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Abstracts

(1) IVAN PARKIN LECTURE: FRESH FOODS WITH LOW NUMBERS OF MICROORGANISMS MAY NOT BE THE SAFEST FOODS

James M. Jay, University of Nevada Las Vegas, Las Vegas Nevada

The largest outbreaks on record of the following foodborne diseases occurred during the past 10 years: Botulism, hemorrhagic colitis, listeriosis, and samonellosis. The first three occurred during the past three years. While there are several contributing factors, it must be noted that they occurred at a time in history when our food supply overall is at its most highly developed stage. We need to ask if here is not some cause and effect here. The hypothesis to be presented is that in our efforts to produce food that contain fewer and fewer microorganisms, the harmless ones that are known to be inhibitory or antagonistic towards pathogens have been reduced to such low levels that pathogens are able to grow. Current concerns over the reduction in fresh meats need to be approached with the above mind.

(2) NCIMS UPDATE AND STRUCTURE OF NCIMS

Dan Rackley, Oklahoma Department of Agriculture, Dairy Service Division, Oklahoma, OK

The National Conference on Interstate Milk Shippers (NCIMS) is a cooperative program between the Food and Drug Administration, state rating and regulatory authorities, and the dairy industry. The Conference holds biennial meetings to discuss proposals from all interested parties on issues that affect conference documents and the dairy industry. The basic structure and function of NCIMS will be discussed and an update on issues that were accepted by the delegates at the 1995 meeting held in May in St. Louis will be presented.

(3) THE 3-A SANITARY STANDARDS PROGRAM - NOW AND IN THE FUTURE

Dr. Thomas M. Gilmore, 3-A Secretary, Dairy and Food Industries Supply Association, 1451 Dolley Madison Boulevard, McLean, VA 22101-3850

The 3-A Sanitary Standards program is a 50 year, voluntary approach to safeguarding public health and product safety through a unique industry-regulatory program of sanitary standards for equipment used in processing dairy and other foods. An overview of pertinent historical events, goals, committee structure are covered. A look at the anatomy, general criteria and the differences between 3-A Sanitary Standards and 3-A Accepted Practices are illustrated. The advantages of the 3-A Sanitary Standards program to processors, equipment manufacturers and regulatory officials are listed. The functions of the 3-A Symbol Council and 3-A Symbol authorization procedures are explained. 3-A's interactions with international standards organizations and predictions for the future are presented.

(4) LAYING THE GROUNDWORK FOR HACCP AND ISO 9000

John B. Adams, National Milk Producers Federation, Arlington, VA

The dairy industry faces significant challenges with regard to food safety and public policy. It is clear that FDA has adopted Hazard Analysis Critical Control Points (HACCP) as a strategy to guide regulation of the U.S. food industry into the future.

Commensurate with a changing regulatory policy for the U.S., new management approaches are being adopted by private industry. Utilizing systematic approaches, such as TQM and ISO 9000 series standards, progress is being made to standardize policy, improve quality and reduce costs.

As the United States adopts the HACCP approach to assure food safety, many countries throughout the world have adopted or are moving to adopt ISO 9000 series standards. The ISO Standard(s) serve as an umbrella under which regimentation can occur to systematically document compliance of a process to a specific set of requirements and/or product standards. Adherence to the ISO 9000 series standards can improve operational control, reduce costs and correct process inadequacies, thus assuring more consistent control over the management and production of any product. ISO 9000 can, therefore, be viewed as a standard and procedure to assure efficient and effective management control over the process of designing, producing and distributing a product of consistent quality.

HACCP can also be utilized to reduce the risk associated with any process by monitoring and controlling the most critical control points. Thus, for the purpose of assuring food safety, HACCP and ISO 9000 could be considered equivalent system approaches. In most cases, adoption and certification under ISO 9000 will include the standard along with a HACCP plan. HACCP could even be considered a subpart of any ISO 9000 system for purposes of assuring food safety.

*Asterisk indicates author to whom inquiries regarding this paper should be addressed.

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Regardless of the approach adopted to assure food safety or reduce the risks associated with food production and processing, the ultimate goal must be to reinforce consumer confidence in the safety of the food supply along the entire continuum from farm to household point of use.

Given the current restrictions on budget at both the Federal and State levels, inspection resources will continue to be limited. Adoption of HACCP, therefore, will require changes for industry as well as regulatory. Industry can expect to assume more responsibility for assuring food safety, while regulatory can be expected to assume more of an audit function related to monitoring industry performance as measured against a HACCP plan.

As HACCP is implemented at the processing level, it is inevitable that the critical control point for the safety of raw materials will force more focused attention at the farm or preharvest level. Thus, it becomes imperative to define critical control points at the preharvest level and design HACCP based quality assurance systems which will reduce risks of food adulteration from chemicals and human pathogens.

As the regulatory transition continues to occur toward HACCP at the processing level, a commitment to preharvest food safety at the farm level will require the development and integration of quality control systems (TQM). In return, the application of TQM can provide a higher degree of product safety and quality for consumers and a greater economic benefit to producers. The transition toward a preharvest HACCP based system will be greatly strengthened through economic incentives which are market driven.

(5) DAIRY PRODUCT SHELF-LIFE TESTS FOR QUALITY CONTROL AND RESEARCH AND DEVELOPMENT

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Shelf-life of dairy products is a critical quality parameter for consumers and processors. The perishable nature of dairy products does not allow processors time to determine whether each lot produced will meet labeled code date. Many test methods have been developed to estimate quality and potential shelf-life of dairy products with varied results. These have ranged from simple bacterial enumeration to newer tests based on detection of metabolites or ATP. Several systems using Bioluminescence-ATP technology are being marketed to food microbiologists and sanitarians for monitoring processing plant hygiene and suggest improved shelf-life will result. This paper reviews the status of these dairy product shelf-life tests.

To be useful to the dairy industry, a test must be technologically feasible, fast, easy to perform and low cost. Then to achieve shelf-life extension, the results must be understood, guidelines developed, corrections made in cleaning practices and all discrepancies investigated and eliminated.

(6) NATIONAL MILK DRUG RESIDUE DATA BASE

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The National Milk Drug Residue Data Base was established to collect data regarding the extent of testing milk for animal drug residues within the United States. This report summarizes the data reported by participating states and their regulated milk industry. These data include the total numbers of tests as classified by family of drug, and the type of analytical procedures used. This report also illustrates the total number of samples analyzed for Grade A and non-Grade A milk and milk products, regulated industry vs. regulatory agency testing, and the sampling and testing of various sources such as producers and bulk milk pick-up tankers. The total number and type of methods analysis utilized is indicated by reporting the incidence and percentage of positive samples.

(7) PRACTICAL SOLUTIONS TO PATHOGENS FROM MILK AND OTHER ANIMAL PRODUCTS

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Raw agricultural products, especially those of animal origin, such as milk, meat, poultry, eggs, fish and shellfish, often contain various pathogenic microorganisms. Elimination of these pathogens depends upon proper pasteurization and sanitation in processing plants or in the home. Recent outbreaks of food-borne disease from *Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes*, and *Campylobacter* indicate that adequate heat treatments are not always applied and/or cross-contamination occurs. Proper pasteurization of milk should ensure the complete destruction of *Listeria monocytogenes*, a heat-resistant, vegetative pathogen found in 3 to 5% of raw milk supplies. Research in our laboratory indicates that *L. monocytogenes* may survive HTST pasteurization and exist in an injured state that is undetectable by current official detection methods. Most food-borne illnesses due to animal products are currently caused by gram-negative food-borne pathogens, such as *Salmonella* spp., *Campylobacter jejuni/coli* and *E. coli* O157:H7. As a result, many practical methods for destroying these pathogens on/in raw animal products have been and are being studied intensively. We

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have recently demonstrated in our laboratory that a combination of high pH (pH greater than 11) and high temperature (greater than 100°F) has a dramatic synergistic effect on the destruction of these gram-negative foodborne pathogens. Recent commercial application of this technology to the washing of shell eggs has resulted in a practical, effective method for destruction of *Salmonella enteritidis* and other gram-negative pathogens. The two critical control points of pH and temperature can be easily integrated into a HACCP plan and may, along with other compounds, allow effective and practical control of gram-negative pathogens and spoilage bacteria on/in raw animal foods.

(8) DESIGN, INSTALLATION AND MAINTENANCE OF PLATE COOLERS

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The proper design, installation and maintenance of plate coolers can make the difference between a profitable and non-profitable process; they can also make the difference between a sanitary and unsanitary process. We will discuss the various designs of plate coolers, making use of water/milk flow diagrams to discuss the advantages and disadvantages of various designs. Proper installation of the properly designed plate cooler is the next critical step. We will discuss the range of problems that can befall an installation and show you ways of solving these problems before they occur.

Once the plate cooler is in place, sanitary, profitable operation depends on the proper and timely maintenance of the system, including the replacement of gaskets. We will outline a maintenance procedure which will ensure years of safe, profitable use of your plate cooler.

(9) SHELF LIFE EXTENSION AND SAFETY OF FRESH PORK TREATED WITH HIGH HYDROSTATIC PRESSURE

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High pressure treatment, a new method of food preservation, has been used extensively in Japan in processing of various fruit products. We examined the use of this technology for improving the safety of fresh meats. Our objectives were: to determine the optimal conditions of pressure, temperature, and processing time required to eliminate *Salmonella typhimurium* and *Listeria monocytogenes* in fresh pork, and to determine the effect of the optimal conditions obtained above on the quality of the pork. Twenty-five gram samples of fresh whole-muscle pork were inoculated with 0.1 ml of an 18-h culture of either of the two organisms. Samples were then subjected to pressures between 60,000 and 120,000 psi, at either 2 or 25°C, for up to 30 min A 5-$\log_{10}$ reduction in both organisms was observed (as determined by MPN and spread plating on selective and non-selective agars) at 20 min of processing. The maximum D-values at 60,000 psi were determined to be 2.13 min at 25°C for *L. monocytogenes*, and 1.48 min at 2°C for *S. typhimurium*. Samples subjected to 60,000 psi to achieve a 6D reduction were evaluated for sensory quality by taste panel using the Triangle Test of Difference. There was a detectable difference ($p < 0.1$) between samples processed at high pressure at 2°C and non-treated controls, and for samples processed at 25°C ($p < 0.05$) and controls. Further analysis showed the controls being ranked as more tender than treated samples. Objective analysis showed no significant difference in moisture content between the control and pressure-treated samples. The controls were found to be darker in color (as measured by Hunter Lab). Further studies are being carried out to determine how the treatment parameters of pressure level and time of processing can be adjusted to achieve a 6D reduction in the pathogens while not affecting the sensory quality of the meat.

(10) MICROBIAL MONITORING OF IRRADIATED COMMERCIALLY-PREPARED CHUB-PACKAGED GROUND BEEF

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Ten pound chubs of coarsely ground beef were irradiated (2.0 - 2.4 kGy surface dose) and stored at 2°C to examine the effects of x-ray irradiation on microbial spoilage of the product. The beef was sampled to determine pH and total microbial load on days 0, 1, 3, and 4 to 7 days thereafter until extensive microbial growth, gas and/or odor production rendered the product spoiled. In trial 1, growth ($\geq 10^4$ CFU/g meat) in the control (unirradiated) samples was detected at day 3, with maximum counts($\geq 10^7$ CFU/g meat) reached at day 13. However, similar levels of growth in irradiated meat did not occur until days 21 and 33, respectively. Trial 2 is currently in progress but results through day 21 are similar to those of trial 1. Initial pH values in both trials for treated and untreated beef were 5.8, and dropped as low as 5.0 as spoilage progressed. In addition, oxymyoglobin stability degenerated noticeably at the onset of exponential growth. Presumptive tests indicate that Gram positive, catalase negative cocci may largely be responsible for spoilage of the product.

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(11) REDUCTION OF *SALMONELLA TYPHIMURIUM* ON CHICKEN CARCASSES USING PULSED ELECTRICITY

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More effective methods are needed to control bacterial contamination in poultry processing. Pulsed electricity has been studied as a method to destroy bacteria in poultry chiller water, scalder water and chicken skin. The objective of this research was to determine the reduction of *Salmonella typhimurium* on chicken carcasses using pulsed electricity and salt water.

Pre-chill chicken carcasses were inoculated with 1 x 10^6 CFU/ml *S. typhimurium* and then dipped in .85% NaCl, 1% Na_2CO_3, 1% $NaHSO_4$ or 1% Na_3PO_4 solution. Electricity at 50 V, 100 Hz and 50% duty cycle was applied for 5 min. The total plate count and MPN tests were performed to detect bacteria. The controls were performed in the same way but no electricity was applied.

The electrical treatments with 1% NaCl and 1% Na_2CO_3 were not significantly different compared to the controls. The electrical treatments with 1% $NaHSO_4$ and 1% Na_3PO_4 reduced *Salmonella* number by 1.0 and 1.4 log. No bacteria were detected in the treatment water. It was concluded that using electrical treatment with 1% Na_3PO_4 or 1% $NaHSO_4$ was effective in reducing *S. typhimurium* on the chicken carcasses.

(12) ISOLATION AND CHARACTERIZATION OF GRAM-NEGATIVE BACTERIA, ISOLATED FROM GROUND BEEF, THAT EXHIBITED INHIBITION OF *ESCHERICHIA COLI* O157:H7

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Anti-microbial peptides, or bacteriocins, produced by bacteria, have been suggested as novel methods of food biopreservation. Recent outbreaks of Haemolytic Uraemic Syndrome, HUS, caused by *E. coli* O157:H7, were associated with frozen ground beef patties. Data generated in our lab and research conducted by Schoeni and Doyle (1984, AEM), indicated survival in frozen ground beef for over six months. The incidence of this organism in ground beef varies. It was hypothesized that this low incidence maybe due to the presence of inhibitory bacteria in the ground beef. Therefore, retail ground beef was screened for the presence of bacteriocin producing organisms. Ground beef samples, stored at 37°C for 24 h and 4°C for 7 d, were surface plated onto Brain Heart Infusion agar (37°C for 24 h and 7°C for 5-7 d). Isolates were than screened for the ability to inhibit the growth of *E. coli* O157:H7, with an agar flip and streak method using Brain Heart Infusion. With this method, 500 isolates were screened. Thirteen isolates, preliminary identified as various *Pseudomonas* spp., exhibited inhibition. Investigation into the mechanism of inhibition, ruled out organic acids, competition, and hydrogen peroxide production. This research has helped us gain insight into the microbial flora of our retail ground beef supply. In addition, the identification of inhibitory microbes, found naturally in ground beef, gives rise to possible new biopreservation methods that maybe effective against gram negative pathogens like, *E. coli* O157:H7.

