BY COMBINATION TREATMENTS OF OZONE AND CHEMICAL PRESERVATIVES

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Inactivation of *E. coli* O157:H7 and *Salmonella* in apple cider and orange juice treated with ozone and dimethyl dicarbonate (DMDC) or hydrogen peroxide (H_2O_2) was evaluated. A four- or five-strain mixture of nalidixic acid-resistant *E. coli* O157:H7 or *Salmonella* spp., respectively, was inoculated (7 log CFU/ml) into apple cider and orange juice. Ozone (0.9 g ozone/h) was pumped into juices (4°C) containing either 250 ppm DMDC or 300 ppm H_2O_2 for 90 min. Samples were withdrawn at 15 min intervals, neutralized with 1.0N NaOH, diluted in 0.1M phosphate buffer, and surface plated onto tryptic soy agar containing 50 ppm nalidixic acid. Combined treatments of ozone with DMDC or H_2O_2 were more effective against both pathogens than treatment with ozone alone, and the ozone + DMDC treatment was more effective than ozone + H_2O_2 ($P < 0.05$). Orange juice supported greater survival of both pathogens than apple cider ($P < 0.05$), regardless of treatment. Treatment of apple cider containing DMDC and H_2O_2 resulted in 4.2 and 2.3 log CFU/ml reductions, respectively, of *E. coli* O157:H7 populations. Treating orange juice with ozone and DMDC or H_2O_2 reduced *E. coli* O157:H7 populations by 3.5 and 2.5 log CFU/ml, respectively. *Salmonella* populations in ozonated apple cider containing DMDC and H_2O_2 decreased by 4.1 and 2.2 log CFU/ml, respectively; populations in orange juice were reduced by 1.5 and 1.2 log CFU/ml, respectively, by the same treatments. While modifications of treatment are necessary, ozone treatment in combination with chemical preservatives may provide an alternative to pasteurization for effective reduction of *E. coli* O157:H7 and *Salmonella* in fruit juices.

T43 HYDROGEN PEROXIDE AND ORGANIC ACIDS AS ANTIMICROBIALS IN FRUIT JUICES

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The economic and sensory drawbacks of pasteurization have led some fruit juice processors to search for alternative methods of pathogen reduction. Our objective was to determine the survival of *E. coli* O157:H7 and *Salmonella* in various fruit juices after direct low level additions of H_2O_2 and organic acids. Preservative-free apple cider, purple grape, and white grape juices were inoculated with approximately 2×10^6 CFU/ml of a 5-strain, acid adapted, nalidixic acid-resistant *E. coli* O157:H7 cocktail. Orange juice was inoculated with a similar *Salmonella* cocktail. 100 ml portions of inoculated juice were treated with one of four possible combinations of two H_2O_2 levels (0.017% and 0.012%) and two organic acid levels (0.3% and 0.1% of the dominant acid: malic/apple cider, tartaric/grape juices, citric/orange juice). Juices were stored for 21 days at 4°C and 25°C. Samples were taken every 24 h, diluted in 0.1% peptone, and pour plated in TSA. Results indicate that both 0.017% H_2O_2 treatments (0.3% or 0.1% acid) reduced bacterial counts to < 1 CFU/ml within 24 hrs at 25°C, and 72 h at 4°C for all juices except purple grape. 0.012% H_2O_2 treatments decreased counts by at least 4-logs within 24 h, with the exceptions of all purple grape juices and one orange juice treatment (25°C, 0.1% acid). Higher organic acid concentration generally increased effectiveness, though by less than 1-log in most cases.The results are positive for use of H_2O_2 and possibly organic acids as antimicrobials in fruit juice.

T44 GROWTH OF *LISTERIA MONOCYTOGENES* AND *ESCHERICHIA COLI* 0157:H7 IS ENHANCED IN READY-TO-EAT LETTUCE WASHED IN WARM WATER

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Ready-to-eat lettuce is routinely washed in cold chlorinated water. This treatment reduces total microbial populations by 1 log CFU/g. Research in our laboratory has shown that reductions approaching 3 log CFU/g are possible in warm (47°C), chlorinated water. The effect of warm water washes on the survival and growth of pathogens such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 during subsequent storage are unknown. Lettuce was dipped in cold (1°C) and warm (47°C) chlorinated water (100 µg /mL total chlorine) for 3 minutes before and after inoculation with both pathogens. The lettuce was packed in oxygen permeable film bags (OTR: 6000-8000 cc/m2/24 h) and stored at ideal (1°C) and abusive (10°C) temperatures. Total microbial populations were reduced by approximately 1 log CFU/g in cold chlorinated water, and 2.5 log CFU/g in warm chlorinated water. *L. monocytogenes* and *E. coli* O157:H7 populations did not increase in warm-water washed lettuce after 14 days in storage at 1°C but decreased in lettuce washed in cold water. Growth of both microorganisms was limited in lettuce processed in cold chlorinated water and stored at 10°C. In contrast, extensive growth was observed in lettuce washed in warm chlorinated water. These results suggest that survival and growth of these pathogens may be enhanced in ready-to-eat lettuce washed in warm water.

T45 APPLICATION OF VAPOR HEAT TO THE EXOCARP OF CANTALOUPE FOR THE REDUCTION OF *SALMONELLA* AND *ESCHERICHIA COLI* PRIOR TO MINIMAL PROCESSING

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Consumption of cantaloupe (*Cucumis melo* L. group Reticulatus) has been linked to outbreaks of illness, primarily caused by *Salmonella* serovars, several times over the past decade. We have demonstrated that vapor heat will substantially eliminate surface contamination of inoculated *Salmonella* and *E. coli* as well as naturally contaminating *E. coli* without negatively affecting sensory quality of minimally processed cantaloupe. Transference of *Salmonella* Typhimurium LT2rif (STR) and *E. coli* 506rif:inaC (EcINA+) from the exocarp to the edible mesocarp tissue was evaluated. Following a single cut through an inoculated exocarp site harboring population densities as low as $\log_{10}$ 2.54 CFU/cm², multiplication of both STR and EcINA+ was detected on melon pieces (48h at 10°C). Vapor heat was applied to the STR inoculated exocarp sites (n = 5) by a single steam orifice at a distance of 3.0cm for up to 4 min (59-63C). STR could not be detected after 3 min. Cantaloupes with EcINA+ or with field-acquired, nonpathogenic *E. coli* were placed in a small retort and treated at 15psi. Three to five melons were placed in the chamber for each time treatment, up to 1.25 min, and the entire experiment was repeated three times. Naturally acquired *E. coli* were not detectable after treatment times of 0.5 min in two tests and 0.75 min in one trial. EcINA+ was detected on 100% of 120 individual melon pieces prepared from inoculated but nontreated cantaloupes (48h at 10°C). EcINA+ was not detected on identically prepared melon pieces from cantaloupes treated for 0.5 min with vapor heat.

T46 EFFECT OF HOT WATER AND HEATED HYDROGEN PEROXIDE TREATMENTS IN REDUCING TRANSFER OF *SALMONELLA* AND *ESCHERICHIA COLI* FROM CANTALOUPE SURFACES TO FRESH-CUT TISSUES

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Cantaloupe melon has been associated with outbreaks of *E. coli* O157:H7 and *Salmonella*. Contamination may have been introduced into the flesh from the rind by cutting or contact of cut pieces with contaminated rinds. Our objectives were to investigate the efficacy of blanching or 5% hydrogen peroxide (70°C) in reducing transfer of *E. coli* ATCC 25922 or *Salmonella* from cantaloupe rind to the fresh-cut tissue. Whole cantaloupes were immersed in 8 $\log_{10}$ CFU/ml inocula containing *E. coli* ATCC 25922 or *Salmonella stanley* (H0558) for 10 min. The inoculated melons were stored at 4°C or 20°C for up to 5 days and at 0, 1, 3, or 5 days were blanched (70°C or 100°C) or washed in 5% hydrogen peroxide (70°C) for 60 s. Hydrogen peroxide treatment (70°C) caused a 4.4 log reduction of *E. coli* or 4.0 log reduction of *Salmonella* from an initial population of 4.6 or 4.4 $\log_{10}$ CFU/cm², respectively. Fresh-cut pieces prepared from the control melons were *E. coli* or *Salmonella* positive while those from the melons exposed to 100°C water or hydrogen peroxide (70°C) were negative throughout storage. Fresh-cut pieces prepared from melons exposed to hot water (70°C) 1 to 5 days after inoculation were also positive for *E. coli* or *Salmonella*. The results of this study suggest that boiling water or heated hydrogen peroxide treatment for 1 min can be used to decontaminate melon surface before fresh-cut preparation.