(13) INHIBITION OF A PSYCHROTROPHIC *CLOSTRIDIUM* SPECIES BY SODIUM DIACETATE AND SODIUM LACTATE IN A COOK-IN-THE-BAG, REFRIGERATED TURKEY BREAST PRODUCT

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A nonpathogenic, nonproteolytic, psychrotrophic *Clostridium* sp. was isolated from a cook-in-the-bag, refrigerated turkey breast product with an intense pink discoloration and off odor. Sodium diacetate (0-0.5%), sodium lactate (0-3.0%), and combinations of both ingredients were subsequently evaluated in this turkey product inoculated with spores (10-30/g) of the *Clostridium* sp. Inoculated turkey was vacuum packaged, cooked to 71°C, chilled, and incubated at 4°C. In the absence of antimicrobials, off odor occurred in 7 weeks and counts reached 7.7 log CFU/g. Turkey containing sodium diacetate (0.25%) or sodium lactate (1.5%) spoiled in 12 weeks and counts reached 7.3 log CFU/g and 6.2 CFU/g, respectively. Turkey containing both sodium diacetate (0.25%) and sodium lactate (1.5%) did not spoil after 22 weeks. Sodium diacetate and sodium lactate can prevent spoilage of cook-in-the-bag, refrigerated turkey due to this *Clostridium* sp.

(14) INHIBITORY EFFECTS OF SUCROSE FATTY ACID ESTERS, ALONE AND IN COMBINATION WITH EDTA AND ORGANIC ACIDS ON *LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS*

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The effects of sucrose esters of fatty acids, alone and in combination with ethylenediaminetetraacetic acid (EDTA) and organic acids, on survival, growth and thermal inactivation of *Listeria monocytogenes*

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and *Staphylococcus aureus* were investigated. At 400 ppm, sucrose monolaurate (SML) was lethal to *L. monocytogenes* and inhibitory to *S. aureus* in tryptose phosphate broth (TPB) and tryptic soy broth (TSB), respectively. The addition of EDTA to broth containing SML had an additive effect on the inhibition of both organisms. The chelator alone had no effect at 100 ppm on either pathogen but was inhibitory at 200 ppm. Inhibition of *L. monocytogenes* was more pronounced as the incubation temperature was decreased from 30°C to 5°C. The addition of 0.1% acetic or lactic acid to TPB minimized the anti-*Listeria* effect of 100 or 200 ppm SML during the first 32 h of incubation. The additive effect on *S. aureus* was not as dramatic. An additive lethal effect of SML and EDTA on heat inactivation was evident for *L. monocytogenes* but not *S. aureus*. Populations of *L. monocytogenes* in ground beef supplemented with sucrose laurate (0.39% and 0.64%) or sucrose palmitate (0.57%) were significantly lower ($P < 0.05$) than those in the control after 1, 2, 4 and 7 d of storage at 5°C. Ground beef supplemented with 0.57% sucrose palmitate and stored at 15°C also contained significantly lower populations of *L. monocytogenes* throughout the 7-day storage period. Survival of *S. aureus* was unaffected by test esters when beef was stored at 5°C. At 15°C, sucrose laurate, sucrose palmitate and sucrose stearate significantly inhibited both pathogens through day 4; sucrose oleate was without affect at 0.16% or 0.27% for both pathogens. Populations at each sampling time were significantly less ($P < 0.05$) in beef containing 0.39% or 0.64% sucrose laurate through day 2, regardless of the storage temperature. Further studies to determine the effectiveness of sucrose fatty acids as sanitizers and preservatives for beef and other foods are warranted.

(15) EVALUATION OF COLICINS FOR INHIBITION AGAINST DIARRHEAGENIC VEROTOXIGENIC *ESCHERICHIA COLI* STRAINS

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Diarrheagenic verotoxigenic *Escherichia coli* (VTEC) strains have clinical, economic, and public health significance and strains such as O157:H7 have been associated with foodborne disease outbreaks and some deaths. Colicins may offer an alternative and specific method for control of VTEC strains. A reference set of 22 standard colicin-producing *Escherichia coli* strains was tested for inhibitory activity against 27 diarrheagenic VTEC from 5 genetically distinct backgrounds which included a variety of serotypes (O157:H-, O26:(H11, H?), O111:(H8, H11, H-) and :O157:H7). Production of colicins by VTEC strains was also assessed. Cross-streak and overlay methods were used to assess inhibitory activity on Luria agar (LA) or LA supplemented with .25 μg/ml mitomycin C (LM) as the growth medium. Results from overlay assays were more reliable. In assays using LM as the growth medium, group A colicins (Col A, E1-E8, K, N, S4 and MccB17) were more inhibitory towards the VTEC strains than group B colicins, (Col B, D, G, H, Ia, Ib, M and V). Col E2, E3, E4, E7, E8, K, and N inhibited 81-96% of the VTEC strains, while the most active group B colicins, Col D, inhibited 30% of the VTEC strains examined. Inhibitory activities of Col G, H, and MccB17 were suppressed on LM, however, they inhibited 78-96% of the VTEC strains on LA. The 11 VTEC strains of serotype O157:H7 tested were sensitive to 11 of 13 group A colicins used and to Col G and H. Ten of the 27 (37%) of the VTEC strains tested produced at least one colicin. Production of and sensitivity to colicins was not restricted to a single VTEC serogroup. While presence of colicinogeny in some VTEC strains may modify their sensitivity to colicins, there is potential for using colicins to inhibit or kill sensitive VTEC strains, especially for serotype O157:H7.

(16) INHIBITION OF *L. MONOCYTOGENES* AND *A. HYDROPHILA* ON COOKED BEEF BY PLANT EXTRACTS COMBINED WITH DRIED WHEY PREPARATIONS OF ANTAGONISTIC BACTERIA

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The influence of each of 3 plant extracts (eugenol, pimento, and bay) combined with dried cultured whey (DW) prepared from each of 3 antagonistic bacteria (*Carnobacterium piscicola*, *Leuconostoc paramesenteroides*, and *Lactobacillus sake*) on growth of *Aeromonas hydrophila* (AH) and *Listeria monocytogenes* (LM) in refrigerated, cooked beef was investigated. Whole sirloin tips were roasted and cut into 25 g pieces. After an extract was added, samples were inoculated with either a low (10^1 CFU/g) or high (10^5 CFU/g) population of AH and LM. DW was then added to the surface of the sample. Inoculated meat was incubated at 5°C for two weeks or 15°C for one week, and pathogen populations were determined periodically using starch ampicillin agar (AH) and modified Oxford agar (LM).

AH was less resistant to the treatments than was LM. At 5° and 15°C, treatments containing either eugenol or pimento reduced growth of AH by 1 to 2 log CFU/g in samples containing high populations of the pathogen (10^5 CFU/g). At the low inoculum level, AH growth was not significantly different from the control at 5°C, but differed by 2 to 5.5 log CFU/g at 15°C for all treatments containing eugenol or pimento. LM growth was either not inhibited (high inoculum) or did not occur (low inoculum) when stored at 5°C. However, high inoculum samples containing eugenol-*L. paramesenteroides* or pimento-*L. sake* inhibited LM

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by 1 log CFU/g when stored at 15°C. Treatments containing eugenol or pimento inhibited growth of LM by 1 to 3 log CFU/g in samples containing low inoculum at 15°C. In general, the most significant reductions in the growth of AH and LM occurred in samples stored at 15°C. Treatments including eugenol or pimento were most inhibitory regardless of DW. However, sensory evaluation would be necessary to determine if these treatments would adversely affect the acceptability of the product.

(17) CONTROL OF *LISTERIA MONOCYTOGENES* ON CATFISH FILLETS *ICTALURUS PUNCTATUS* USING FOOD GRADE ANTIMICROBIALS

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The following food grade antimicrobials were evaluated in synthetic media for their effects on *Listeria monocytogenes* (4 strain cocktail; ca. 10^5 CFU/ml); Alta™2341 (2%), citric acid (2%), nisin (N; 0.1%), trisodium phosphate (2%), sodium diacetate (2%), and dehydroacetic acid (DA; 0.5%). Only N alone or N with DA significantly reduced (2.2 and 3.0 $\log_{10}$ CFU/ml, respectively) populations. Subsequently, solutions of N, DA, or N with DA were prepared in ddH_20 or in sodium alginate (1% w/v) carrier and applied to inoculated (4 strain cocktail; ca. 10^5 CFU/ml) retail catfish fillets via dipping. Fillets were packaged, stored at 2°C and sampled (25g portions) for viable *L. monocytogenes* at 0, 2, 4, and 6 days. On fillets dipped in ddH_20, sodium alginate, or DA (≤ 2%), populations fell about 1 $\log_{10}$ CFU/g within 1 day and remained static over 6 days. On fillets dipped in N alone (0.4%) or N (0.2%) plus DA (2%), counts decreased 2 to 3 $\log_{10}$ CFU/g within 1 day and remained suppressed over 6 days. Application via a sodium alginate carrier resulted in an additional 0.5 to 0.8 $\log_{10}$ unit decrease in counts. These results demonstrate the potential for reducing levels of *L. monocytogenes* on catfish fillets using alginate:antimicrobial dips.

(18) MICROBIAL DECONTAMINATION OF FECALLY CONTAMINATED CARCASSES AS AFFECTED BY VARIOUS TEMPERATURE WATER SPRAYS AND STEAM

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The effectiveness for reducing a range of bovine fecal bacteria populations on sheep carcasses using various temperature water spray washes (W) and wash/steam combinations (WS) was determined. Water spray washes of 15.6, 54.4, and 82.2°C were administered using a hand held spray nozzle. For steam application, a cabinet capable of holding a hanging carcass side was constructed. Bacterial populations of approximately 2.5, 4, and 6 $\log_{10}$ CFU/cm² on a carcass were subjected to all treatment conditions. The $W_{82.2}$ and $WS_{82.2}$ treatments reduced bacterial populations by as much as 4.01 $\log_{10}$ CFU/cm² when contamination levels were initially ≥ 6 $\log_{10}$ CFU/cm². The achievable end point bacterial level for fecally contaminated carcasses subjected to the $WS_{82.2, 54.4, 15.6}$ and $W_{82.2}$ treatments, was observed to be in the range of 2.72 - 3.25 $\log_{10}$ CFU/cm². It appears that moist heat interventions can reduce bacterial populations effectively to a distinct end point, regardless of the initial contamination level or method of application. However, when the initial carcass bacterial population was 2.5 $\log_{10}$ CFU/cm², $WS_{82.2, 54.4, 15.6}$ and $W_{82.2}$ treatments all produced a 1.5 $\log_{10}$ CFU/cm² population reduction. It appears that the extended hydration of a carcass before and during moist heat interventions affords some protection to a limited bacterial population. Thus a distinctly different, non-hydrating intervention administered prior to moist heat might be successful in reducing bacterial populations to very low levels on red meat carcasses.

(19) DISINFECTION OF CUTTING BOARDS BY MICROWAVE ENERGY

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Cutting boards are seen as potential sources of cross-contamination of foods. New wood and plastic cutting boards have been shown to be relatively easy to clean. However, used boards (with numerous knife marks), particularly those made of polymers, are much more difficult to disinfect manually. Plastic cutting boards have been preferred to wood because they can be washed in dishwashers and used in microwave ovens. Our study suggests that, at least for home use, this preference may not enhance food safety. Challenge tests using microwave ovens have shown that bacteria are much more readily killed on wood than on plastic. In fact, on wood, almost total elimination of vegetative cells is possible with exposure times of 4-6 min on typical medium-sized wood boards (depending on board size, bacterial load, and moisture level). On plastic, microwave energy has almost no lethal effect on bacteria: 15 min of exposure did not reduce the number of bacteria significantly. Factors such as increased moisture level (wetness) elevated kill efficiency on wood, but were negligible on plastic. Temperatures inside wood exceeded 200°C within the first 4 min, whereas

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plastic surfaces reached no more than 40°C. Aside from the high temperatures, drying due to microwave heating (wood lost 30% of its initial weight due to desiccation during 3 min of heating) appeared to have a bactericidal effect. On plastic surfaces, meanwhile, the original pools of inoculated cells remained wet, and viability remained almost 100%. Our study indicates that brief "cooking" of wooden boards in a microwave oven is an effective way to kill bacteria, and thus a very simple and cheap method to protect food against cross-contaminating pathogens. Because plastic is a relatively inert medium to microwaves, disinfection of plastic boards in a microwave oven is impractical.

(20) GENERAL PRINCIPLES OF ISO 9000 AND ISO 45000: HACCP, TQM AND ISO LINKS

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The International Organization for Standardization (ISO) has developed standards for quality assurance - the ISO 9000 series. These include the guidelines for quality management (ISO 9004), and the models for quality assurance in design/development, production and installation (ISO 9001), in production and installation (ISO 9002), and in final inspection and testing (ISO 9003). The European Community is promoting the adoption of ISO 9000 as a way to improve free trade and the opening of foreign markets. The ISO 9000 series standards and guidelines are based on system and process quality, and are not specific for the food industry. However, in application to the agrifood industries, quality assurance programs based on the ISO 9000 standards and food safety systems based on HACCP should be and are easily integrated. These systems, together with continuous improvement and responsibility to society as a whole, lead to a Total Quality Management (TQM) approach in the food industry.

(21) AN INTEGRATED SYSTEM OF ISO 9000 AND ISO 45000 CERTIFICATES IN THE CONTROL OF FOOD HYGIENE

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The quality systems described by the ISO 9000 series of standards demand a commitment by management to improve and maintain the quality of products and services. The implementation of the ISO quality standards, and certification to the ISO 9000 series, must include efforts to ensure that a company's suppliers meet procurement specifications. In Europe, this aspect is one of the ten most frequently identified noncompliance items during ISO certification audits. Particularly in the food industry, supplier surveillance is often challenging since the majority of suppliers are small and medium-sized operations that do not always readily adopt additional quality assurance activities. The European approach to resolving this problem will be discussed. In addition to the ISO 9000 series of quality standards, the issue of the new ISO standard 45004 for inspection services will facilitate the global harmonization of standards in the food production industries and the move towards eliminating trade barriers.

(22) PROCUREMENT AND QUALITY ASSURANCE OF MEATS BY THE DEPARTMENT OF DEFENSE

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The Department of Defense procures millions of pounds of beef and pork annually. Primarily, the Defense Personnel Support Center (DPSC) contracts for all red meat purchases that arrive at military bases in the Continental United States. This paper is designed to give the reader a basic understanding of the red meat procurement system and the quality assurance provisions that these products are inspected by. Since the Department of Defense is unique in its own right, as compared to a large commercial chain store, it has developed its own set of requirements based on the Institutional Meat Purveyors Specifications (IMPS). These are called the DIMP's, which is an acronym for Defense Commissary Agency Modified IMP's. These are also discussed in this paper.