T47 LETHALITY OF 5 MEV E-BEAM TO *STAPHYLOCOCCUS*, *SALMONELLA* AND *LISTERIA* IN SLICED CANTALOUPE AND TOMATO

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Although irradiation of fresh-cut produce and fruit is not currently approved by FDA at levels exceeding the 1 kGy approved many years ago for insect control, petitions for approval for pathogen control are imminent. The use of 5 MeV irradiators offers many advantages over 10 MeV machines in terms of cost, size and convenience but the lower power levels also decrease the ability of e-beam to penetrate foods. This study was undertaken to evaluate the efficacy of 5 MeV e-beam pasteurization in sliced cantaloupe and tomato. Cantaloupe was pealed, quartered and sliced into 1 cm thick slices. Fresh green tomatoes were cored and sliced into 1 cm thick slices. All slices were individually vacuum packaged using a proprietary film. Each package was individually inoculated with log 6.0 CFU/g produce using a cocktail of 5 species of *Listeria*, *Salmonella* Typhimurium and *Staphylococcus aureus* and irradiated to 1 or 3 kG (surface energy) using a Surebeam 5 MeV irradiator. Uninoculated packages served as controls. *Listeria* and *Salmonella* were

recovered and enumerated by direct plating on differential agar and also by FDA BAM enrichment methods immediately after irradiation and after 10 days storage at 4°C. APC, coliforms and *S. aureus* counts (coagulase positive) were also performed. Both *Salmonella* and *Listeria* were recovered at low levels (<log 3.0 CFU/g) from fruit irradiated with 1 kGy but not in cantaloupe or tomatoes irradiated with 3 kGy by direct plating. However, enrichment methods showed that both *Salmonella* and *Listeria* were recovered by enrichment in approximately 30% of samples irradiated with 3 kGy. *Staphylococcus aureus* was recovered at 1 kGy but not at 3 kGy. Irradiation at 1 and 3 kGy had no significant effect on spoilage microorganisms (APC). These data show that even in 1 cm thick slices of fruit, levels higher than 3 kGy will probably be needed to ensure that product is free of pathogens such as *Listeria* and *Salmonella*.

T48 FOOD SAFETY BEGINS ON THE FARM: A NATIONAL EDUCATION AND EXTENSION PROGRAM FOR GROWERS AND PACKERS

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In the past two decades, there has been a doubling of foodborne illnesses associated with fresh fruits and vegetables. Once contamination with harmful microorganisms such as bacteria, viruses, and parasites has occurred, it is difficult to remove the pathogens. Therefore, preventing contamination is paramount. Our program on Reducing Microbial Risks in Fresh Fruits and Vegetables with Good Agricultural Practices in the US is funded by the CSREES-USDA Food Safety Initiative and the US FDA. Our goal is to educate growers and packers about the importance of produce safety and provide resources to assist in assessing and preventing microbial risks within each operation.

With collaborators from 16 states across the nation, the Good Agricultural Practices (GAPs) Program has developed a comprehensive education and extension program. In the last year, GAPs colleagues have presented and exhibited at over 40 grower and industry meetings. In addition, the GAPs project has designed several education materials; a booklet entitled Food Safety Begins on the Farm: A Grower's Guide, a pamphlet entitled Reduce Microbial Risks with Good Agricultural Practices, and a CD-ROM containing ready-to-use PowerPoint presentations.

The GAPs program also emphasizes the importance of awareness. Whether growers sell their commodities to commercial retailers or directly market to consumers, produce food safety is an important issue. With public concern about food safety increasing, growers and packers need to be prepared to answer food safety related questions. By combining good agricultural practices with awareness, the GAPs Program hopes to increase the safety of fresh fruits and vegetables.

T49 DEATH KINETICS OF *LISTERIA MONOCYTOGENES* IN MARGARINE, YELLOW FAT SPREADS, AND TOPPINGS

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The foodborne transmission of *Listeria monocytogenes* (LM) has been linked to human outbreaks of listeriosis involving the consumption of coleslaw, raw vegetables, and meat products, i.e., hot dogs, luncheon meats, pate and undercooked chicken. Dairy products including milk, Mexican-style cheese, and most recently butter have also been implicated. The ability of LM to grow at refrigerated temperatures, and survive at relatively low pH values, underscores the concern that various "like" foods could serve as vehicles for LM mediated foodborne infection, particularly those subject to multiple use and chill abuse. This study was undertaken to determine the fate of LM in 7 commercial "water-in-oil" based food emulsions representing the margarine, reduced fat spreads, and liquid topping food categories, the principal risk concerns being incidental contamination and temperature abuse in use. The aqueous phase pH of the products ranged from 3.8 to 5.5, salt from 1.5 to 5%, and oil content from 35 to 80%. All contained sorbate or sorbate and benzoate as preservatives. To assess LM "clearing" ability the products were challenged with approximately 1×10^5 CFUs/g of a 5 strain mixture of *L. monocytogenes* and incubated at 5, 10 and 23°C. Sample populations were assayed using MOX and Standard Methods Agars. LM did not grow in any of the products. In all instances, at the least, a 1-4 log drop in LM numbers was observed by day 3 and a 2-5 log drop by day 7. Die-off was most rapid at ambient (23°C) and "chill-abuse" (10°C) temperatures. Aqueous phase pH (< 4.7), and acidulant type (lactic>citric>phosphoric) appeared to exert the greatest impact. Increasing sorbate levels or combinations of benzoate/sorbate, present at levels ranging from 0.12-0.25%, enhanced lethality at higher pH values. LM die-off also appeared to vary indirectly with increasing oil concentration.

T50 SURVEY OF PASTEURIZED MILK AT RETAIL IN THE UNITED STATES FOR *LISTERIA MONOCYTOGENES*

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This survey was undertaken due to a lack of recent data on the prevalence of *Listeria monocytogenes* in pasteurized milk in the United States. Pasteurized milk near code date in gallon, half-gallon, quart, pint and half-pint containers (paperboard and plastic) was collected from retail locations in four FoodNet cities (Baltimore, MD; Atlanta, GA; Minneapolis/St.Paul, MN; and San Francisco, CA). Samples were arranged in such a way that results were not traceable to specific brand name or manufacturer of the milk. Sampling occurred during a 5-week period from June 5 to July 8, 2000, and included large and small retail stores in urban and suburban locations. *L. monocytogenes* was prescreened in samples through the AOAC approved rapid VIDAS ELFA method. Positive prescreening samples were cultured according to the Bacteriological Analytical Methods (BAM) and samples were enumerated by Most Probable Numbers (MPN) technique and confirmed by biochemical characterization. The frequency of isolation and confirmation of *L. monocytogenes* was 0/1897 (0%) whole milk, 1/1846 (0.05%) nonfat milk, 0/1669 (0%) chocolate milk (various fat levels), 0/107 (0%) other milk samples (reduced fat and low fat milk). Overall, *L. monocytogenes* was confirmed in 0.018% of pasteurized milk samples (1/5519). Enumeration of the single confirmed positive sample in nonfat milk (1-gallon plastic container) resulted in *L. monocytogenes* at <0.3 MPN/g of nonfat milk at 5 days past code date.