(23) INTEGRATED QUALITY CONTROL IN THE PIG SECTOR

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Food producing industries must be consumer responsive to continue to serve the marketplace. Consumers are increasingly becoming concerned about what takes place in all segments of the food chain. This includes on-farm production methods, animal care practices and the chemical and microbiological quality of meat. To provide assurances to consumers about the quality and safety of pork, several countries have implemented quality control programs which start at the farm and extend to various levels of the food chain. Examples of these programs are the Pork Quality Assurance Program (U.S.), Integrated Quality Control Scheme (The Netherlands) and ISO 9002 Certification (Denmark).

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(24) QUALITY SYSTEMS IN CANADIAN MEAT PROCESSING OPERATIONS

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The Canadian meat industry is responding to ongoing changes in provincial and federal inspection systems. These changes have been driven by domestic and international pressure to move from an inspection system based on visual parameters to one based on scientific risk assessment in conjunction with the principles of HACCP. Approaches taken by Agriculture and Agri-Food Canada, Health Canada, and provincial governments in Ontario, Saskatchewan and Alberta, and the responses of the Canadian meat industry to these changes will be presented.

(25) APPLICATION OF HACCP PRINCIPLES AND BEYOND: BEEF SLAUGHTER AND FABRICATION

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In the late 1980's, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA), together with the United States meat industry, initiated efforts to gradually develop and introduce the Hazard Analysis Critical Control Point (HACCP) system approach in meat and poultry inspection programs. To facilitate these plans, the United States National Advisory Committee on Microbiological Criteria for Foods developed a generic HACCP model for beef slaughter and fabrication, which was then included in the FSIS/USDA proposed rule on "Pathogen Reduction; HACCP Systems." Based on this model, we developed a plan which we have presented in workshops to a large number of United States beef slaughter and fabrication operations. An important component of a HACCP system should be development of standard operating procedures (SOP) for the critical control points. These SOPs can also be used for auditing of the HACCP plan. Establishment of HACCP principles in these operations is viewed as a fundamental step preceding the adoption of TQM and ISO principles, which should lead to harmonization of international trade.

(26) A GENERAL INTRODUCTION TO THE HOWS AND WHYS OF MOLECULAR TYPING

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Until recently, determination of the relatedness of bacterial isolates was done solely by testing for one or several phenotypic markers. This included methods such as serotyping, phage typing, biotyping, antibiotic susceptibility testing and bacteriocin typing. However, there are problems in the use of many of these phenotype-based methods. For example, phage and bacteriocin typing systems are not available for all bacterial species and serotyping can be labor intensive and costly. In addition, phenotypic markers may not be stably expressed under certain environmental or culture conditions. In contrast, some of the newer molecular typing methods involving the analysis of DNA offer many advantages over traditional techniques. One of the more important advantages is that since DNA can always be extracted from bacteria, all bacteria should be typeable. Another is the increased discriminatory power of DNA-based methods over phenotypic procedures. As an introduction to the more detailed talks on specific typing methods, the basics of molecular typing will be discussed, along with the advantages and disadvantages of several of the newer molecular typing techniques. Some of the methods that will be covered are pulsed-field gel electrophoresis, ribotyping and its variations, and random amplified polymorphic DNA analysis.

(27) THE RIBOPRINT™ SYSTEM - A NOVEL AUTOMATED METHOD FOR MOLECULAR TYPING OF FOODBORNE MICROORGANISMS

John Webster, Dupont, Wilmington, DE

RiboPrint™ patterns provide accurate species and type descriptions of foodborne microorganisms without a presumptive identification step. In the RiboPrint™ System's automated process, cells are lysed, DNA fragments are generated, and patterns are created using a labeled ribosomal RNA operon. These patterns are imaged using chemiluminescence and a CCD camera. Through the use of algorithms for data normalization, reproducible strain-dependent pattern types are obtained. These patterns are compared to patterns in a database linked to current nomenclature. The scientific basis for the automated process is a database of normalized patterns, which was derived from over 10,000 strains of more than 250 species using a semi-automated process. Using the semi-automated process, accurate identification of unknown strains was seen through broad groups such as *Listeria*, *Carnobacterium*, *Brochothrix*, *Enterococcus*, the lactic acid bacteria, *Staphylococcus*, *Escherichia coli* and *Salmonella*.

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This technology has enabled the recognition of not previously described *Staphylococcus* species isolated from foods. Identification was extended to the type level using the pattern diversity seen within most species. For example, 50 types of *L. monocytogenes* have been observed. In many instances type designations were associated with other attributes of the strains. Some types of *Staphylococcus* species were host specific which enabled use, for example to distinguish *S. aureus* isolated from a variety of animal species. Pattern type analysis also enabled us to recognize and differentiate *E. coli* O157:H7 from other *E. coli* serotypes. Most serotypes of *Salmonella* studied were easily distinguished. RiboPrint™ patterns represent information and they are the keys to accessing knowledge.

(28) USE OF PFGE FOR THE MOLECULAR TYPING OF FOOD-BORNE PATHOGENS

John B. Luchansky, Ph.D., University of Wisconsin - Madison

The prevalence of pathogenic bacteria in foods and food processing environments and the attendant increase in producer recalls and human illness have fostered efforts to develop more rapid, sensitive and specific typing methods. The current literature substantiates that genomic fingerprinting using pulsed-field gel electrophoresis (PFGE) is the most reproducible and discriminating method available for several food-borne pathogens. This presentation will provide an overview of the conduct of PFGE and discuss applications of this technology for the food industry. Applications discussed will include the use of PFGE to correlate genomic fingerprints with relevant phenotypic traits, discriminate among isolates involved in outbreaks, and identify unrecognized outbreaks. The advent of molecular typing methods, notably PFGE, has already made an appreciable impact on epidemiologic studies. Improvements in the acquisition and management of PFGE fingerprint data in the next few years will augment efforts to substantially reduce food-borne pathogens from our food supply.

(29) METHODS FOR DATA CAPTURE, ANALYSIS, AND INTERPRETATION OF DNA FRAGMENT PATTERNS FOR MOLECULAR SUBTYPING OF BACTERIAL PATHOGENS

Bala Swaminathan,* Susan Hunter and Timothy Barrett, Centers for Disease Control and Prevention, Atlanta, GA

Molecular epidemiology in cases of human diseases caused by pathogenic bacteria currently involves analyzing DNA fragment patterns generated from chromosomal and/or extrachromosomal DNA by restriction endonuclease digestions or in vitro amplification methods. Whether pattern analysis can be performed visually or requires the use of a computer-based image acquisition and analysis system depends on the type of answers sought from the subtyping data in a particular study. Visual interpretation of data is sufficient and acceptable for projects involving a few bacterial isolates and questions confined to a narrow window in time, such as a small foodborne disease outbreak. Long-term studies of pathogen demographics involving large numbers of isolates will require computer-based image acquisition, normalization of DNA fragment patterns against reference DNA fragments, analysis of normalized patterns, and generation of dendrograms that provide an indication of genetic diversity between subtypes. Criteria for determining whether two or more closely related patterns represent the same or different type(s) have not yet been fully determined. Often, the criteria for interpretation must be based on epidemiologic relatedness among the strains.

Optimization and standardization of molecular subtyping protocols for each species of interest, when combined with appropriate computer-based analysis, will facilitate inter-laboratory comparisons of subtyping data and enable the establishment of national or international databases of subtyped for each bacterial pathogen. On-line retrieval of geographic and temporal incidence data on specific subtypes of specific pathogens from these databases should be possible by setting up client-server systems that can communicate through the Internet. The WHO-sponsored international collaborative study of subtyping *Listeria monocytogenes* and the ongoing NCID/ASTPHLD project for setting up Area Laboratories for macrorestriction analysis of *Escherichia coli* O157:H7 are two examples of application of this approach to foodborne pathogenic bacteria.

(30) USE OF MOLECULAR TYPING IN FOODBORNE OUTBREAK INVESTIGATIONS: PITFALLS AND ADVANTAGES

J. Rocourt - WHO Collaborating Center for Foodborne Listeriosis, Institut Pasteur, Paris, France

Investigations of foodborne outbreaks are crucial 1) to identify and monitor the sources 2) to identify groups at higher risk so they may be targeted by control and preventive measures. Identification of the vehicle rely on both case-control study results and the detection of the epidemic strain in the suspect food. Implicating a particular food, especially a processed food, at the origin of human cases has multiple consequences, including economic losses for the brand, long adverse publicity (possibly extended to a segment of the food production) and sometimes lawsuits. Epidemiological investigations need therefore to be as reliable as possible and statistical data have to be supported by typing results of strains from human and food origin.

Phenotypic methods, like serotyping and phage typing, have been used for decades and were the only methods providing epidemiological markers until recently. They are still valuable for routine purposes and for rapid screenings of large numbers of strains during outbreaks. Over the past few years, molecular methodologies have been successfully applied to type bacteria, providing additional new markers able to distin-

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guish strains within serovars and/or phagovars. A number of methods have been developed based on the characterization of proteins (multilocus enzyme analysis) or DNA [plasmid analysis, DNA micro- and macrorestriction analysis, ribotyping, RAPD (Random Amplification of Polymorphic DNA)...].

Serotyping and phage-typing are usually restricted to a small number of laboratories because of the use of specific biological material like sera and phages. In contrast, genomic methods are widely utilized since they are based on general molecular methods for proteins and DNA characterization. As a consequence, numerous laboratories are now involved in molecular characterization of foodborne pathogens.

The profusion of published data obtained with various molecular methods is rapidly generating confusion in the classification of strains. To circumvent this problem regarding *Listeria monocytogenes*, an international multicenter study was launched in 1990, under the auspices of the World Health Organization (Food Safety Unit, Geneva, Switzerland) to select the most appropriate methods for epidemiological investigations of listeriosis, to standardize these methods and to define a common nomenclature of varieties. The first step consisted of typing a common set of 80 strains in 27 laboratories in 14 countries.

(31) MOLECULAR AND CONVENTIONAL TYPING METHODS FOR *LISTERIA MONOCYTOGENES*: THE UK APPROACH

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The considerable interest in both human and animal listeriosis and the widespread availability of methods to isolate *Listeria* from the environment (including food) has led to an increased demand for subtyping of *L. monocytogenes* by the Public Health Laboratory Service in England and Wales.

Molecular methods offer typing systems of high reproducibility and good discrimination. However, these are too labor intensive for the available resources to process the 2-4000 cultures of *L. monocytogenes* submitted to this laboratory each year. A combination of methods based on phenotypic markers (serotyping, phage typing and susceptibility testing) together with molecular methods (plasmid analysis and DNA restriction fragment analysis) are currently used. Strategies for the application of these methods will be presented.

(32) GROWTH OF *LISTERIA MONOCYTOGENES* AND LISTERIOLYSIN O SECRETION IN BROTH CONTAINING SALTS OF ORGANIC ACIDS

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Growth and listeriolysin O (LLO) secretion by *Listeria monocytogenes* were studied in tryptic soy broth supplemented with 0.6% yeast extract (TSBY), containing potassium sorbate (0.05-4%), and sodium lactate, citrate, acetate or propionate (0.1-8%). Measurements were after aerobic and anaerobic incubation for 24 h at 35°C and 20°C. Growth in anaerobic conditions was about 20% lower than in aerobic incubation. Inhibitory effects of the salts were propionate>sorbate>acetate>lactate>citrate. Although *Listeria* growth was suppressed under anaerobic conditions, LLO secretion was enhanced. Citrate, acetate and lactate enhanced LLO secretion during incubation at 35°C, while sorbate sharply suppressed it. LLO secretion was also suppressed when sorbate (0.4%) was combined with each of the other salts. Overall, observations at 20° were similar to those at 35°, although enhanced LLO secretion at 20° was observed only with acetate and citrate. Deactivation of the sulfhydryl-activated LLO by sorbate was confirmed by subjecting the concentrated cell extracts to electrophoresis (SDS-PAGE), electroblotting the proteins and detecting the hemolysin with anti-LLO antibody. These findings confirm that the mechanism of microbial inhibition by sorbate involves deactivation of thiol-containing enzymes.

(33) HEAT-RESISTANCE OF *LISTERIA MONOCYTOGENES* INCREASES WHEN PRODUCTION OF OSMOPROTECTANTS IS INDUCED

Yuqian Lou,* Graduate Student, and Ahmed E. Yousef, The Ohio State University, Dept. of Food Science & Technology

Treating *Listeria monocytogenes* with high concentrations of NaCl may induce the production of osmoprotectants such as betaine, carnitine, proline. Thermotolerance of *L. monocytogenes*, subjected to conditions inductive to osmoprotectants production, was investigated in this study. *L. monocytogenes* was grown in Trypticase Soy Broth Yeast Extract (TSBYE) at 35°C. Cells were harvested and suspended in TSBYE or TSBYE + 6.0% NaCl. Cell suspension (ca 10^{10} CFU/ml) was incubated at 20°C for 2 hours and transferred into a heating medium which contained low (0.5%) or high (6%) NaCl concentration. Cells that were suspended and heated in the high-salt media (expected to contain the highest level of osmoprotectants) had the highest heat resistance. D-values were 300 and 33 min at 55 and 60°C, respectively. The cells that were suspended and heated in the low-salt media had the lowest heat resistance; D-values were < 10 min at

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55 or 60°C. The cells that were suspended and then heated in media with different NaCl concentrations showed intermediate resistance to heat. In conclusion, thermal treatments of salt-containing foods should be designed to account for the increased resistance of *L. monocytogenes* to heat.

(34) GROWTH AND SURVIVAL OF *LISTERIA MONOCYTOGENES* IN MINIMALLY PROCESSED GREEN BEANS AS INFLUENCED BY MODIFIED ATMOSPHERE PACKAGING, NaCl TREATMENT AND STORAGE TEMPERATURE

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Minimally processed vegetables (MPV) have increased tissue injury which increases respiration rates and enhances bacterial growth. Modified atmosphere packaging (MAP) is a method used to extend the shelf-life of vegetables. Many studies have shown that environmental changes inside packages can change microbial populations and may affect the growth of pathogenic bacteria such as *Listeria monocytogenes*. This study was undertaken to determine the survival and growth of *L. monocytogenes* in minimally processed green beans as affected by MAP, NaCl and storage temperature. Green beans were inoculated with 10^2-10^3 CFU/g of *L. monocytogenes* Scott A, packaged in air or vacuum and stored at 5, 10 and 15°C. *L. monocytogenes* were not detected in non-inoculated samples. *L. monocytogenes* counts in inoculated beans increased to 10^5 CFU/g at 5 and 10°C after 9 days of storage. At 15°C, *L. monocytogenes* counts in green beans were 10^6 CFU/g after 8 days of storage. Growth of aerobes, psychrotrophs, lactics, coliforms, yeast and molds was significantly affected by temperature but not by NaCl treatment or atmosphere. Green beans remained bright green in both air and vacuum packages at 5°C after 15 days, while at 15°C after 4 days of storage, green beans turned yellow. Generally, non-salt treated beans had better appearance than salt-treated beans. These data suggest that additional barriers are needed to prevent the growth of *L. monocytogenes* in minimally processed green beans.

(35) RADIOSENSITIVITY OF *LISTERIA MONOCYTOGENES* FOLLOWING SPLIT-DOSE APPLICATION OF GAMMA RADIATION

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Among food-borne pathogens, *Listeria monocytogenes* is one of the most resistant to food processing methods, including irradiation. USFDA approval for irradiation of processed meat and seafood products will likely be limited to a maximum of 3 kGy gamma radiation. With *L. monocytogenes* present in up to 30% of raw food products at levels approaching 10^4 CFU/g, and with this bacterium's known ability to proliferate at refrigeration temperatures, it becomes imperative to maximize control processes. The purpose of this study was to determine if split-dose application of gamma radiation would alter the radiosensitivity of *L. monocytogenes*. Concentrations of 10^6 and 10^9 CFU/ml *L. monocytogenes* were suspended in tryptic soy broth and exposed to 0-5 kGy gamma radiation at 20, 4, and -80°C with equal split doses at 0-2 h time intervals (TI). Regression analysis of survival plots were used to calculate irradiation D-values. Irradiation D-values for split doses of 1 and 2 h TI were significantly lower ($p < .05$) at 20°C but not at 4° or -80°C. During the 1 and 2 h TI for 20°C, the bacterium was near its generation time of 1.2 h, possibly accounting for the increased sensitivity. Results of this study indicated that further investigation into split dose application of irradiation is warranted.

(36) PRESENCE OF *LISTERIA* SPECIES IN MARKET BEEF

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The presence of *Listeria* species in market beef was evaluated by using the USDA, FDA, Malthus, and Modified cold enrichment methods. Samples of the domestic and imported market beef were collected from local meat shops at Seoul, Korea. A total of two hundred and six *Listeria* spp. were isolated and identified from beef samples. *L. welshimeri* was the most abundant *Listeria* species in market beef. Among 206 isolates, the number of *L. welshimeri* was one hundred and twenty-one (58.7%). The numbers of isolated *L. innocua*, *L. murrayi*, *L. monocytogenes*, *L. grayi*, *L. seeligeri*, and *L. ivanovii* were 49 (23.8%), 14 (6.8%), 12 (5.8%), 6 (2.9%), 2 (1%), and 2 (1%), respectively. Detection rates of *Listeria* spp. varied among the four methods. The highest detection rate of *Listeria* spp. in market beef was found with the USDA method and that of *L. monocytogenes* was found with the Malthus method.

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(37) THE INCIDENCE OF PATHOGENIC MICROORGANISMS IN AQUACULTURED RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

T. J. McAdams,* Graduate Student, R. G. Reinhard, G. L. Flick, G. S. Libbey and S. A. Smith, Food Science & Tech. Dept., Virginia Tech., Blacksburg, VA 24061-0418

Newly proposed legislation by health regulatory agencies necessitates scientific research on microbial hazards associated with aquacultured products. This study determined psychrotrophic, mesophilic, total coliform, *Escherichia coli*, and *Listeria* species counts in filleted and whole aquacultured rainbow trout, and compared pathogen levels (*Vibrio*, *Salmonella* and *Listeria* species) of whole trout to two culturing systems (flow-through and recirculating).

Samples from five farms were analyzed. A total of twenty whole fish from flow-through and recirculating culture systems were examined for *Listeria*, *Vibrio*, and *Salmonella* species with five fish being analyzed for psychrotrophic and mesophilic counts. Water samples were also analyzed from each farm for coliforms and *Escherichia coli*. Twenty fillets from three flow-through systems were tested for *Listeria* species with five also analyzed for aerobic plate, *Escherichia coli*, and coliform counts.

In fillets analyzed for microbial pathogens, *Listeria* species were found in 43 of 60 (71.7%) samples. *Listeria* was also found in 59 of 100 (59%) whole fish samples. Counts ranged from < 0.36 MPN/g to 24 MPN/g for fillets and < 0.36 MPN/g to 15 MPN/g for whole fish. Species isolated included *Listeria monocytogenes*, *Listeria innocua*, and *Listeria seeligeri*. Psychrotrophic and mesophilic counts ranged from 10^3 to 10^6 for a whole and filleted trout. *Escherichia coli* was not found on filleted trout. *Salmonella* and *Vibrio* species were not detected in whole trout. There was no significant difference in *Listeria* species levels on whole and filleted trout (> 0.05). No significant difference was observed in culturing systems for *Listeria* counts on whole fish ($p > 0.05$). A significant difference was not present in total coliform levels of water samples and filleted fish ($p > 0.05$), however, a significant difference was observed with *Escherichia coli* and coliform counts for water in the different culturing systems ($p < 0.05$).

(38) THE INCIDENCE OF PATHOGENS IN AQUACULTURE RECIRCULATION WATER SYSTEMS AND A COMPARISON OF THEIR PRESENCE TO FISH SIZE AND STOCKING DENSITIES

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Proposed legislation by governmental agencies concerning microbial hazards associated with aquacultured products, and the industries desire to establish a HACCP program has led to a need to identify pathogenic microorganisms in aquaculture growing waters. This study was to identify the presence of selected microbial pathogens in water and compared their presence to the general microbial quality of the water, fish stocking density and fish size from Tilapia aquaculture facilities with a recirculating water system. Water samples from different facilities were collected and analyzed. One liter of water was filtered and tested for pathogenic microorganism presence, while unfiltered water was tested for general microbial quality. Pathogenic organisms tested included *Listeria*, *Vibrio*, *Yersinia*, and *Salmonella* species, and *Clostridium botulinum*, while general microbial quality was indicated by aerobic plate count, coliforms, and *Escherichia coli* levels. A total of two tanks were tested at each facility three times. Two of the tanks analyzed contained large fish (280-440 g) with stocking densities of 11.4 g fish/gal. and 381.2 g fish/gal. The other tanks contained young fish (9-23 g) with stocking densities of 16.0 g fish/gal. and 34.2 g fish/gal., respectively. Aerobic plate counts, coliform, fecal coliform and *Escherichia coli* levels for the tank holding large fish with a stocking density of 11.4 g fish/gal. were 2.1×10^6 CFU/mL, 76.2 MPN/mL, 5.7 MPN/mL, and 0.91 MPN/mL, respectively.

Aerobic plate counts were 6.3×10^6 CFU/mL, coliform counts of 1,100 MPN/mL, fecal coliform counts of 460 MPN/mL, and *Escherichia coli* counts of 15 MPN/mL were found for the tank holding the same size fish and a high stocking density (381.2 g fish/gal.). No *Listeria*, *Vibrio*, *Yersinia*, and *Salmonella* species, and *Clostridium botulinum*, were found in the tank of lower stocking density, and only *Listeria* species were isolated from the tank with a high stocking density in two of the three trials. The two tanks with young fish (9-23 g), aerobic plate count, coliform, fecal coliform, and *Escherichia coli* levels were 5.1×10^5 CFU/mL, 250 MPN/mL, 46 MPN/mL, and 9.1 MPN/mL for the tank with a higher stocking density (34.2 g fish/gal.) and 2.7×10^5 CFU/mL, 980 MPN/mL, 46 MPN/ml and 10.3 MPN/mL for the tank with a lower stocking density. The only pathogen isolated from either tank with young fish was *Listeria*. One of three samples from each tank was found to be *Listeria* positive. Species of *Listeria* isolated from all tanks were *Listeria innocua* and *Listeria monocytogenes*. A significant difference was observed for *Listeria* presence and general microbial quality in the tanks with large fish and different stocking densities ($p < 0.05$).

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(39) SURVIVAL/GROWTH OF GRAM POSITIVE BACTERIA IN RECONDITIONED, POTABLE, AND NON-CHLORINATED WATER

Jeffrey E. Call,* Microbiologist, Samuel A. Palumbo, Binh Huynh, Joseph Fanelli and Paul Jackson, USDA, ARS, ERRC, East Reg. Research Ctr., 600 E. Mermaid Ln., Philadelphia, PA 19118

This study determined the growth/survival potential of *Enterococcus faecium* [*Ef*] (ATCC 19433), *Staphylococcus aureus* [*Sa*] (196E), and *Listeria monocytogenes* [*Lm*] (Scott A) in potable, reconditioned, and non-chlorinated reconditioned water from a local meat processing plant. Sodium thiosulfate (final level of 10 mg/100 ml) was added to samples of potable and reconditioned water to neutralize residual chlorine. Each bacterium was added to a starting count of $\log_{10}3$ CFU/ml and held from 5° to 28°C. At intervals, aliquots were plated on appropriate selective agars. Regardless of water type or temperature, *Ef* did not increase in numbers after 24 days. *Lm* slowly increased ($\log_{10}1.5$) in non-chlorinated water at 5°C over 24 days and at 28°C within 6 days. *Sa* also increased in non-chlorinated water at 19°C within 6 days. Without added thiosulfate, *Sa* and *Lm* survived up to 12 days in potable water but were undetectable immediately after inoculation in reconditioned water. *Ef* survived a maximum of 9 days in potable water. Survival was longer in potable and reconditioned waters with added thiosulfate and at lower temperatures. These results emphasize the importance of residual chlorine in preventing the growth of these Gram positive bacteria in potable and reconditioned water.

(40) POTENTIAL FOR GROWTH, INJURY, AND SURVIVAL OF FOODBORNE PATHOGENS IN A MODEL PROPYLENE GLYCOL PRODUCT COOLING SYSTEM

Arthur J. Miller,* Research Leader, Carol A. Paska and Brian S. Eblen, USDA, ARS, ERRC, East Reg. Research Ctr., 600 E. Mermaid Ln., Philadelphia, PA 19118

Recycled propylene glycol (PG) can be used to cool thermally processed ready-to-eat foods if cold temperatures and low water activity are maintained. There are no quantitative guidelines, however, to define food safety risks. Here, a model PG system was used to evaluate growth and survival potential of *Yersinia enterocolitica*, *Listeria monocytogenes* and *Staphylococcus aureus*. Overnight brain heart infusion (BHI) cultures of each organism (ca 10^6 CFU/mL) were inoculated into BHI broth flasks containing 0-35% PG. Flasks were incubated aerobically at -12° to 28°C for 0 hr to 30 days. Periodic samples were plated to assess injury using dual agar dishes, consisting of BHI + 1% pyruvate and BHI + 2 or 5% NaCl. Injury was defined as $>2 \log_{10}$ CFU difference between the two media. No growth was observed at <5°C and >8% PG. Survival was demonstrated at -12°C and 35% PG for 35 days. Little injury was detected. These data indicate that growth of these pathogens can be controlled in cooling solutions. However, their survival indicates a potential risk if transferred to the food surface.

(41) A COMPARISON OF PROCESSING FACILITY SIZE AND SANITATION TO THE PRESENCE OF *ESCHERICHIA COLI* O157:H7, *KLEBSIELLA PNEUMONIAE*, *CAMPYLOBACTER*, AND *SALMONELLA* IN FRESH BLUE CRAB *(CALLINECTES SAPIDUS)* MEAT

R. G. Reinhard,* Graduate Student, T. J. McAdams, G. J. Flick, A. A. Diallo, R. E. Croonenberghs and R. J. Wittman, Dept. of Food Science and Tech., Virginia Tech., Blacksburg, VA 24061-0418

The possibility of the FDA adopting increased microbial standards in the seafood industry has led to a need for sound scientific data on the microbial content in fresh crabmeat. This study compared levels of *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella*, *Campylobacter jejuni*, *Campylobacter coli*, and *Escherichia coli* O157:H7 to plant size and sanitation indicated by Virginia Department of Health inspection scores. Handpicked crabmeat samples from 12 blue crab processing facilities were analyzed based on production levels (large, medium, and small) and Virginia Department of Health inspection scores (excellent > 94.5; acceptable < 94.5). Twenty samples of crabmeat were tested to determine the qualitative and quantitative levels of pathogenic bacteria. Of 240 samples of fresh crabmeat, 52 (21.7%) were found to contain *Klebsiella pneumoniae*. Counts ranged from less than 0.30 to 4.3 MPN/g (geometric). Thermophilic Campylobacters were isolated from 50 (20.8%) samples with counts less than 0.30 MPN/g (geometric). *Campylobacter jejuni* was isolated from 36 (15%) of the samples and *Campylobacter coli* from 14 (5.8%). Crabmeat produced in plants with high inspection scores were found to have 23.3% and 30% of the samples positive for *Klebsiella pneumoniae* and *Campylobacter* species respectively. Plants with low inspection scores had 18% of crabmeat contaminated with *Klebsiella pneumoniae* and 13.3% *Campylobacter* species. *Klebsiella pneumoniae* and *Campylobacter* species were found in small (16.3%, 33.0%), medium (22.5%, 8.8%) and large (26.3%, 21.3%) facilities. No significant differences ($p < 0.05$) between size and inspection scores were observed for coliforms, *Escherichia coli*, *Klebsiella pneumoniae*, or *Campylobacter* species. A correlation model of plant size and inspection scores based on general microbial and pathogenic organism level was also insignificant ($p < 0.05$). *Salmonella* and *Escherichia coli* O157:H7 were not detected in any of the 240 crabmeat samples analyzed.

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(42) SURVIVAL AND GROWTH OF *ESCHERICHIA COLI* O157:H7 ON PRODUCE

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The objective of this project was to observe the growth pattern of a mixed culture containing three strains of *Escherichia coli* O157:H7 inoculated onto cucumbers, broccoli, and green peppers. Produce was inoculated at a high level, 10^6-10^7 CFU/g, and a low level, 10^3-10^4 CFU/g. After inoculation, produce was held both whole and in chopped form at 15°C for 7 days and at 4°C for 14 days. Chopped vegetables at 4°C were held on ice to simulate procedures used in a salad bar. Samples were selected on days 0, 3, 7, 10, and 14 for enumeration on Sorbitol MacConkey's/MUG agar. *E. coli* O157:H7 counts on all the chopped vegetables were higher after 7 d storage at 15°C than *E. coli* O157:H7 counts obtained on day 0. *E. coli* O157:H7 counts remained unchanged on whole cucumbers and peppers inoculated at the high level and stored at 15°C for 7 days. A 2 log decrease was observed after 14 days for whole peppers, whole cucumbers, and chopped broccoli inoculated at the high level and stored at 4°C. Results indicate that *E. coli* O157:H7 was able to survive at both 15°C and 4°C.