T51 THE THERMAL RESISTANCE OF *LISTERIA MONOCYTOGENES* AS AFFECTED BY THE PH AND WATER ACTIVITY OF THE HEATING MENSTRUUM

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Thermal treatments such as pasteurization reduce the risk of contamination by foodborne pathogens such as *Listeria monocytogenes*. The food matrix is known to affect the rate at which microoorganisms are killed by the thermal treatments. This study determined the impact pH and water activity (a_w) have on the thermal resistance of *Listeria monocytogenes*. Brain Heart Infusion Broth (BHI) was used as the heating menstruum. Its water activity and pH were modified using NaCl and HCl respectively. The BHI water activity studied ranged from 0.987 to 0.960, while the pH range was from 3.0 to 7.0. The times for one log of inactivation (D Value) observed ranged from 201 to 15,309 seconds. Thermal resistance decreased with decreasing pH and increasing water activity (i.e. decreasing salt concentration). The greatest thermal resistance for the four water activities examined was seen between pH 6.0 and pH 6.5. These results will allow a food processor to more accurately design appropriate thermal treatments for foods that have varying pH and water activities.

T52 FOODWORKERS AS A SOURCE FOR SALMONELLOSIS

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Point source foodborne outbreaks of salmonellosis are typically associated with foods of animal origin. However, extended restaurant outbreaks of salmonellosis have occurred in restaurants, suggesting a persistent reservoir of contamination. Therefore, we characterized *Salmonella* infections in restaurant employees during outbreaks.

During restaurant salmonellosis outbreaks, the Minnesota Dept. of Health and local public health agencies interviewed restaurant employees and collected stool specimens. Duration of *Salmonella* shedding was calculated from date of illness onset until the last positive stool specimen result or from the first to the last positive result for asymptomatic employees. Outbreak strains were characterized by pulsed-field gel electrophoresis to establish a common source, rather than multiple independent sources.

From 1997 through 2000, 157 confirmed foodborne outbreaks were investigated in Minnesota. Sixteen (10%) of these were due to *Salmonella*, 11 of which occurred in restaurants. The median duration of these outbreaks (i.e., transmission to patrons) was 32 days (range, 1-146 days per outbreak). Six hundred and sixty-three employees submitted stool specimens for testing. Sixty-four (10% overall; range, 0-35% per outbreak) employees tested positive for *Salmonella*. Of the employees with positive results, 34 (53%) were asymptomatic. The median duration of shedding was 8 days (range, 1-126 days).

The proportion of foodhandlers infected with *Salmonella* and their duration of shedding provides a persistent source of contamination in outbreaks in restaurants. Foodworker infections may play an important role in transmission of *Salmonella* during outbreaks.

T53 YEAST INACTIVATION KINETICS DURING THERMO-ULTRASONICATION TREATMENTS

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Low frequency ultrasound can be included in the formulation of combined methods of food preservation to diminish the intensity of traditional factors. The combined effect of simultaneous application of heat (45, 50 or 55°C) and low frequency ultrasound (20 kHz) at 90 microns amplitude on *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii* and *Z. bailii* viability was evaluated. Laboratory broth formulated at selected a_w (0.99, 0.97 or 0.95) and pH (5.5, 4.5 or 3.5) was inoculated with 10^6 yeast CFU/mL. An ultrasonic horn (13 mm) was submerged into the broth, then thermal (TT) and/or thermoultrasonication (TUT) treatments were applied. Samples were taken periodically and survivors were determined by surface platting. Survival curves followed first order inactivation kinetics; therefore D and z values were calculated. At constant a_w, D values decreased with pH reduction, being more noticeable for TT. At constant pH, D values were lower for a_w 0.99 than for 0.97 or 0.95. D values were significantly ($P < 0.05$) lower for TUT than for TT. At pH 3.5, *Z. bailii* D values at 45°C were reduced from 15.4 (TT) to 7.4 (TUT) min for a_w 0.99; from 26.8 (TT) to 8.6 (TUT) min for a_w 0.97; and from 43.5 (TT) to 12.9 (TUT) min for a_w 0.95. TUT significantly ($P < 0.05$) reduced the heat-protective effect of reduced a_w. *Z. bailii* was more resistant to the evaluated conditions than *Z. rouxii* or *S. cerevisiae*. Thermoultrasonication treatments can be applied to inactivate yeast cells, reducing heat treatment intensity, especially for a_w reduced media.

T54 THE BIOCIDAL EFFICACY OF HIGH RETENTION GEL OXIDANT SANITIZERS ON VERTICAL AND IRREGULAR SURFACES

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The inability of aqueous sanitizers to adhere to vertical and irregular surfaces prevents the sanitizer from having sufficient contact time with the target microbes adhering to the food/non-food contact surfaces. A promising alternative is to utilize food grade gel systems that thicken certain oxidant sanitizers to provide a higher level of adherance and biocide contact time on vertical and a number of irregular surfaces while minimizing sanitizer volumes.

The enhanced efficacy of such gel oxidant systems on such surface types is readily seen with a vertical surface test model we developed. Stainless steel coupons inoculated with either a *Listeria monocytogenes* or *Escherichia coli* O157:H7 broth suspension with a serum load were permitted to dry at 37°C for 60 min. After exposing the vertically hung inoculated coupons to sprayed liquid or gel oxidant systems for 60 s, we utilized a sponge swab-broth sampling system to reconstitute and neutralize the test biocides. Aliquots were plated utilizing IsoGrid membranes.

With 10 s applications and 60 s contact times we found that the Gel peroxygen at 85 ppm totally eliminated a 5.6 log challenge of *E. coli* O157:H7 while the liquid peryoxygen had only a 3 log reduction. Against *L. monocytogenes* the gel peroxygens achieved at 85 ppm a 2.6

log reduction while the liquid peroxygen had only a 1.5 log reduction. Additional studies utilizing other gel oxidant systems including field trial data will also be discussed.

T55 ASSESSING AND REDUCING THE RISK OF CROSS CONTAMINATION IN FOOD SERVICE

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Food service establishments are recognized as a major location for outbreaks of food poisoning. Outbreak investigations in the UK indicate cross contamination as a major risk factor and US studies that contaminated equipment in food service show many to be out of compliance. The present investigation concerns the degree of contamination of food service hand contact and environmental surfaces in relation to hand actions.

Four hundred sixty-seven food contact and environmental surfaces, post cleaning, were assessed in 15 businesses across the food service sector. Cleanliness was assessed using ATP bioluminescence, microbiological methods and visual methods. Visual assessment was a poor indicator of cleanliness with overall 50% of surfaces considered unacceptable compared to 75% for ATP and 79% using microbiological methods. ATP results ranged from <100 - 500 000 RLUs. Microbial counts ranged from <2.5 to >250 CFU/cm^2. After cleaning, surfaces were quickly recontaminated, with 63% considered unacceptable within one hour. The nature of the surface was an important determinant of cleanliness with smooth surfaces significantly cleaner ($P < 0.05$). Significantly higher ($P < 0.05$) microbiological counts were obtained from moist surfaces. Sites most likely to fail included a range of hand contact surfaces including tap, door and fridge handles which were omitted from most cleaning regimes.

Food handling actions, in relation to the surfaces tested, were observed and recorded using notational analysis. Over 2,500 hand actions were notated of which nearly 10% involved touching ready to eat food. Sequences of hand actions with the potential for cross contamination were analyzed. Contaminated surfaces touched by hands and attempts at decontamination are discussed in relation to management efforts to minimize cross contamination.