(43) COMPETITIVE GROWTH OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN GROUND BEEF AT 9.5°C

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Since ground beef has been implicated in numerous outbreaks of foodborne illness due to enterohemorrhagic *Escherichia coli* (EHEC), the growth and adherence characteristics of six EHEC strains, *E. coli* K-12, and *Pseudomonas fragi* (ATCC 4793) was monitored in ground beef for 12 days to determine whether: (1) numbers of organisms changed from initial inoculation levels; (2) indigenous microflora influenced these levels; (3) viable organisms were present after 12 days; and (4) adherence characteristics to ground beef and contact surfaces varied over time. Ground beef containing low initial levels of microflora (< 70 CFU/g) were made into 25g patties, inoculated with 10^2-10^3 organisms/g, placed on sterile polycarbonate filters (0.2 m) in sterile petri dishes and incubated at 9.5°C. The same levels of each strain were also inoculated into 25 mls filter-sterilized Beef Extract Broth (BEB) containing a sterile filter membrane and incubated at 9.5°C. Each day, 2 g beef was remove for microbial enumeration. Mesophiles and psychrotrophs were enumerated on Trypticase Soy Agar (TSA) at 24 and 9.5°C. Enumeration of *E. coli* was done on both MacConkey's (MAC) and Eosin Methylene Blue (EMB) at 35°C, EMB showed consistently higher numbers of *E. coli* (≥ 0.5 logs) than MAC. Enumeration of *E. coli* from BEB was done on TSA and EMB. Small pieces of beef and filters were also removed each day and fixed by standard techniques for low voltage scanning electron micro scopy (LVSEM).

In general, less than 1 log change in numbers of EHEC were observed over the 12 days. Gram negative contaminants continually increased from day 4 through day 12. Numbers of EHEC enumerated from BEB showed the same levels as EHEC enumerated from ground beef. Apparently, the presence of other microflora in the ground beef did not affect EHEC levels. *E. coli* remained near initial inoculum levels throughout the study and viable organisms were still present after 12 days. An immediate biofilm build-up (bacteria, beef fibers, and fat) was seen by LVSEM of filter membranes.

(44) SUSCEPTIBILITY OF PRE-EVISCERATION WASHED CARCASSES TO CONTAMINATION BY *ESCHERICHIA COLI* O157:H7 AND SALMONELLAE

J. S. Dickson, Associate Professor, Dept. of Food Science and Human Nutrition, 2312 Food Science Bldg., Iowa State University, Ames, IA 50011

Pre-rigor bovine cutaneous truncii muscle was subjected to a simulated pre-evisceration wash procedure ten minutes after the hide was removed from the carcass. Five minutes after washing, the washed tissue and unwashed control tissues were contaminated with a direct application of either bovine manure, or bovine manure which had been inoculated with five strain mixtures of either *Escherichia coli* O157:H7 or salmonellae to simulate fecal contamination. The contamination was allowed to adhere to the tissues for 10 minutes, and then washed off with distilled water. Surface free energy of the pre-evisceration washed tissue was significantly lower than that of the control tissue. Scanning electron micrographs of the tissue samples revealed plant material adhering to the surface of the control tissues, but not to the pre-evisceration washed tissues. Microbial populations of both total aerobic bacteria and enterobacteriaceae were approximately 0.7 $\log_{10}$ greater on the control tissue. Pre-evisceration washing may be beneficial in reducing the susceptibility of animal carcasses to further contamination.

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(45) NEBRASKA SURVEY OF ORGANIC AND CONVENTIONALLY GROWN PRODUCE FOR THE PRESENCE OF PATHOGENIC BACTERIA

Susan S. Sumner,* Assistant Professor, Kristine J. Richert, Julie A. Albrecht and Lloyd B. Bullerman, University of Nebraska, 356 Food Industry Complex, Lincoln, NE 68583-0919

Organic and conventionally grown produce were surveyed for the presence of pathogenic bacteria, specifically *Escherichia coli* O157:H7, *Salmonella* spp., and *Shigella* spp. Thirty cucumbers and thirty tomatoes were collected from each of three organic growers and three conventional growers during the 1994 growing season. Farms were located in central and eastern Nebraska. Produce was washed for two minutes with 100 ml Phosphate buffer. Wash water was placed into Buffered Peptone water and EC Medium for enrichment. After 24 hours further selective enrichment from Buffered Peptone water was achieved with Selenite Cystine broth and Tetrathionate broth. Organisms were enumerated on Sorbitol MacConkey's/MUG agar and MacConkey's agar. Approximately 200 colonies presumptive for *E. coli* O157:H7 from Sorbitol MacConkey's/MUG agar were further tested for agglutination. From MacConkey's agar 712 colonies presumptive for *Salmonella* spp., or *Shigella* spp. were inoculated into TSI slants. Organisms that demonstrated properties typical of *Salmonella* spp., and *Shigella* spp. were identified using BBL Crystal ID kits. Results indicated the presence of a variety of organisms typically associated with soil *Providencia* spp., *Enterobacter* spp., *Pseudomonas* spp., *Escherichia coli*, *Klebsiella pneumoniae*, and *Morganella morganii*. Organism types found on organic and conventionally grown produce were similar; however, 12% more presumptive pathogenic colonies were found on organic produce.

(46) GROWTH OF *YERSINIA ENTEROCOLITICA* ON OSMOTICALLY DEHYDRATED BROCCOLI PACKAGED IN MODIFIED ATMOSPHERES AND STORED AT 10°C

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Minimally processed vegetables (MPV) such as broccoli, carrots and shredded lettuce are widely accepted by the consumer. Osmotic dehydration (OD) is a novel process that uses solutes, such as NaCl, to improve the shelf life of MPV, through a reduction in respiration. The microbiological quality of minimally processed broccoli (MPB) treated by OD and stored at 10°C in modified atmospheres was studied by examining the fate of spoilage and pathogenic bacteria. Broccoli was inoculated with 10^4 CFU/g *Yersinia enterocolitica*, osmotically dehydrated and packaged in film PD941 (Cryovac) under vacuum, in air and in a modified atmosphere. *Y. enterocolitica* increased to 10^8-10^9 CFU/g after 15 days storage at 10°C. Counts of *Y. enterocolitica* were not significantly ($p > 0.05$) affected by OD or type of packaging. Lactic acid bacteria increased to 10^7-10^8 CFU/g in OD treated product contributing to raised CO_2 and reduced O_2 levels in all packs. The shelf life of MPB was at least 6 days in all treatments.

(47) EFFECT OF HIGH pH ON THE SURVIVAL OF *SALMONELLA TYPHIMURIUM*, *SALMONELLA NEWPORT* AND *CAMPYLOBACTER JEJUNI* IN POULTRY SCALD WATER AT 55°C

Aubrey F. Mendonca,* Research Scientist, and Joyce N. Njoroge, Microbial Food Safety, North Carolina A&T State University, A-9 C.H. Moore Ag. Research Facility, Greensboro, NC 27411

The effect of high-pH poultry scald water at 55°C on the survival of *Salmonella typhimurium* ATCC 14028, *Salmonella newport* ATCC 6962, and *Campylobacter jejuni* was studied. Scald water from a commercial poultry processing plant was sterilized and then adjusted to pH 9, 10, 11, and 12 by addition of sodium hydroxide. *S. typhimurium*, *S. newport*, and *C. jejuni* were suspended in scald water at pH 7, 9, 10, 11, or 12 to give a final cell concentration of approximately 1.5 x 10^8 CFU/ml and then held at 55°C for 15 min. D_{55} - values for all three organisms decreased as pH increased form 7 to 11. Scald water at pH 12 caused instantaneous cell death and D_{55} - values could not be determined. The use of high-pH scald water at 55°C may offer a simple and highly effective way to reduce cross-contamination of poultry carcasses by destroying *Salmonella* and *Campylobacter* during the scalding process.

(48) GROWTH OF *SALMONELLA* AND *VIBRIO CHOLERAE* IN RECONDITIONED WATER

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Increased emphasis to reduce the biological oxygen demand (BOD) and total solids (TS) of waste water before entering the municipal system has forced food plants to recondition water thereby lowering

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the BOD and TS. Food companies are looking to use reconditioned water for initial cleaning (vegetables) and scalding water (meat/poultry). An initial survey showed that the reconditioned water from a local meat plant could support bacterial growth. Bioassay for the assimilable organic carbon and coliform growth response also suggest that this reconditioned water contained sufficient nutrients to support bacterial growth. Growth potential of *Salmonella* and *Vibrio cholerae* (starting level of 10^3 CFU/ml) in various water from this plant (with/without added thiosulfate) was studied at temperatures from 5 to 42°C. Both bacteria grew in the reconditioned water containing 10 mg/100 ml thiosulfate to neutralize residual chlorine, and declined rapidly in water without thiosulfate. The results of this study emphasized the importance of maintaining residual chlorine levels in both reconditioned and potable levels to prevent pathogen growth.

(49) THERMAL RESISTANCE OF *AEROMONAS HYDROPHILA* IN LIQUID WHOLE EGG

J. D. Schuman,* Ph.D. Candidate, and B. W. Sheldon, Dept. of Food Science, N.C. State University, Box 7624, Raleigh, NC 27695-7624

Aeromonas hydrophila (AH) is a psychrotrophic spoilage bacterium and potential pathogen which has been isolated from a variety of refrigerated foods of animal origin, including raw milk, red meat, pork and poultry products. Decimal reduction times (D-values) of 4 strains of AH (1 egg isolate, 2 egg processing plant isolates, 1 ATCC type strain) were determined in raw liquid whole egg (LWE) using in immersed sealed capillary tube (ISCT) procedure. Initial populations (7.0 to 8.3 log CFU/tube in 0.05 ml LWE) were heated at 48, 51, 54, 57 and 60°C, and survivors were plated onto starch-ampicillin agar (48 h at 28°C). D-values ranged from 3.62 to 9.43 min (48°C) to 0.026 to 0.040 min (60°C). Both processing plant isolates were more heat-resistant than the ATCC strain. Thermal death time curves ($r^2 \geq 0.98$) yielded z-values of 5.02 to 5.59°C, similar to other nonsporeforming bacteria. D-values of the most heat-resistant AH strain were also determined in LWE at 48, 51 and 54°C using a conventional capped test tube procedure (10 ml/tube). AH cells heated in test tubes yielded non-linear (tailing) survivor curves and larger ($P \leq 0.05$) apparent D-values at each temperature than those obtained using the ISCT method. This study provides the first thermal resistance data for AH in LWE and the first evidence that straight-line, semilogarithmic thermal inactivation kinetics may be demonstrated for *Aeromonas* using the ISCT procedure.

(50) THE POTENTIAL OF DANISH MARKET CHEESES TO SUPPORT GROWTH OF FOOD-BORNE PATHOGENS

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The potential of Danish market cheeses to support growth of the food-borne pathogens *Yersinia enterocolitica*, *Listeria monocytogenes*, *Salmonella typhimurium* and psychrotrophic *Bacillus cereus* was evaluated. Seven different cheeses were purchased, surface inoculated and incubated at 5°C for 21 days and 15°C for 14 days, respectively. Two firm cheeses did not support growth at any temperature and the number of the bacteria tested, apart from *L. monocytogenes*, was significantly reduced. Of three semi-soft cheeses, Danablue caused significant reductions at all combinations while *L. monocytogenes* and *Y. enterocolitica* survived or grew slightly on Esrum and Mozzarella. Of the two soft cheeses, a high pH, blue-veined cheese supported growth of all bacteria at 15°C and *L. monocytogenes* and *Y. enterocolitica* at 5°C, while soft, fresh, smoked cheese caused both rapid and significant reductions at all combinations. The study confirms that most cheeses do not support growth of food-borne pathogenic bacteria upon contamination post-opening with the exception of some high pH and low lactate and salt containing.

(51) INFLUENCE OF TEMPERATURE ABUSE ON GROWTH OF *CLOSTRIDIUM PERFRINGENS* FROM SPORES IN COOKED TURKEY

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Clostridium perfringens growth from a spore inoculum was investigated in vacuum-packaged, cook-in-bag ground turkey that included 0.3% (w/w) sodium pyrophosphate, and sodium chloride at 0, 1, 2 or 3% (w/w). The packages were processed to an internal temperature of 71.1°C, ice chilled and stored at various temperatures. The total *C. perfringens* population was determined by plating diluted samples on tryptose-sulfite-cycloserine agar followed by anaerobic incubation for 48 h at 37°C. At 28°C, an addition of 3% salt was effective in delaying growth for 12 h. At 15°C, growth occurred within 4 days in samples with 2% salt, but vegetative cells were not observed even after 28 days of storage in the presence 3% salt. *C. perfringens* growth was not observed at 4°C regardless of salt levels. The D-values ranged from 23.1 min (no salt) to 17.7 min (3% salt). Cyclic and static temperature abuse of refrigerated products for 8 h did not permit growth of *C. perfringens* from a spore inoculum.

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(52) VALIDATION OF PREDICTIVE MATHEMATICAL MODELS TO DEMONSTRATE APPLICABILITY TO FOODS

Isabel Walls, Research Microbiologist, V. N. Scott and D. T. Bernard, National Food Processors Assoc., 1401 New York Ave., NW, #400, Washington, DC 20005

Mathematical models may be used to predict the growth of microorganisms under different conditions. Before use, models must be validated to demonstrate their applicability to foods. A central composite statistical design based on the parameters of the *Listeria monocytogenes* model developed at the USDA-ARS was used to investigate growth of *L. monocytogenes* at different temperatures, pH values and sodium chloride levels. Growth studies were performed in a commercially available, sterile, homogeneous food at 12°C, 25°C and 30°C with 1.5%, 2.5% and 3.5% added NaCl at pH values of 5.3, 6.0, and 6.8. Growth data were fitted to the Gompertz equation and resulting growth kinetics were compared with predictions from the model developed at the USDA-ARS. Good agreement was obtained between observed and predicted values for growth rates, while lag phases ranged from 58% shorter to 153% greater than predicted. The overall effect was that for most growth conditions, the model failed safe. Based on these studies, this model may be used with more confidence to assess the safety of foods. Further research is needed to determine whether this is true for nonsterile foods.

(53) CHARACTERIZATION OF *CAMPYLOBACTER* STRAINS, COMPARATIVE SENSITIVITIES TO DNA GYRASE INHIBITORS AND OTHER AGENTS USED FOR THE CONTROL OF THIS FOODBORNE PATHOGEN IN THE POULTRY INDUSTRY

Ana Jimenez,*[1,2] Jorge B. Velazquez,[1] Joaquin Rodriguez,[3] Benilda Chomon[4] and Tomas G. Villa,[2] [1]Laboratory of Food Technology, Escuela Politecnica Superior, University of Santiago at Lugo; [2]Department of Microbiology, University of Santiago, Spain; [3]Laboratory of Microbiology, Hospital Juan Canalejo, Coruna; and [4]Hospital de Conxo, Santiago de Compostela, Spain

One-hundred and two *Campylobacter* strains were characterized in both their biochemical profiles and their in vitro resistance to erythromycin (1.9%), three fluoroquinolones (31.3-34.3), tetracycline (43.1%), kanamycin (4.8%), ampicillin (18.6%) and other 16 antimicrobial agents. Conjugative transfer of tetracycline and kanamycin resistances among these strains was achieved and small plasmids of 4.3, 4 and 1.9 kb were observed in kanamycin-resistant *Campylobacter coli* strains. The high rate of isolation of spontaneous fluoroquinolone-resistant strains (to our knowledge, the highest so far reported) and the apparent relationship between fluoroquinolone resistance and that to unrelated antibiotics, such as ampicillin and novobiocin, might suggest that more than one mechanism of resistance to fluoroquinolones is involved. Mutations affecting passive permeability might lead to resistance to fluoroquinolones and unrelated agents, although these mutants have a reduced growth rate, and in the absence of selective pressure, have clear ecological disadvantages, being rapidly outgrown by wild type strains. The lack of seasonal variation in the distribution of the fluoroquinolone-resistance *Campylobacter* strains as well as the differences in their biochemical profiles and antibiogram patterns does not suggest spread of a single fluoroquinolone-resistant strain. Enrofloxacin, flumequine and other quinolones are widely used in our region in broiler and reproductive chickens and laying hens to prevent chronic respiratory disease and enteritis. Such a high rate of fluoroquinolone-resistance in *Campylobacter* in relation to other genera suggests that the source of fluoroquinolone-resistant *Campylobacter* mutants might be the poultry exposed to high levels of quinolones and that these pathogens could infect humans through the food chain. Additionally, it seems likely that the transfer of resistance to kanamycin and tetracycline by conjugation operates among *Campylobacter* strains, and may have potentially serious consequences if transference takes place in food animals or in the environment.

(54) TECHNICAL CHALLENGE IN PROGRESSING FROM CONVENTIONAL MILK PROCESSING TO ASEPTIC PROCESSING

Barry Ritschard, Parmalat Inc., Spring City, PA

Through changes in technology and equipment, it is now possible to achieve a fresh-tasting milk product that can sit on your shelf for six to eight months. The challenges in doing so can be painstaking and very expensive.

The time-honored methods of producing fresh milk products involved conventional HTST with standard gable-top packages or assorted bottles. The results were products with a standard 12 day code.

The next generation of products were the extended shelf life. Certainly higher temperatures were a big factor here, but many other variables come into play. The question of sterility from processor to package is of prime importance. The packaging itself must not only be sterile but must also be able to hold up under the extended life of 35-60 days, depending on the end product.

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Today's product is aseptic packaged milk. The convenience of no refrigeration is combined with an ability to be on the shelf for 6-8 months. Far greater challenges are presented on producing these products. Besides the issues of sterility and specialized equipment, a processing authority takes a place in the picture with a variety of tests depending on the specific application. The results can be worth the effort when the taste equals that of familiar fresh products.

(55) ISSUES IN USING RECLAIMED WATER

R. Tassone, Diversey Water Technologies, Inc., Chagrin Falls, OH 44022

The food industry is the largest industrial consumer of water in the USA. Therefore, food plants are increasingly urged to look at innovative methods that this scarce resource can be conserved, reclaimed or recycled in their facilities. Several issues become paramount: the economics of the cost of water, discharge, energy man hours and chemicals must be evaluated against capital required for reclaiming, and testing, monitoring, data collection, and any attendant risks of product contamination. The environmental costs of discharging water must be evaluated against the capital required to build and operate polishing treatment systems, waste water systems, or specific removal equipment, and the effort needed to stay ahead of both the regulations and POTW costs curves. Regulatory concerns in using reclaimed versus "fresh city" water requires close coordination and examination, possibly including legal, with both governmental bodies and also QA standards.

(56) CRISIS MANAGEMENT AND PRODUCT RECALLS

Gale Prince, Kroger Co., Cincinnati, OH

A food company must have in place a crisis management program to deal with any unexpected problems which could develop within a plant. The crisis management program shall be designed to work seven days per week, 24 hours per day and be inclusive of handling product recalls, threats of product tampering or other crisis situations. The plan shall identify the crisis management team and their back ups in order to maintain it to be operational 24 hours per day, seven days per week. The team shall be made up of the appropriate expertise to evaluate problems, determine the risk and implement the appropriate corrective action. Production, processing, laboratory and distribution records are key elements in implementation of an effective product recall. A system must be available to systematically provide a road map in gathering information related to a product recall. Communication of product recalls must be focused and all inclusive to be effective. A recall is not complete until corrective action has been taken and the situation brought under control. How well you handle a crisis situation will determine the future of a product or of your company.

(57) INNOVATIONS IN PLANT DESIGN AND PROCESSING

Dale Sieberling, Sieberling Associates, Inc., Roscoe, IL

For more than fifteen years, our company has been involved with the application of automation to increase production capacity, both via the construction of new plants and by renovation of existing facilities. Whereas the driving force for the more than 50 projects handled in fluid milk, ice cream and cultured products facilities during this time has been increased or added production, substantial emphasis has also been placed on the application of a high-degree of automation to (a) improve control and reporting and (b) reduce product loss. Quality improvement is always an objective, but generally a lesser driving force for substantial capital investment. Emerging concepts and technologies to be considered at the present time would include (a) CIP cleaning of the floor drains in the packaging areas, (b) membrane processing, (c) CIP chemical recovery and re-use, and (d) mix-proof valves. The presentation will be based on slides of recent projects including new facilities and renovation of existing facilities. The impact of the FDA initiatives will be considered.

(58) PUBLIC HEALTH AND REGULATORY ASPECTS OF EMERGING MILK PLANT TECHNOLOGY

Steve Sims, FDA-Milk Safety Branch, Washington, DC

The presenter will explore public health concerns and regulatory requirements associated with single body double seat "block-and-bleed" valve technology in traditional milk and cheese plants and potential alternatives for use of similar technology in aseptic and near aseptic systems. The presenter will also provide information and incites into changing regulatory concerns and requirements associated with rapidly evolving existing technologies such as increasing computer control of pasteurization public health safeguards, and new developments in aseptic and near aseptic milk processing systems. Time will be provided to respond to presubmitted questions.

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(59) DAIRY FARMSTEAD EVALUATION AS A RESPONSE TO ENVIRONMENTAL ISSUES

Les E. Lanyon, Department of Agronomy, Penn State, 116 Agricultural Sciences and Industries Bldg., University Park, PA 16802

Increasingly significant environmental issues are facing the dairy industry. Responses to these issues will need to be larger in scope than the individual farm management decisions made by dairy producers. This is because agriculture has evolved rapidly from a local resource-based activity to a far-reaching, interconnected network of farms, businesses and markets. Milk handlers and others who are involved directly with milk producers as part of this network should be contributing to a dairy industry that balances the emerging production system with environmental protection. If they do not take the challenge, they run the risk of being blindsided by the changes. Those businesses which have a large number of clientele in a limited geographic area are mostly likely to be affected by unforeseen changes. Dairy farmstead evaluation using new criteria can be a tactic within a strategic response for businesses, government and farmers to address environmental issues proactively. Dairy farm inspectors using criteria that reflect the environmental impact of farmstead conditions could play a critical role in the success of such an effort.

(60) ENVIRONMENTAL ISSUES - DAIRY PRODUCER VIEWPOINT

Lester C. Jones, Lester C. Jones & Son, Massey, MD

I am a dairy farmer whose family has just completed the construction of a new dairy for 600 milking cows in Massey, Maryland. Why did we choose to relocate from New Jersey to Maryland? What were the major issues? In New Jersey we were becoming surrounded by houses. Our land base was shrinking and we did not have the ability to expand because of the environmental issue. Without an adequate land base it was becoming a problem to dispose of the manure. Controlling the runoff and odor was almost beyond our control without spending a tremendous amount of money.

The family, consisting of my wife, our three sons and their wives, made the decision to relocate some 10 years ago. This relocation project was put off several times because of our inability to sell the property in New Jersey.

The many delays were not all bad - they gave us time to plan and evaluate our different options. In this planning state we discovered that planning the dairy to be environmentally friendly and to meet all of the regulations of the Chesapeake Bay was a major challenge. Let me stop here and state that even though there were many challenges we have not found anything to be unreasonable or unachievable.

The criteria used by us allowed us to meet the requirements at no additional cost but I must add there was a tremendous cost involved. We decided that we wanted a totally roofed dairy facility which allows us to have control of all runoff. The soil conservation service worked with us designing the clean water runoff and keeping it separated from any that could be contaminated (i.e., runoff from the bunker silo). The fact that this was a brand new dairy allowed us to incorporate all of the drainage rather easily. All manure is collected with alley scrapers and brought to the center where it is supposed to go by gravity to a 12 acre lagoon. The lagoon will be agitated by a floating pump and agitator. The pump will move the slurry to the underground irrigation system, then back to the ground and be applied by injection. This method will give us the best use of the manure and created the least amount of odor.

Prior to even starting to build it was necessary to develop a nutrition management plan. This was done in cooperation with the Maryland Extension Service. This requirement should not be a burden on anyone provided they have enough land to properly dispose of the manure. This plan, if properly implemented should make us money. This ideas was not new to us as we were already doing it in New Jersey even though it was not required.

Not everyone will have the opportunity to build a complete new facility. Therefore, you as a leader in the industry must take a realistic approach as critical problems have to be solved. Many state and federal cost sharing programs are available and these should be continued and expanded. Dairymen need to be honest about the problems and look to realistic ways to solve them. As dairies get bigger so do the problems but most all of the dairymen I know are attempting to cooperate and solve environmental problems.

(61) DESIGN CHALLENGES IN MODERN MILKING EQUIPMENT

Stephen B. Spencer, Professor of Dairy Science, Penn State University, University Park, PA

Milking system design is more complex as systems become larger and accessory equipment such as electronic meters are included. Larger systems require larger pipe diameters which increase the complexity of cleaning. Distribution of cleaning solution equally among many units can be difficult to accomplish. Air injector volume, timing and placement are critical to a successful cleaning program.

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As larger herds are assembled, water supply and quality, waste disposal of manure and spent cleaning solutions will be critical in all parts of the country. Computer controlled systems will become more prevalent and will require a high level of power quality with an absence of neutral to-earth voltage, harmonic distortion and voltage variability. On-line quality control and cow condition features such as milk conductivity, milk progesterone, pregnancy specific protein, infra-red analysis and automatic sampling will require a high degree of operator skill. The economic position of the robotic milker is unknown while adequate performance characteristics are now reasonably perfected. The present milking machine liner is in need of design optimization with the cow as the central core. Less restrictive rubber formulation requirements could greatly enhance longevity and liner performance.

Milk cooling must be energy efficient and ultra filtration may appear on some farms to reduce hauling costs. Now, and in the future, system design must be cow oriented and there will be no compromise to milk of the highest quality.

(62) CURRENT ENZYME SYSTEM CLEANING

Dave Simyak, Diversey Corp. Livonia, MI (800-441-9855 X-2497)

It seems as though dairy farmers have been using chlorine to clean milk contact surfaces of protein and fatty soils since the beginning of time. Indeed, chlorine does a very effective job of ridding surfaces of these films. Chlorine is often added to alkaline cleaners to improve performance resulting in "chlorinated alkalis."

Enzymes have been playing a bigger role in detergent solutions in recent years. Enzymes are those naturally occurring compounds found in all living organisms that facilitate the breakdown of specific materials into smaller pieces.

For years, industry and the general public have been asking for a safer product to use, one that complies with the regulatory agencies, is lower in pH and works to remove damaging films on milk contact surfaces. Enzymes can do this and more. The basic advantages of enzymes are that they contain: NO PHOSPHATES; NO SODIUM; NO CHLORINE; AND INCREASED SAFETY.

Even though chlorinated alkalis are very effective in removing protein and fatty soils, the use of enzymes for cleaning will continue to grow. The fact is that enzymes do clean. They have minimal alkalinity, no phosphates, no sodium, no chlorine and are much safer to use by handlers. Environmentally friendly, these safer products give the consumer a much desired product.

(63) FUTURISTIC DAIRY FARM DESIGN

Doyle Waybright, Mason Dixon Farms, Gettysburg, PA

The future of the dairy industry will be dependent upon our ability here in the present to build and manage efficient dairy operations. We, as individual dairymen have little influence upon the cost of input components for milk production such as feed, supplies and labor. As well on the other end we have little influence on the price of milk. What we do have is a great deal of control over the quality of the product we produce and the efficiency with which we produce it. Quality and efficiency of production will determine whether we have profitable successful businesses of ones that are here today and gone tomorrow. We cannot become complacent with today's well run operations but must always be looking towards how we can make improvements so that we will continue to deliver a high quality milk product to the American public in spite of whatever economic conditions are present at the time. To me, production agriculture is in exciting times!

(64) RAPID, MULTIANALYTE IMMUNOASSAY TO SCREEN FOR ANTIBIOTIC RESIDUES IN MILK

Amit Kumar,* Ph.D., New Technology Project Manager, K. Hara, S. Kharadia, D. Leung, M. Piani, R. Rocco and C. Yu, Idetek, Inc., 1245 Reamwood Ave., Sunnyvale, CA 94089

We describe a solid-phase, fluorescence immunoassay that can screen for multiple antibiotics in a single, rapid (less than three minutes, two steps) test. For example, a single test can screen milk for all six of the beta-lactam antibiotics for which the FDA has set tolerance/safe levels. The components are a unit-dose, disposable cartridge, containing all required reagents, and a portable (less than 10 pounds), dedicated reader based on a semiconductor-diode laser. Antibiotics at the parts per billion level can be detected in a total assay time of less than three minutes. Sensitivity and selectivity of the assay will be presented as well as performance under conditions of varying pH, temperature, matrix, presence of potentially cross-reactive drugs, somatic cell counts, etc. will be presented. The potential for the use of this system for other types of assays will also be detailed.

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(65) THE RAPID CHARM PHOSPHATASE TEST CONFORMS WITH USDA REQUIREMENTS FOR COOKED MEAT AND GAUGES MICROBIAL LOG REDUCTION

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A simple procedure has been developed for rapid estimation of phosphatase activity in raw, comminuted meat. Thermal stability of the meat acid phosphatase enzyme has been established and found to correlate well with USDA required cooking temperatures. Enzyme activity was found to be consistent and stable in raw meat and shows a linear decrease in activity upon heat treatments at 63° to 75°C. All four main meat sources surveyed (pork, beef, chicken and turkey) exhibited high phosphatase activity with similar heat stability properties.