T56 EXPOSURE ASSESSMENT FOR HUMAN PATHOGENS TRANSMITTED BY POOR HANDLING PRACTICES OF READY-TO-EAT FOODS

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Many cases of foodborne disease are caused by the consumption of ready-to-eat (RTE) foods contaminated by poor handling practices such as inadequate cooking, temperature abuse, use of contaminated raw ingredients, cross-contamination, and poor personal hygiene of infected food handlers. As part of a broader project to estimate the degree of risk posed to human health by poor handling practices of RTE foods, the purpose of this study was to characterize the concentration of the pathogens transmitted by cross-contamination through various food handling scenarios. Bacterial (*Salmonella*, *Campylobacter jejuni*) and viral (Norwalk, hepatitis A virus) pathogens were chosen to model the magnitude of individual exposure based on variables including prevalence and initial level of contamination, as well as the rate of survival and transfer of pathogens during various food handling scenarios. Using Monte Carlo simulation in conjunction with supporting laboratory data on pathogen transfer and survival, it was estimated that <1% (range 0.1% to 0.5%) of viral or bacterial contaminants are transmitted during food handling, although these estimates varied widely with different handling scenarios. While seemingly small, these levels of contamination may be significant, considering that infectious doses for many pathogens are quite low (1-100 infectious units). This study demonstrated that exposure levels are greatly influenced by survival and transfer rates during pathogen transmission, which in turn are influenced by extrinsic factors such as time and temperature. Further research will combine these exposure models with dose-response models to quantify individual and population risks associated with consumption of contaminated RTE foods.

T57 PHYSICIANS' ATTITUDES TOWARD FOOD SAFETY EDUCATION

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The safety of the United States food supply continues to be a high profile issue among consumers, government leaders, health professionals, educators and the media. In recent years, the need to target food safety information to specific "at-risk" groups has been identified. These "at risk" groups include infants and children, pregnant women, older adults, and individuals with weakened immune systems such as HIV/AIDS patients. Therefore, it is critical that physicians are well equipped to deliver food safety education and risk reduction to patients.

In 1999 and 2000, the International Food Information Council (IFIC) conducted 8 focus groups in Baltimore, Boston, Chicago and San Francisco with obstetricians, pediatricians, oncologists, geriatricians and HIV/AIDS specialists to understand physicians' views of food safety issues to target educational techniques and materials. This was achieved by investigating physicians' understanding of foodborne illness, and their priorities in educating patients, and determining which methods they use for patient education. The key findings from this research include: physicians share a limited time with patients; patients' specific health concerns determined nature of visit, and the small prevalence of foodborne illness in the United States was of little concern. Overall, research participants provided little, if any, patient information on foodborne illness and general food safety. In the majority of instances, obstetricians were the least knowledgeable about foodborne illness and disease.

The need for better food safety understanding and education among physicians is paramount especially for those at higher risk of contracting a foodborne illness, such as *Listeria*, among pregnant women.

T58 EFFECT OF PEROXY ACID SANITIZERS AGAINST BACTERIOPHAGE ASSOCIATED WITH CULTURED DAIRY PRODUCTS

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Significant development of new technology sanitizers has occurred over the past few years and their effectiveness against bacteriophage is not well known. Peroxyacetic acid (POAA), and peroxy acid/carboxylic acid were tested and compared against chlorine at the recommended concentration, one-half the recommended concentration, and one and one-half times the recommended concentration. Four bacteriophage common to cultured dairy products were used to evaluate sanitizer effectiveness. POAA was found effective at higher than the recommended usage level. Peroxy acid/carboxylic acid was effective against all four bacteriophage. It was also found effective at half the recommended use level. Chlorine was effective against all four bacteriophage but was not found effective at half the recommended use level. POAA at half the recommended concentration and one minute contact time killed, i.e., no survivors, two of the four bacteriophage, and all four at one and one-half the recommended concentration. Peroxy acid/carboxylic acid was effective at killing all four bacteriophage at one-half the recommended use concentration, whereas chlorine was not effective at one-half the recommended use concentration but did kill all four at the recommended use concentration. The peroxy acid/carboxylic acid sanitizer was found to be equal to chlorine in effectiveness against the four bacteriophage at the recommended usage level, but was found to exhibit superior activity over chlorine when tested at half the recommended usage concentration.

T59 MOLECULAR EPIDEMIOLOGY OF NORWALK-LIKE VIRUS OUTBREAKS IN MINNESOTA

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Norwalk-like caliciviruses (NLV) are the leading known cause of foodborne illness in the United States. In 1996, the Minnesota Dept. of Health began using reverse transcription-polymerase chain reaction for laboratory confirmation of NLV gastroenteritis. During 1996-1999, 133 confirmed foodborne outbreaks were identified in Minnesota; 71 (53%) met epidemiologic criteria for NLV gastroenteritis. NLV RNA was detected in specimens from 24 (63%) of 38 NLV outbreaks for which stool specimens were collected for testing. Of 123 stool samples tested from the 38 NLV outbreaks, 54 (44%) were positive for calicivirus RNA. In 2000, Minnesota implemented sequencing of the viral polymerase gene from positive specimens. From January through November, 43 NLV RNA products representing 35 specimens from 15 outbreaks (foodborne and person-to-person) and eight specimens from sporadic cases were sequenced. Sixteen distinct gene sequences were identified; all sequences from the same outbreak were identical. In two instances, apparently unrelated simultaneous NLV outbreaks were linked by gene sequencing. In the first instance, epidemiologic investigation identified an ill worker who prepared food for both a nursing home and a catered wedding reception. Sequences from ill nursing home residents and reception guests were identical. In the second instance, a caterer prepared food at a different nursing home during a NLV outbreak, became ill, and subsequently prepared food for a wedding reception. Sequences from a nursing home resident, the caterer, and a reception guest were all identical. Gene sequencing of NLV has the potential to elucidate NLV transmission in the community.

T60 TECHNOLOGY REQUIREMENTS AND TECHNOLOGY TRANSFER IN THE WELSH FOOD INDUSTRY

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The food processing sector in Wales primarily comprises small to medium enterprises (under 250 employees/less than £64million turnover) with an average work force of 20. These frequently emanate from 'kitchen industries' where a small number of usually traditional products form the basis for the first stage of entrepreneurship. Rapid growth of quality products often results in the company's economic growth outstripping its technical capabilities, resulting in a technical gap. A range of schemes have been developed to fulfil the technology transfer needs of the companies.

Study of Welsh food processors was undertaken to determine specifically the needs/requirements of companies in the food processing sector. The study encompassed the whole of Wales geographically and targeted 600 companies ranging in size and sector (e.g. dairy, meat, etc.). Twenty percent of companies responded to the questionnaire with over 60% confirming that they had sought expertise outside the company in the previous year. Interest in a wide range of specific expertise was identified with over 70% stating an interest in at least one 'technical area', e.g., HACCP, nutritional analysis, food hygiene. There was also strong evidence that current levels of consultancy in some areas was perceived as inadequate.

To meet the needs of the companies specified in the study, the Food Industry Centre used the Teaching Company Scheme program (a three-way partnership between the university, the company and a recent graduate) to facilitate technology transfer in key technical areas. The case study examined in this abstract is the problem experienced by a vegetable processor when the Soil Association removed the ability of the company to decontaminate its vegetables via hypochlorite dipping. The study investigates the efficacy of alternative decontamination processes such as lactic acid, UV light and organic acid combinations. The results are discussed within the context of technology transfer and the links between food industry and academia.

S01 MOVING BEYOND HACCP — RISK MANAGEMENT AND FOOD SAFETY OBJECTIVES, SESSION I

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Currently, HACCP (Hazard Analysis Critical Control Point) is the most appropriate food safety system where a critical control point can be defined, i.e., a step at which control can be applied and is essential to prevent or eliminate a food safety hazard, or reduce it to an acceptable level. ILSI North America (ILSI N.A.) Technical Committee on Food Microbiology is sponsoring a project to help define a scientifically supportable food safety system to minimize microbial risk to consumers that will go beyond HACCP to integrate microbial risk assessment, HACCP, and food safety objectives or "FSOs." At the same time, the International Commission on Microbiological Specifications for Foods (ICMSF) is writing a monograph on managing microbial food safety risks that includes use of FSOs. FSOs can be defined as the maximum level of microbiological hazard in a food considered consistent with an acceptable level of consumer protection. They are developed as a result of deliberations of risk managers in consultation with risk assessors, the affected industry, and consumers. They must be technically feasible based on current knowledge of good manufacturing practices and HACCP, and ideally, should be quantifiable and verifiable. Their public health impact should be assessed.

The objective of the two-part symposium on food safety objectives is to present information on the upcoming ICMSF document (morning session) and then to consider where there is consensus and where there are controversies with the use of risk analysis, FSOs, and microbial testing in establishing food safety systems (afternoon session). The ICMSF combines the expertise of scientists from throughout the world with the objective of enhancing the microbiological safety of foods in international trade. The presentations in the morning session will address the steps of a sound strategy for managing microbial risks, outlining the role of risk assessment, FSOs, process and product criteria to meet the FSOs, acceptance, and microbial sampling criteria. Relevant examples of applying the integrated scheme will be discussed.

To answer the consensus versus controversies question, the ILSI N.A. Technical Committee on Food Microbiology has invited scientists representing academia, government, and industry, as well as representatives of the international food microbiology community and the public sector, to discuss the application of these concepts to improve food safety. This highly interactive session is designed not only to inform the audience about the issues, but also to foster debate between the audience and the speakers regarding the components of a comprehensive, scientific, and supportable food safety system.

S02 IMPACT OF WATER QUALITY ON FOOD SAFETY

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While waterborne diseases are typically considered to be problems of underdeveloped countries with inadequate sanitary practices, there is increasing recognition that developed countries also have significant public health problems caused by use of untreated, partially treated, or inadequately treated domestic water supplies. The incidence of major outbreaks of the classical waterborne bacterial diseases, such as: typhoid fever and cholera, has declined in developed countries since the initiation of chlorination of domestic water supplies. However, outbreaks of waterborne diseases still occur.

The use of chlorine compounds as a disinfectant for the conventional contact time is not adequate to control many enteroviruses and is significantly deficient in controlling cyst-forming and oocyst-forming pathogenic protozoa that are ubiquitous in domestic raw water supplies. The practice of flocculation and filtration as part of municipal water treatment of surface waters significantly reduces the numbers of cyst-forming and oocyst-forming protozoa and enteroviruses and minimizes the frequency of major outbreaks of waterborne disease from those organisms in drinking water subjected to those processes. However, failures in the performance of those systems, and inadequate treatment provided for other supplies continue to allow significant outbreaks of waterborne disease.

The presence of enteric pathogens in domestic water supplies represents a potentially significant threat of foodborne diseases where inadequate water treatment is practiced. Major outbreaks of disease, associated with inadequate treatment of domestic water supplies, continue to periodically occur in developed countries. In the past few years *Cryptosporidia* and *Giardia* have caused major outbreaks of disease in every province in Canada, in many states in the USA, as well as in the global community. The number of reported outbreaks is grossly underestimated since *Cryptosporidiosis* and *Giardiasis* are not universally reportable. *Cyclospora* outbreaks, associated with fresh fruit and produce, have occurred in both Canada and the United States. Outbreaks associated with drinking water

caused by *Escherichia coli* O157:H7 have caused death and significant morbidity in Cabool, Missouri (1989), Grampian, Scotland (1991), a summer camp in Minnesota (1995), Washington, Illinois trailer park (1997), Alpine, Wyoming (1998), Albany, New York (1999), and Walkerton, Ontario (2000).

One of the fundamental problems with current regulatory approaches for addressing water supply-associated waterborne diseases is that the coliform standard that is used for evaluating the "sanitary quality" of treated water does not reliably assess the presence of cyst-forming protozoa or enteroviruses.

There is obviously a need to redefine our concepts of "potability". We must significantly improve our ability to determine the adequacy of municipal water treatment to prevent microbial pathogens from causing epidemic waterborne and foodborne illness.

S03 IMPROVING LABORATORY QUALITY ASSURANCE IN THE REAL WORLD

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In the food industry, quality assurance is used to describe a system of processes and measurements that instill confidence in a product or service. Microbiology laboratories are faced with a growing number of challenges to deliver a consistent reliable product–their lab results.

Technology and rapid method development have caused an explosion of paths to give customers results. As the need for speed continues to push laboratories, so does the importance of developing a strong quality assurance program. This symposium will use a variety of real world experiences and theoretical knowledge to help laboratories develop, implement and improve laboratory quality assurance programs.

S04 FOOD ALLERGENS — CURRENT ISSUES AND CONCERNS

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This symposium will attempt to provide an overview of current issues and concerns related to dairy and food allergens. The session will be initiated by providing a consumer perspective of the issues: frequency and severity of reactions, consumer adjustments to the issues, and consumer desires from industry. The session will then provide the current status of analytical procedures, sensitivities and a summary of results garnered from review of products. Two speakers from GMI will then provide the "How-To's" of managing supplier issues and in-plant practical methods for Allergen Crossover Control. Regulatory will provide an update regarding classifications, recent regulatory actions, and perspectives regarding crossover plans and labeling. Wrap-up will be a discussion of legal implications relating all of the above elements.

S05 MOVING BEYOND HACCP — RISK MANAGEMENT AND FOOD SAFETY OBJECTIVES, SESSION II

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S06 USDA COMPETITIVE GRANTS IN FOOD SAFETY AND THE AWARDS PROCESS

ETTA SALTOS, USDA-CSREES, Room #2438, Waterfront, Washington, D.C. 20024, USA; JAN SINGLETON, USDA-CSREES, Mailstop 2220, Washington, D.C. 20250, USA; DAMANNA RAMKISHAN RAO, USDA-CSREES, Mailstop 2220, Washington, D.C. 20250, USA; SUSAN S. SUMNER, Virginia Tech., Department of Food Science and Technology, Blacksburg, VA 24061, USA; PATRICIA A. KENDALL, Colorado State University, Dept. of Food Science and Human Nutrition, Fort Collins, CO 80523-1571, USA

The objective of this symposium is to share information on the USDA-CSREES competitive grants in food safety and the process of grants administration and seek input from the audience to improve the process of awards. The target audience is primarily researchers, extension specialists, educators and industry professionals in food safety. In addition, the symposium could be useful for the other stakeholders such as consumers and press who may be attending the IAFP Annual meeting.

The symposium will consist of five presentations by experts followed by a question and answer/discussion session. The first part of the symposium will address various grant programs administered by the CSREES. These include National Integrated Food Safety Initiative (NIFSI), Initiative for Future Agriculture and Food Systems (IFAFS), and Enhancing Food Safety and Epidemiological Approaches to Food Safety Programs of the National Research Initiative (NRI). In NIFSI and IFAFS presentations emphasis will be placed on the multifunctional approaches (integration of research, education and extension) aimed at delivering the end product to the stakeholder, while NRI programs address primarily research activities. The second part of the symposium will explain the process of gathering information on the needs from stakeholders prior to formulation of the request for proposals (RFPs), the process of awards, and the elements of a winning proposal. The final part of the symposium will engage the audience by answering any questions and entertaining suggestions for improving the process of administration of grants.

S07 FOOD SAFETY IN THE DIGITAL AGE

KAREN MULLERY, 3M Microbiology, 3M Building 275 5W-05, St. Paul, MN 55144-1000, USA; JOHN GRIGGS, GSC Mobile Solutions, 4660 South Hagadorn, Suite 110, East Lansing, MI 48823-5353, USA; ARTHUR P. LIANG, CDC, Mailstop G-24, Atlanta, GA 30333, USA; JOHN LARKIN, FDA, 6502 S. Archer Road, Summit-Argo, IL 60501, USA; RICK BRENNER, USDA-ARS-CMAVE, 1600 S.W., 23rd Drive, Gainesville, FL 32608, USA; MARK CARTER, McKee Foods, 10606 McKee Road, Collegedale, TN 37315, USA; LORI LEDENBACH, Kraft Foods, 801 Waukegan Road, Glenview, IL 60025, USA

Information technology will have a profound impact on the food industry in the 21st century. The CDC FoodNet has already advanced the detection and communication of foodborne illness outbreaks. The food industry faces increasing pressure to employ new strategies and use innovative tools to prevent and manage these outbreaks. In this digital age a growing number of companies apply information management tools that help them store, track, analyze and better understand variables that affect food safety and quality. Better knowledge of these measures enhances their business decisions. As businesses develop information technology tools, awareness of the 1997 Federal regulations regarding electronic record keeping will be important. Hearing about information technology currently implemented in the food industry, and about new and emerging technologies can provide a catalyst for thinking of new ways to manage food safety and quality in your own organization.