The Charm Phosphatase Test uses a bioluminescent substrate which, when exposed to phosphatase activity, releases instantaneous luminescence detected by the Charm II Analyzer or Charm Luminometer. The extent of luminescence directly correlates with the total phosphatase activity in the meat.

In a series of pilot experiments, beef phosphatase activity dropped exponentially in raw beef exposed, for up to 3 minutes, to temperatures between 63 and 69°C (145°F-156°F). Reduction in phosphatase activity also correlated with log reduction of bacteria *E. coli* exposed to the same treatment. A 99% drop in phosphatase activity correlated with a 6 log reduction of *E. coli* at the four temperatures tested. Detection level of raw meat in cooked meat is better than 0.01%, providing a reliable monitoring tool for cooked meat consistent with regulatory requirements. Monitoring phosphatase activity on processing areas and equipment (swab assay) has great potential for sanitation programs and cross-contamination control.

(66) SPECIFICITY OF FOUR MONOCLONAL ANTIBODIES PRODUCED AGAINST *SALMONELLA TYPHIMURIUM*

Ziad Jaradat,* Graduate Student, and Jerzy Zawistowski, Economic Innovation and Technology Council, University of Manitoba, Food Science Dept., Winnipeg, MB R3T 2N2 Canada

Four MAb's have been produced by a fusion of myeloma cells and splenocytes of a mouse immunized with attenuated *Salmonella typhimurium* cells. MAb's 5A5 and 5B2 were of IgM class, MAb 4A8 was of IgG2a while MAb 1D6 was of IgA class; all with k light chains The specificity of MAb's was determined by ELISA for 36 Enterobacteriaceae, including 24 salmonellae representing ten serogroups. Results indicated that MAbs 5A5, 5B2 and 4A8 were reactive only to *S. typhimurium*, and other B-serotypes: *S. heidelberg*, *stanley* and *schottmuelleri* without any cross-reaction to other organisms tested. Further analysis revealed that all three MAbs recognized LPS O-5 antigen unique to B-group of *Salmonella*. In contrast, the fourth MAb (1D6) was reactive with ten *Salmonella* serovars representing 4 groups. Electrophoresis (SDS-PAGE) followed by immunoblot analysis of outer membrane proteins indicated that MAb 1D6 reacts with a single protein band with a molecular weight of 35 kDa present in *Salmonella* species.

(67) ANTIGENICITY OF 35 AND 24 kDa OUTER MEMBRANE PROTEINS OF *SALMONELLA*

Ziad Jaradat,* Graduate Student, and Jerzy Zawistowski, Economic Innovation and Technology Council, University of Manitoba, Food Science Department, Winnipeg, MB R3T 2N2 Canada

Identification of antigenic surface components of *Salmonella* genera may facilitate the development of reagents to diagnose and prevent salmonellosis. Outer membrane proteins (OMP's) of *Salmonella* and other enterobacteriaceae were prepared by Sarcosyl extraction and were studied by electrophoresis and immunoblot. Marked similarities were found in the OMP profiles of *Salmonella* serovars tested. Immunoblot with antisera revealed two major protein bands with molecular weight of 35 and 24 kDa. The 35 kDa protein was detected only in all *Salmonella* serovars while the 24 kDa OMP was present in all bacteria tested. Culture media and heat treatment did not affect antigenicity of the 35 and 24 kDa proteins, although some differences in the OMP profiles were observed. Immunoblots of OMP extracts probed with the monoclonal antibody 1D6 revealed that the 35 kDa OMP was antigenically different than the 24 kDa protein. Glycoprotein immunoblot indicated that carbohydrates were absent in the epitope of 35 kDa OMP. Our findings suggested that this OMP is a surface exposed and conserved protein among the *Salmonella* serovars tested.

(68) A NEW PETRIFILM™ METHOD FOR ENTEROBACTERIACEAE TESTING

Patrick A. Mach,* Microbiology Specialist, and Gregory P. Sandberg, 3M Company, St. Paul, MN 55144-1000

Methods have been developed to detect bacterial pathogens identified as sources of morbidity and mortality from inadequate food processing methods. These methods must be augmented with systems to enumerate indicators of the pathogens to achieve adequate food safety due to sensitivity concerns. Classi-

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cally, coliform organisms have been used as indicators of enteric bacterial contamination. Although coliforms do include some pathogenic strains, they exclude most of the pathogenic species. Investigators have found that a better pathogen indicator system is the enumeration of Enterobacteriaceae (EB). In the past, this has been done using a modified MacConkey agar system, substituting glucose for lactose as the fermentable sugar. A new format has been developed that utilizes Petrifilm™ technology, together with a medium that allows detection of acid and gas-producing EB. Comparisons of the new Petrifilm™ Enterobacteriaceae Count Plate (PEB) with violet red bile glucose agar have yielded the following results:

Samples	Number Tested	MLD[1]	t-value[2]	Corr. Coeff.
EB	66	-0.08	-1.31	0.96
Food Samples	149	-.03	-.63	0.97

[1]Mean Log Difference for the two methods
[2]Paried, two-tailed t-test for the two methods

Seventy-two non-EB species were tested with equivalent results for the two systems. These results indicate the PEB gives at least equivalent results with the petri dish/agar based system and affords many of the labor productivity and efficiency benefits found with Petrifilm™.

(69) ONTARIO'S INSPECTION PROTOCOL FOR UNDRAWN DRESSED POULTRY (UDP): A MODEL FOR STANDARDS DEVELOPMENT FOR ETHNIC MARKETS

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The Ontario Ministry of Agriculture, Food and Rural Affairs has developed and implemented a modified inspection protocol for uneviscerated poultry being marketed in ethnic communities. Regulatory changes required this previously exempt product to be inspected. A 1993 research study comparing inherent risks of UDP with those of traditional eviscerated poultry was conduced in conjunction with federal authorities and industry representatives. Based on favorable results, a protocol was developed, tested and circulated for comment. As a result, pre-harvest production data and statistical sampling of eviscerated birds determines acceptability of each lot for this type of processing, and external inspection of all carcasses detects conditions which may have a public health significance. The results of the first two years suggests this is a successful program. The process provides a model for future standards development in the area of ethnic foods.

(70) RE-ENGINEERING OF LICENSING AUDIT FOR ONTARIO ABATTOIRS

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The Meat Industry Inspection Branch (MIIB) of the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) has introduced a risk based operational audit system. After receiving expert training in standardized audit development, an interdisciplinary team of Ministry staff reviewed applicable legislation, policy, codes of practice, etc., and identified inherent and control risks in various categories of Ontario abattoir (e.g., red meat, poultry, further processing). The control risks were each assessed by the team and assigned a weight, relative to the threat that non-compliance would pose to food safety, animal welfare, or occupational health and safety. A further component of the audit verifies uniform program delivery. A one-page "Standard of Compliance" was developed for each of 635 control risk elements. Audit frequency and scope are based on inherent risks such as volume, type of product, compliance history, etc. and action plans are based on audit results. The development and implementation process for the new licensing audits will be presented.

(71) THE APPLICATION OF RISK ASSESSMENT AND STANDARD AUDIT PRINCIPLES FOR COMPLIANCE VERIFICATION IN ONTARIO INSPECTED ABBATOIRS

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Food safety audits performed by regulatory agencies have been criticized as subjective, "bricks and mortar" and of little use to plant operators attempting to achieve process control. With HACCP programs likely to become mandatory, it is critical that audits verify that all pre-requisites are in place and any critical

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or serious deficiencies are quickly addressed. The Ontario Ministry of Agriculture, Food and Rural Affairs has developed standardized audit procedures for application in the 310 abattoirs under its jurisdiction using internationally recognized audit principles. Standards of compliance for risk elements in all relevant legislation were developed and weighted according to risk. Inherent, control risks and audit risks are assessed during the planning, conducting and reporting phases of the audit process. In addition, a commitment has been made to apply risk analysis methods to the development of any new audit standards. Advantages of the new program include better utilization of scarce resources, improved consistency among auditors and the full participation of plant operators. The methods used in developing this risk based audit system can be applied to any food processing environment.

(72) ADVANCES IN LABORATORY INFORMATION MANAGEMENT SYSTEMS (LIMS) IN DAIRY QUALITY CONTROL LABS

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Over the last several years, dairy quality control laboratories have begun to implement powerful laboratory information management systems (LIMS). A survey of several dairies using a LIMS was conducted to determine how they were using the new technology available and they felt they could improve their system.

Current users have resolved quality problems, modified formulations, and controlled costs using their LIMS systems. The most advanced users have integrated their LIMS with Supervisory Control and Data Acquisition (SCADA) packages that control operation of their entire plant.

One area that is continually improving is data entry systems. Four different faster methods (optical character recognition, voice recognition, bar coding and handwriting recognition) offer hope for the future, but none are currently the answer for all situations.

(73) A COMPUTER PROGRAM FOR MANAGING A FOODBORNE DISEASE SURVEILLANCE NETWORK & COMPILING SURVEILLANCE DATA

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This paper will describe a free standing computer program written by the New York State Department of Health that is menu driven, provides extensive help screens and run on DOS based PCs. The program facilitates the tracking of ongoing investigations and automatically generates three different status reports. The program compiles ten summary tables from completed investigation information, which summarize data on agents, cases, contributing factors, methods of preparation, significant ingredients and places where food was mishandled. Use of this program by other surveillance agencies to categorize and compile their data would facilitate sharing and comparisons, as well as use of their data for HACCP risk assessment.

(74) INTERNATIONAL TRENDS IN HACCP

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HACCP-based systems are becoming the international Standard for Food Safety. However, there is a wide range of forms in which HACCP is applied within the food industry of different countries. In the U.S., HACCP will probably not be mandatory until 1996, while in Mexico the Official Mexican Standards (NOM) are currently being issued as an effort to introduce an objective measure of fairness in the regulatory process, support the denomination of origin and foster competitiveness. A preliminary HACCP standard has already been issued and will make a HACCP plan mandatory to all food processing establishments when it comes into effect. The HACCP standard will have a strong impact on all food imports coming into Mexico. This paper will review the most important aspects of HACCP as affected by NAFTA and other international trade agreements.

(75) ANTIMICROBIAL ACTION OF A NISIN-BASED TREATMENT AGAINST *SALMONELLA TYPHIMURIUM* IN FRESH PORK LOIN

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The antimicrobial activity of a nisin-based treatment against *S. typhimurium* (ST) in fresh pork loin was evaluated. Pork loin samples (25 g) were inoculated with a nalidixic acid-resistant (NAr) strain of ST (3.3 to 3.9 log CFU/g of pork) and dipped for 30 min in 25 ml of a nisin-based solution (pH 3.5, HCl) containing

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100 µg/ml of nisin, 5 mM EDTA, and 0.5% Tween 20. Controls were dipped in distilled water (pH 3.5, HCl). Following treatment, each sample was sealed in a Whirl Pak bag containing 5 ml of either treatment solution or distilled water. Packaged loin samples were stored at 4°C for either 24, 48, 72, or 96 h. Following storage, samples (n = 3) were stomached for one min and the number of surviving organisms enumerated on BHI agar containing 800 ppm of NA. The study was replicated three times. The nisin-based treatment was significantly more effective than water at reducing the number of ST organisms on fresh pork loin. Meat treated with the nisin formulation resulted in a 2.1 to 3.4 log reduction after 24 h. A 2.5-to-3.7 log reduction was achieved over the 96 h period. No detectable ST cells were recovered from the nisin-containing treatment dip, whereas 1.5 to 2.4 log CFU/ml were recovered from the water dip after 96 h. These results indicate that nisin-containing formulations may be effective in reducing the level of ST on fresh pork loins during refrigerated storage.

(76) *NANNOCYSTIS EXEDENS* AS A POTENTIAL BIOCOMPETITIVE AGENT AGAINST TOXIGENIC *ASPERGILLUS FLAVUS* AND *ASPERGILLUS PARASITICUS*

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Preharvest aflatoxin contamination, which results from the growth of toxigenic strains of *Aspergillus flavus* or *Aspergillus parasiticus* on susceptible field crops, poses a serious threat to humans and domestic animals. Aflatoxins are potent toxic carcinogens that cause illness or death when foods or feeds contaminated by them are consumed. Crop management techniques are insufficient in controlling the growth of *A. flavus*, *A. parasiticus* and subsequent aflatoxin contamination of field crops. This study examined the potential for controlling the growth of aflatoxigenic molds by biological means. The bacteriolytic bacterium, *Nannocystis exedens* was screened as a potential biocompetitive agent for controlling growth of *A. flavus* and *A. parasiticus* on 0.3% trypticase-peptone yeast extract agar for 14 days at 28°C. A clear zone of inhibition of mold growth was observed when *N. exedens* was grown in close proximity to *A. flavus* or *A. parasiticus*. When *N. exedens* was inoculated onto the center of an *A. flavus* or *A. parasiticus* colony, lysis of the colony was observed. Microscopic observations revealed that *N. exedens* grew parasitically on spores, hyphae and sclerotia of the molds. These results indicate that *N. exedens* may be effective in interfering with or inhibiting growth of toxigenic strains of *A. flavus* and *A. parasiticus*.

(77) FEASIBILITY OF USING FOOD GRADE FOOD ADDITIVES TO CONTROL THE GROWTH *CLOSTRIDIUM PERFRINGENS*

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Previously, it was demonstrated that the combination of sucrose laurate (SL) + EDTA (E) + BHA (B) was an effective antimicrobial agent against both gram-negative (aerobes) and gram-positive (facultative anaerobes) food-borne bacteria. As a result of some concern raised about inherent differences between aerobic and anaerobic bacteria, an investigation was initiated to determine the effectiveness of SLEB against the growth activities of a strict anaerobe, *Clostridium perfringens* ATCC 12916.

At SLEB conc. (1:1:1, v/v/v) of 100, 200 and 300 ppm in fluid thioglycollate, outgrowth of spores of *C. perfringens* was inhibited at 200 ppm of SLEB during 192 hr of incubation (37°C). These observations seem to be consistent with an earlier finding that established the minimum inhibitory concentration (MIC) of SLEB against *C. perfringens* grown in tryptose-sulfite cycloserine agar was 150 ppm.

(78) EFFECT OF TRISODIUM PHOSPHATE ON *LISTERIA MONOCYTOGENES* ATTACHED TO RAINBOW TROUT

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Rainbow trout (*Oncorhynchus mykiss*) fillets were inoculated with 10^2 or 10^6 colony forming units (CFU) of mixed strains of *Listeria monocytogenes* (Scott A, V-7 and Brie). Inoculated fillets were dipped in 10 and 20% trisodium phosphate (TSP) solutions with and without adding lactic acid for 10 min. All treated fillets, overwrapped with polyvinylidene chloride films, were stored at 4°C for 9 to 12 days and tested at 3-day intervals. *L. monocytogenes* levels of fillets inoculated with 10^6 CFU were significantly ($p < 0.05$) reduced by 2 logs when the 20% TSP dips were used as compared to those of fillets without dips. The 10% TSP plus 1.0% lactic acid and 20% TSP dips significantly reduced the total psychrotrophic population by 1 and 2 logs, respectively, throughout the entire storage period as compared to tap water dips.