S08 DAIRY PLANT HACCP — WHERE ARE WE AND WHERE ARE WE GOING?

WILLIAM SVEUM, Kraft Foods, 910 Mayer Ave., Madison, WI 53704, USA; SUSAN CRAWFORD, Michigan Dept. of Agriculture, 1255 Cedarhill Drive, East Lansing, MI 48823, USA; JOHN RUSHING, North Carolina State University, P.O. Box 7624, Raleigh, NC 27695, USA; REBECCA PISTON, Garelick Farms, Division of Suiza Foods, P.O. Box 1659, Bangor, ME 04401, USA; STEVE SIMS, FDA, Milk Safety Branch, 200 C St., S.W., Washington, D.C. 20204, USA; KATHY GOMBAS, FDA, Division of HACCP, 200 C St., S.W., Washington, D.C. 20204, USA

The National Conference on Interstate Milk Shipments (NCIMS) HACCP Committee has been overseeing the implementation of the NCIMS HACCP Pilot program for dairy plants since the 1999 conference. To date, the volunteer pilot plants and state regulators have shown that product manufactured under this program was as safe as under the traditional NCIMS PMO program. In addition, there was a strong feeling by state regulators that they understood the operation of the plant in more detail under the HACCP pilot and the industry personnel were more knowledgeable and better trained regarding their public health responsibilities. The result is that reciprocity between states for plants under the HACCP pilot has been maintained.

S09 JOINT FAO/WHO INITIATIVE ON MICROBIAL RISK ASSESSMENT

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Risk assessment of microbiological hazards in foods was identified as a priority area for the Codex Alimentarius Commission (CAC). In response, FAO and WHO jointly set an objective of providing expert advice on risk assessment of microbiological hazards in foods to member countries and the CAC. Working groups were established to examine three pathogen-commodity combinations: *Listeria monocytogenes* in ready-to-eat foods, *Salmonella* spp. in poultry, and *Salmonella* Enteritidis in eggs and prepare exposure assessments (EAs) and hazard characterisations (HCs) which were reviewed by a group of experts last July, and the revisions put on the Web site for public comment. The EAs described the prevalence and concentration data for each pathogen from retail to consumption taking into account time, temperature and the food matrix, as well as amounts eaten. The HC described dose response assessments using outbreak data, volunteer feeding experiments and animal studies. As a result of the reviews, revised documents were prepared and the EAs were combined with the HCs to give risk characterizations early in 2001. The assessments involved probability distributions and Monte Carlo analysis and generated examples of the probablity of illness arising from meals eaten in selected countries.

S10 ORGANIC FOODS: UNIQUE CHARACTERISTICS AND GROWTH POTENTIAL

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The organic market grew from $178 million in 1980 to $2.3 billion in 1994, and $6.7 billion in 2000. In 1995 the organic industry represented 0.5% of US farmers and included over 5,000 certified and up to 6,000 uncertified producers. In 2000, organic produce was estimated to represents approximately 2% of retail produce sales and is projected to increase 10 to 12% yearly. This growth has been facilitated by improved distribution channels and entrée into up scale supermarkets and restaurants. With establishment of the USDA organic guidelines in 2000, this method of production may increase substantially.

This symposium will provide an overview of the underlining philosophic approach to organic production, review challenges and opportunities of organic applied to different food products, address safety issues, and assess the national and international market potential. The first speaker will review the philosophy and beliefs of those advocating organic production. Next, unique attributes of organic production methods applied to plant and dairy foods will be presented. While the organic approach extends beyond food safety considerations, speakers will review chemical and microbiological safety issues related to organic production. Finally, organic production in an international market will be reviewed through discussion of uniformity of regulations and market potential.

S11 INDICATOR MICROORGANISMS — WHAT DO THEY INDICATE, AND IS IT OF ANY USE?

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Most of the food industry uses some type of testing to help assure product safety. Testing methods range from general analyses (such as standard plate count); to tests for microbiological indicators (such as fecal coliforms and Enterococci); to pathogen-specific assays (*Salmonella*, *L. monocytogenes*, *E. coli* O157:H7). This symposium will focus on indicator organisms, and how they might be used as process control indicators (or in other ways) to reduce risk and improve safety.

Practical examples of the use of indicator organism testing in the food industry will also be covered in this symposium. The food industry's rationale for the use of indicator testing, and examples of how it is currently being used will be explained. The use of microbial indicators by the FDA will also be addressed. Specifically, the topic of how the FDA decides upon which indicator organisms might be considered appropriate and the circumstances in which they might be used will be covered. The New Zealand Meat Industry Standards Council and the MAF Regulatory Authority have jointly developed a standardized microbiological monitoring program (including information on indicator organisms) that utilizes national targets indicative of required levels of hazard control. The role of indicator organisms in the context of this microbiological monitoring program will be discussed. Research on the statistical relationship between microbial and nonmicrobial indicators of fecal contamination and fecal bacteria will also be presented.

Finally, it should be noted that the act of sampling for indicator organisms has no inherent value unless decision-making is affected. Value-of-Information approaches for

indicator sampling programs will be discussed. These approaches provide an objective measure of the value of sampling results (i.e., information) in improving the expected value of outcomes prior to making decisions. This approach requires a quantitative description of the relationship between sampling results and decisions using quantitative risk assessment.

S12 ENSURING THE QUALITY AND SAFETY OF EXTENDED SHELF-LIFE MILK PRODUCTS

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Extended shelf-life fluid dairy products are becoming more common within the US and internationally. "Extended Shelf-Life" (ESL) may be used to describe any process that lengthens product shelf-life beyond what is normally expected, though in the US, ESL is most often used to describe the Ultra-Pasteurization (UP) process and resultant milk products. UP-ESL milk products are expected to last from 30 to 90 days under refrigeration storage. While the general regulatory requirements for UP-ESL milk products fall under the same guidelines as for pasteurized milk, much more stringent conditions are required to ensure that these products are safe, wholesome and of lasting good quality. UP-ESL milks are manufactured under near sterile conditions with much higher processing temperatures, requiring equipment and procedures that go well beyond conventional pasteurization requirements. Deviations resulting in post processing contamination in any form can be devastating, especially if contamination includes potential pathogens that may thrive in the absence of competing microflora. This symposium is designed to give an overview of ESL processing and products as it differs from conventional pasteurization and its products. Emphasis will be placed on the importance of validation and monitoring of equipment design and function, processing parameters, and product safety, quality and shelf-life from both manufacturing and regulatory perspectives. The goals and accomplishments of an industry wide task force formed to address issues of quality and safety of ESL products will also be discussed along with an international view of ESL processing and products.

S13 IRRADIATED PASTEURIZATION: REALIZING THE FOOD SAFETY POTENTIAL

ROB TAUXE, CDC, Division of Bacterial and Mycotic Diseases, Atlanta, GA 30333, USA; FRITZ KAFERSTEIN, FDA-USDA, Joint Institute for Food Safety and Applied Nutrition, 200 C St. S.W., Washington, D.C. 20204, USA; PAT ADAMS, IBA Advanced Applications, 1661 International Drive, Suite 350, Memphis, TN 38120-1415, USA; ROD CHURCH, Minnesota Dept. of Health, Disease Prevention and Control, 717 Delaware St. S.E., P.O. Box 9441, Minneapolis, MN 55440-9441, USA; MICHAEL WRIGHT, Supervalu and Cub Food Stores, P.O. Box 990, Minneapolis, MN 55440, USA; WILLIAM MARLER, Marler Clark Attorneys at Law, Bank of America Tower, 701 5th Ave., Suite 4301, Seattle, WA 98104, USA

Preventing pathogens from entering the food we eat is the key to reducing foodborne illnesses and deaths from foodborne pathogens. However, even with current state-of-the-art procedures and when following all regulations and laws, it is not possible to completely eliminate pathogens from the foods we eat. The consequences of ingesting a few pathogens can be extremely serious and even life-threatening for some individuals. Therefore health professionals should encourage use of safe, scientifically reviewed, approved procedures to assist in decreasing the risk of foodborne illness, especially to high-risk populations.

Irradiation of food is a proven, safe, effective tool and will prevent numerous foodborne illnesses and deaths when used in accordance with prescribed regulations and guidelines. Consumer research has long shown that people will purchase irradiated foods with endorsement by health and regulatory community being an important factor, which increases the likelihood to buy. Consumers should be provided with science-based information about irradiation and they should have the opportunity to select irradiated food such as hamburger and poultry if they wish to decrease their risk of foodborne illness. Although the benefits of irradiation are compelling, availability of irradiated food continues to be limited.

This symposium will update participants on the magnitude of foodborne illness, emphasize how irradiation processing can reduce risk, review features of gamma and e-beam irradiation, outline steps taken in Minnesota to prepare for successful launching of irradiated food, feature actual experiences of the retailer, and highlight the legal issues involved with food safety and irradiated food.

S14 *MYCOBACTERIUM PARATUBERCULOSIS* — VILLAIN OR BYSTANDER?

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Mycobacterium paratuberculosis is the etiologic agent of Johne's disease, a severe intestinal wasting disease in cattle. Surveys have shown that up to 41% of US dairy herds carry the organism. This organism has a high tolerance to heat and some studies have suggested that it may survive pasteurization conditions used for fluid milk. There are some similarities between Johne's disease in cattle and Crohn's disease in humans, which afflicts over 400,000 people in the US alone. This has led researchers to examine the association of this organism with human Crohn's disease and some to postulate its role as a causative agent of the disease. This symposium brings together some of the leading experts to examine the evidence for and against the association of the organism with human Crohn's disease, implications for the future, and the future research direction needed to definitively address the issue.

S15 ZERO TOLERANCE: BOON OR BUST?

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When health-related problems have arisen from the presence of a contaminant in foods, several regulatory responses are considered as approaches to risk management. One approach that has been used in certain instances is adoption of a so-called zero tolerance for a particular contaminant. In instances where a control step can be applied that is designed to eliminate a particular contaminant, e.g., a pasteurization treatment designed to kill vegetative pathogens, a zero tolerance policy presents few problems. However, in those instances where steps to preclude contaminants are not readily available (e.g., recontamination of cooked products with *Listeria monocytogenes*) applications of zero tolerance as a regulatory position raise questions of practicality and effectiveness of control.

Speakers in this session will explore various aspects and applications of zero tolerance and express their views of the advantages and disadvantages of zero tolerance as a regulatory policy.

S16 COMMUNICATING SCIENCE EFFECTIVELY

CHRISTINE M. BRUHN, University of California-Davis, Center for Consumer Research, Davis, CA 95616-8598, USA; SUSAN S. SUMNER, Virginia Tech., Dept. of Food Science and Technology, Blacksburg, VA 24061-0418, USA; RHONA S. APPLEBAUM, National Food Processors Association, 1350 I St. N.W., Suite 300, Washington, D.C. 20005, USA; NANCY PETERSON, Kansas State University, Agriculture Communications, Kansas State Research and Extension, Room 119 Umberger Hall, Manhattan, KS 66506, USA; DOUG POWELL, University of Guelph, Dept. of Plant Agriculture, Guelph, Ontario N1G 2W1, Canada

Public interest in food science has increased dramatically over the past few years. Food science is continually in the news, associated with product recalls, scientific studies revolving around controversial technologies, emerging microbial pathogens, and new food products. A common thread throughout these studies is the need to inform, educate, and instruct the public regarding healthy lifestyle choices, brought about by food science research. How does one go about communicating food science effectively to the public? Information presented to the public must be concise but factual, and comprehensible enough to educate but not cause alarm. This is not an easy task. Research scientists who serve as spokespersons for the general public must use legitimate resources and have reliable references to back up their statements. A public composed of consumers with varying educational background and expectations may accept or reject research findings based upon the method used to present such findings. Therefore scientific research can provide the basis for consumer attitudes and perceptions. Adequate resources are required to fulfill these duties, but how does one obtain funding and successfully pitch a food safety research project? Before starting a research project one must first write an acceptable grant and obtain funding. After obtaining such funding, and after correctly presenting your data and gaining an understanding of liability and support issues you are ready to inform the public and possibly engage the public in a scientific discussion. This symposium will demonstrate the steps necessary for communicating food science effectively to the general public.

S17 EDUCATING FOOD SERVICE WORKERS

CLARA LAWHEAD, Pasco Co. Health Dept., 10841 Little Road, New Port Richey, FL 34654, USA; RICHARD BARNES, FDA, Division of Federal-State Relations, HFC-150,5600 Fishers Lane, Room 1207, Rockville, MD 20857, USA; ANGELA FRASER, North Carolina State University, Box 7605, NCSU Cooperative Extension Service, Ricks Hall Annex-F-5, Raleigh, NC 27695-7605, USA; LISA WRIGHT, Foodmaker, Inc., 9330 Balboa Ave., San Diego, CA 92123, USA; ROY COSTA, Sanitary Environmental Monitoring Labs (SemcoLabs), 100 Powerline Road, Suite 108-A, Deerfield Beach, FL 33442, USA; MARTHA SMITH PATNOAD, University of Rhode Island, Cooperative Extension Education Center 3 East Alumni Ave., Kingston, RI 02881, USA

The purpose of the symposium is to "train the trainer" in effectively delivering food safety messages/education to a very diverse food service work force. This will be accomplished by using social marketing principles to motivate and engage the workers, overcome barriers, and affect positive behavior changes, ultimately preventing and reducing the risk of foodborne illness. The session will target three specific employee groups within food service–teenage employees, older workers, and English-as-a-Second Language (ESL) employees with cultural difference–for the purpose of addressing their specific needs/barriers/challenges. The presenters will use a social marketing approach in identifying these barriers and share their expertise in developing strategies for effectively delivering food safety messages. The importance and necessity of forming partnerships to accomplish this will also be discussed. The risk factors from the "FDA Retail Food Program Database of Foodborne Illness Risk Factors" (August 2000) in need of the greatest attention will be presented, along with interventions or strategies for dealing with them. This is in keeping with one of the objectives of Healthy People 2010 which is to improve food preparation practices and food employee behaviors at institutional food service establishments, restaurants, and retail food stores.

S18 DETECTION AND CONTROL OF HUMAN PATHOGENS IN FRESH FRUIT AND VEGETABLES

PINA M. FRATAMICO, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; BASSAM A. ANNOUS, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; WILLIAM F. FETT, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; DIKE O. UKUKU, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; ROBERT E. MANDRELL, USDA-ARS-WRRC, 800 Buchanan Street, Albany, CA 94710, USA; LARRY R. BEUCHAT, University of Georgia, 1109 Experiment Street, Griffin, GA 30223, USA

Outbreaks of foodborne illness, associated with the presence of human pathogens on fruit and vegetables and in unpasteurized juices, have led to increased concern about the prevalence of pathogens in the environment and the vulnerability of fresh produce to contamination by these pathogens.

This symposium will discuss research on ensuring the microbial safety of fruit and vegetables. Topics will include up-to-date data on risk factors that can be avoided during growth, harvesting, and processing of fresh produce to help prevent or minimize contamination; problems associated with sampling of produce and types of pathogen detection systems that are being used; and efficacy of chemical, physical and biological methods used in decontaminating fresh produce. Also, presentations will address research on the interactions of pathogens with plant tissues and with natural plant epiphytes on leaf and root surfaces, biofilm formation, and the development of new approaches for minimizing the presence of human pathogens in produce. Products discussed will include apple, unpasteurized apple cider, basil, lettuce, berries and sprouts. Pathogens discussed will include *Campylobacter*, *Listeria monocytogenes*, *Salmonella* species, *Escherichia coli* O157:H7, and *Cyclospora cayetanensis*.

S19 HACCP: HOW TO EVALUATE SUCCESS

THOMAS BILLY, USDA-FSIS, 1400 Independence Ave., S.W., Room 331-E, JLW Bldg., Washington, D.C. 20250-3700, USA; ROBERT L. BUCHANAN, FDA-CFSAN, Office of Science, HFS-006, 200 C St., S.W., Washington, D.C. 20204, USA; ROBERT V. TAUXE, CDC, 1600 Clifton Road, Atlanta, GA 30333, USA; R. BRUCE TOMPKIN, ConAgra Refrigerated Prepared Foods, 2001 Butter-field Road, Downer's Grove, IL 60515, USA; CAROLINE SMITH DEWAAL, Center for Science in the Public Interest, 1875 Connecticut Ave., N.W., Suite 300, Washington, D.C. 20009-5728, USA

Since 1995, meat, poultry and seafood industries have been required to implement HACCP systems to prevent foodborne hazards. FDA and USDA HACCP regulations contain many similarities and also some striking differences. For example, USDA is required by law to visit meat processors on a daily basis, while FDA inspection visits for seafood plants occur once a year or less. USDA also uses information from both industry and government microbial testing to evaluate HACCP's effectiveness. Government speakers will discuss various approaches to measure the success of required HACCP programs in controlling food safety hazards. CDC will evaluate foodborne disease data as one potential measure of the impact of HACCP implementation of food safety risks. Industry and consumer representatives will address other indications or measures of whether HACCP is working to improve the food production process and to reduce food safety risks.

S20 ILSI NORTH AMERICA-SPONSORED RESEARCH UPDATES

EDWARD R. ATWILL, University of California-Davis, Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, 18830 Road 112, Tulare, CA 93274, USA; MICHAEL P. DOYLE, University of Georgia, CFSQE, 1109 Experiment St., Griffin, GA 30223-1797, USA; DALE D. HANCOCK, Washington State University, Dept. of Veterinary Clinical Sciences, Pullman, WA 99164, USA; SOPHIA KATHARIOU, North Carolina State University, Food Science Dept., 339 Schaub Hall, Raleigh, NC 27695, USA; ALAN G. MATHEW, University of Tennessee, Dept. of Animal Science, P.O. Box 1071, Knoxville, TN 37901, USA; CORNELIUS POPPE, Health Canada, WHO Reference Laboratory for Salmonellosis, 110 Stone Road West, Guelph, Ontario N1G 3W4, Canada

A primary objective of the ILSI North America Technical Committee on Food Microbiology is to promote improved understanding of microbial food safety hazards by sponsoring research. In this symposium, researchers will report the results to date of recent committee-funded projects in several important areas.

S21 THE BENEFITS OF BETTER GOVERNMENT AND INDUSTRY RELATIONS IN ASSURING FOOD SAFETY

RON HICKS, USDA-FSIS, Room 347E, Whitten Bldg., Washington, D.C. 20250, USA; JOHN KVENBERG, FDA-CFSAN, HFS 600, 200 C St. N.W., Washington, D.C. 20204, USA; MARK DOPP, American Meat Institute, 1700 North Moore St., Suite 1600, Arlington, VA 22209, USA; MARTHA ROBERTS, Florida Dept. of Agriculture and Consumer Affairs, The Capitol P110, Tallahassee, FL 32399, USA; STEVEN GROVER, National Restaurant Association, 1200 Seventeenth St. N.W., Washington, D.C. 20036, USA

Assurance of Food Safety is unquestionably a multifaceted endeavor. Most authorities now recognize that this involves appropriate recognition of roles and responsibilities throughout the food chain as well as a regulatory structure that re-enforces the importance of addressing important aspects of food safety. It is also recognized that compliance with appropriate regulations and norms is an essential element in the food safety matrix. Compliance to these norms and to the applicable regulations depends on recognition on the part of industry of its responsibilities along with re-enforcement of these expectations through regulatory oversight. Thus the relationship between the inspected industry and the official agency(s) with jurisdiction is an important factor which can influence the success (or lack thereof) of government programs designed to assure food safety. This session will explore ways in which the dynamic relationship between regulators and the regulated industry can help or hinder the objective of assuring safe food. The panelists will also explore solutions that have shown promise and success and will explore prospects of enhancing these relationships.

Addendum

as of July 30, 2001

The following changes were made to the IAFP 2001 Program and Abstract Book.

MONDAY MORNING — AUGUST 6, 2001

(S03) **Title Change — Laboratory Quality Assurance — A Personal Perspective on an International Concern** — MICHAEL BRODSKY, Brodsky Consultants, Thornhill, Ontario, Canada.

(S03) **Speaker Change — Good Laboratory Practices: The Foundation of an Effective Quality Assurance Program** — KAREN BATISTA, Kraft Foods, Inc., East Hanover, NJ, USA will replace Suzanne Tortorelli.

(P02) **Title Change — Development of a Standard Method to Detect *Giardia* on Lettuce and *Cryptosporidum* on Raspberries** — N. Wilkinson, K. L. Barker, C. A. Paton, R. A. B. Nichols, H. V. Smith, and N. COOK, Central Science Laboratory, York, N. Yorks, UK.

MONDAY AFTERNOON — AUGUST 6, 2001

(S07) **Title/Speaker Change — Enlisting Wireless Handheld Technology for Audits** — JOHN SCHULZ, Marriott International, Washington, D.C., USA will replace Rick Brenner.

(T16) **Speaker Change — A Microbial Survey of Household Can Openers, Food and Beverage Can Tops, and Cleaning Methodology Effectiveness** — BARRY MICHAELS, Georgia Pacific Corporation, Palatka, FL, USA will replace Troy Ayers.

(T24) **Speaker Change — Pathogenic and Indicator Bacteria Associated with Handwashing and Drying Contact Surfaces** — BRIAN SMITH, Virginia Tech, Blacksburg, VA, USA will replace Barry Michaels.

(P72) **Withdrawn — Gel Peroxygens as Barrier and Treatment Systems for Beef Carcasses.**

TUESDAY MORNING — AUGUST 7, 2001

(P90) **Withdrawn — SimPlate for Yeast and Mold — Color Indicator: A New Method for Rapid Enumeration of Fungi in Food.**

(P102) **Speaker Change — Resuscitation and Growth of Heat- and Freeze-injured *Escherichia coli* 0157:H7 in Selective Enrichment Broths** — Authors are as follows: LAWRENCE RESTAINO, Elon W. Frampton, Hans Spitx, and William C. Lionberg.

WEDNESDAY MORNING — AUGUST 8, 2001

(T38) **Speaker Change — Efficacy of Disinfection Methods against Caliciviruses on Fresh Fruits, Vegetables, and Food-contact Surfaces** — P. B. ALLWOOD, Minnesota Dept. of Health, St. Paul, MN, USA will replace B. R. Gulati.

WEDNESDAY AFTERNOON — AUGUST 8, 2001

LATE-BREAKING SESSION

1:30 p.m. – 5:00 p.m.

Ballroom E

Fresh Cantaloupe Research: What We Know and What We Still Need to Know

Convenors: Donna Garren, United Fresh Fruit and Vegetable Association, Alexandria, VA; Larry Beuchat, University of Georgia, Griffin, GA

WEDNESDAY AFTERNOON — AUGUST 8, 2001

(S21) **Speaker Change — Current State of Federal Government/Industry Food Safety Relations: FDA/CFSAN Perspective** — JOSEPH BACA, Office of Field Programs, FDA/CFSAN, Washington, D.C., USA will replace John Kvenberg.

(T57) **Speaker Change — Physicians' Attitudes toward Food Safety Education** — ANTHONY FLOOD, International Food Information Council, Washington, D.C., USA will replace David Schmidt.

(T58) **Speaker Change — Effect of Peroxy Acid Sanitizers against Bacteriophage Associated with Cultured Dairy Products** — LOIS T. BRANCH, Ecolab Inc., Mendota Heights, MN, USA will replace Jerome Keller.

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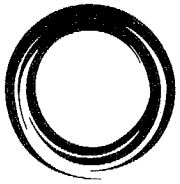

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