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(79) EXPANDED MODELS FOR PREDICTING THE NON-THERMAL INACTIVATION OF *LISTERIA MONOCYTOGENES*

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Previously, a set of response surface models was developed to describe the effects and interactions of temperature, % lactic acid (or pH), % NaCl, and $NaNO_2$ on the non-thermal inactivation of *Listeria monocytogenes* under aerobic and anaerobic conditions. These initial models did not distinguish between the effects of pH and lactic acid concentration. Additional experimental data were acquired where the pH levels of BHI were modified to various values after incorporation of lactic acid or sodium lactate. The new data were appended onto the original database and new models were generated for time to achieve a 4-D inactivation (t_{4D}). The models provided good agreement between predicted and observed t_{4D} values (R^2 values: aerobic/quadratic = 0.87, aerobic/cubic = 0.90, anaerobic/quadratic = 0.89, anaerobic/cubic = 0.93). This approach also allowed calculation of undissociated lactic acid concentration, and a second set of models were generated. Considering that most foods are highly buffered, these new models should allow more effective prediction of *L. monocytogenes* inactivation in acidified and dried foods.

(80) INFLUENCE OF FAT CONTENT IN PORK LIVER SAUSAGE ON GROWTH OF *LISTERIA MONOCYTOGENES* AND ITS INHIBITION BY LACTATE AND SORBATE

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Behavior of *Listeria monocytogenes* Scott A in pork liver sausage containing 22 to 67% fat, and influence of the fat on the efficacy of the preservatives sodium lactate, sorbic acid and potassium sorbate were studied during storage at 5°C and 10°C. Commercial pork liver sausage emulsion (basic formulation, 22% fat) and additions of lard (15, 30, and 45% by weight) were tested. The preservatives (1.8% sodium lactate and 0.1% sorbate as the acid and the potassium salt) were added, and all samples were heat sterilized, cooled, inoculated with 24 h culture of the organism (10^4 CFU/g) and stored at 10°C for 14 days and 5°C for 50 days. Fat content alone produced small decreases in growth of the organism by the end of the storage periods, from log CFU/g of 9.9 to 9.4 at 10°C and from 7.3 to 6.9 at 5°C in the basic sausage formulation and in sausage with 45% added fat, respectively. The inhibitory activity of lactate increased with increase in fat content, and was more pronounced at the lower storage temperature where the effects were listericidal. Inhibition by sorbic acid was not influenced by the fat content at 5°C, and increased with increase in fat content at 10°C. Potassium sorbate was less effective than sorbic acid.

(81) DESTRUCTION OF *LISTERIA MONOCYTOGENES* ON CATFISH FILLETS USING LACTIC ACID AND MONOLAURIN

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This study determined the sanitizing effects of monolaurin and lactic acid, singly or combined, on *Listeria monocytogenes* attached to catfish fillets. Skinless catfish fillets were inoculated with *L. monocytogenes* and dip treated in monolaurin and/or lactic acid solutions for various time periods (0-60 min). Results showed that monolaurin up to 400 µg/ml was not sanitizing. Conversely, lactic acid sanitizing ability increased with increasing concentration. Dipping in 0.85, 1.70, or 2.55% lactic acid for 30 min reduced counts by 0.9, 1.4, or 1.3 logs, respectively. Extending dipping time to 60 min resulted in little additional decrease in counts. Combining monolaurin with lactic acid resulted in an additive interaction. Hence, the sanitizing ability of the two compounds resides with lactic acid and not monolaurin.

(82) SENSITIVITY OF SIX STRAINS OF *LISTERIA MONOCYTOGENES* TO NISIN IN BROTH AT pH 5, 6 AND 7

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Sensitivity of six *Listeria monocytogenes* strains to nisin was studied and compared at pH 5, 6 and 7. The strains included V-7 (serotype 1A), Scott A (4b), and ATCC 19111 (1), 19112 (2), 19114 (4a), and 35152 (NCTC 7973). Nisin (Nisaplin), 6 µg/ml, was added to sterile tryptic soy broth with 0.6% yeast extract (TSBY), and media were inoculated with about 10^8 CFU/ml of a 24 h culture of each of the strains. Cell numbers were determined after one and 24 h of incubation at 35°C. Populations of all strains dropped after one h at pH 5, and differences (log CFU/ml) between controls and treated samples were strain dependent, ranging from 1.0 to 4.4. In four of the strains these differences increased after 24 h (range of 2.3 to

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7.2), while in the remaining two they decreased, but still exceeded 2 log CFU/ml. At pH 6, log counts were 1.3 to 4.1 lower than in controls after one h, and 0 to 2.7 after 24 h. While at pH 7 populations in treated samples were lower than in controls after one h, with differences (log CFU/ml) of 0.1 to 2.4, these differences disappeared after 24 h, and log cell numbers in all samples exceeded 9.0.

(83) EFFECT OF POLYVALENT METAL IONS ON GROWTH INHIBITION OF *LISTERIA MONOCYTOGENES* BY SODIUM POLYPHOSPHATE

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Addition of sodium polyphosphate (SPP, average chain length = 13) to Brain Heart Infusion Broth (BHI) increased the lag time of *Listeria monocytogenes* Scott A (Lm). Previous experiments suggest that the probable mechanism of inhibition involves the binding of essential metal ions by SPP. Experiments were conducted to determine the ability of added metal ions to reverse inhibition of Lm by SPP. Growth kinetics parameters at 19°C were determined for Lm in BHI, pH 6.0, containing 0.5% (nominal 3.6 mM) SPP and sulfates or chlorides of the metals (0.0 to 10 mM) studied. Addition of 10 mM Ca^{2+} or Mg^{2+}, 5 mM Fe^{3+}, 2 mM Mn^{2+} or 1 mM Zn^{2+} resulted in growth comparable to that in control cultures without additives. Fe^{2+} (2 mM) partially restored growth, while Ni^{2+}, Co^{2+} or Al^{3+} were not effective. The activity of SPP against Lm in foods depends on the mineral content (Mg, Ca, Fe, Zn) of the food. Thus, addition of 0.5% SPP to mineral-rich foods such as meat, milk or squash did not delay the growth of Lm.

(84) DETERMINATION OF PURGING RATE OF *ESCHERICHIA COLI* O157:H7 CONTAMINATION FROM A MODEL MEAT GRINDING SYSTEM

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The purging rate of ground beef contaminated with *E. coli* O157:H7 from a laboratory scale grinder was determined in a two part study (3 replications/part). In part 1, a 3 lb. chub of coarsely ground beef was inoculated with ca 10^6 CFU/g rifampicin resistant *E. coli* O157:H7 and 10 subsequent 3 lb. uninoculated chubs were ground. Direct plating of each ground chub on TSA + rifampicin showed that *E. coli* O157:H7 was still detectable after the last grinding. With corresponding ELISA assays of enrichments, *E. coli* O157:H7 was undetectable after the 5th grinding, however ELISA assays of enrichment broth used to rinse the grinder housing and internal parts showed that residual *E. coli* O157:H7 persisted. In part 2 (inoculation level ca 10^2 CFU/g and 20 subsequent chubs were ground) direct plating and ELISA tests yielded positive results only for the initially inoculated chub; all subsequent samples were free of *E. coli* O157:H7. An immunomagnetic bead assay yielded confirmed positive results for the inoculated chubs, and in one replication, for the grinder housing after grinding of the final chub. A meat grinder will likely purge itself of *E. coli* O157:H7 when a low level of contamination is encountered. However, with lean trim containing moderate levels of contamination, product immobility at specific points within the grinder may continuously produce low-level contamination during grinding.

(85) A MODEL FOR THE EFFECTS OF TEMPERATURE, pH AND LACTATE ON THE SURVIVAL OF *ESCHERICHIA COLI* O157:H7

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Escherichia coli O157:H7 has caused infections and deaths in children after consumption of as few as 100 organisms. Because it is sporadically present in foods, particularly beef and including fermented beef products, it is very important to understand the environmental factors that allows even a limited amount of growth or permits the survival of the few indigenous cells. To model the critical ranges that govern survival, BHI broth was adjusted with various combinations of pH (3.0 to 4.5) and sodium lactate or lactic acid (0 - 2%). After inoculation with a cocktail of 24 hr cultures, the broths were stored at various temperatures (4 - 37°C) for up to 106 days. Samples were taken at appropriate times, diluted in peptone, plated on TSA and counted after incubation at 37°C. The data were fitted to a logistic survival model and the resulting parameter values were modeled by polynomial regression. The three environmental factors interacted. Qualitatively, higher temperatures increased the rate of inactivation. Inactivation was slow at pH > 4.0, lactate < 0.5% and refrigeration temperatures. Thus, *E. coli* O157:H7 must be considered as surviving indefinitely in most refrigerated or semi-stable foods.

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(86) SENSITIZATION OF *ESCHERICHIA COLI* TO NISIN AND LYSOZYME BY HIGH HYDROSTATIC PRESSURE, EDTA AND CHITOSAN

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Food processing by High Hydrostatic Pressure (HHP) requires expensive equipment operating at pressures up to 5000 bar and more. The technique has therefore not yet found widespread application, in spite of several advantages over heat processing. The potential of a mild- pressurization step in a hurdle approach to food preservation has been suggested, and prompted us to investigate the effect of HHP on the sensitivity of *E. coli* to different compounds useful as food preservatives.

As a working hypothesis, we assumed that one of the prime targets of HHP would be the outer membrane. Two types of compounds were chosen: (i) lysozyme and nisin, for which *E. coli* is insensitive because they can not penetrate the outer membrane barrier to reach their site of action; (ii) EDTA and chitosan, which destabilize the outer membrane by complexation of bivalent metal ions and electrostatic binding to the negatively charged lipopolysaccharides respectively.

Freshly grown *E. coli* cells resuspended in phosphate buffers containing different combinations of the above mentioned compounds, were subjected to different pressure levels, and surviving cells were counted by plating. Under the conditions used, the presence of lysozyme and nisin increased the lethality of pressurization at 1500 bar and above. This effect increased strongly with the applied pressure level, and was enhanced in the presence of EDTA. Addition of chitosan enhanced strongly the effect of nisin, but reduced the effect of lysozyme. This antagonistic effect of lysozyme and chitosan may be due to inactivation of lysozyme by the polycation chitosan.

We conclude that HHP sensitizes *E. coli* for nisin and lysozyme, probably by increasing the permeability of the outer membrane. Under our conditions, this permeabilization was transient, because several minutes after the release of pressure, the cells lost their sensitivity to lysozyme. Further, this permeabilization of the outer membrane was synergistically enhanced by chemical agents that interfere with outer membrane integrity.

(87) EFFECTS OF LACTATE, SPICE OIL, AND pH LEVELS ON THE GROWTH AND SURVIVAL OF *E. COLI* O157:H7 AT 35 AND 4°C

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Tryptic soy broth adjusted to pH 4, 5, 6 and 7 with HCl and inoculated (10^4/ml) with *E. coli* O157:H7 (EHEC) was held at 35°C or 4°C. Up to 0.2 M lactate was ineffective to control growth of EHEC at pH 6 and 7 at 35°C. Cells survived at pH 4 and populations decreased in presence of 0.1 or 0.2 M lactate. Cell populations at 4°C were essentially static for up to 168 hrs at pH 5, 6 or 7 regardless of lactate level. At pH 4, CFU/ml decreased from log 3.8 to 0 in 96 hrs at 4°C in the presence of 0.2 M lactate. The effect of oil of clove was mainly bacteriostatic with a level of 750 ppm needed to get significant growth inhibition at pH 5 and above (35°C). Oil of origanum was effective against EHEC and demonstrated bactericidal properties. A level of 500 ppm was necessary at optimum pH and temperature to decrease populations to <10 CFU/ml within 8 hr. Combinations of low pH or 4°C with 250 or 500 ppm oil of origanum were effective against EHEC.

(88) COMPARISON OF MATHEMATICAL MODELS TO ESTIMATE GROWTH RATE OF *ESCHERICHIA COLI* O157:H7 AT FLUCTUATION TEMPERATURES

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Growth of *Escherichia coli* O157:H7 in a mixed culture was determined as a function of fluctuating (abuse) temperatures in brain heart infusion broth with initial pH adjusted to 5, 6, or 7. Five 6-hour "square-wave" fluctuating temperature regimes were used: 4-12, 4-19, 4-28, 8-19, and 12-28°C. Similar studies were done in broths with various NaCl concentrations (1, 2 and 3%). Averaged growth rates (GR) were calculated by fitting the Gompertz equation and the effect of cycling temperature on the GR then modeled with these four functions: linear, Arrhenius, square root, and "response surface". For some measurements obtained during log growth, the plotted curves were non-linear (step-like). The "square-root" and Arrhenius model showed the poorest fit. The linear and "response surface" models estimated growth closer to the actual measurements. None of these models estimated the growth for the various temperature fluctuating period. Rather than developing one mathematical model for the entire growth period with the Gompertz, the lag and GR should be measured and modeled separately for growth at fluctuating temperatures.

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(89) EFFECT OF TIME OF EXPOSURE OF BEEF FAT FASCIA TO *ESCHERICHIA COLI* ATCC 11370 ON ITS REMOVAL BY SPRAY-WASHING WITH CHEMICAL SOLUTIONS AND 35° OR 74° WATER

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This study examined the effect of exposure time of beef fat fascia to a fecal paste, containing *Escherichia coli* ATCC 11370, on the efficiency of spray-washing with 2% acetic acid (AA), 5% hydrogen peroxide (HP), 12% trisodium phosphate (TSP), and 35°C or 74°C water to remove bacteria. Beef brisket fat samples (20 cm x 20 cm) were inoculated, in their surface geometric center, within 15 min post-mortem, with four 0.64 cm diameter loops of a bovine fecal paste containing 10^8 colony forming units (CFU)/ml. Samples were then spray-washed in a pilot automated spray-washer at 20.7 bar, for 12 sec, with AA, HP, TSP and 35°C or 74°C water, after 0, 2 or 4 h of inoculation. Spray-washing at time 0 with TSP, 35°C water, HP, AA and 74°C removed 3.0 ± 0.40, 3.5 ± 0.55, 3.6 ± 0.67, 3.7 ± 0.72 and 4.2 ± 0.55 $\log_{10}$ CFU/cm^2, respectively. Spray-washing 2 or 4 h after exposure to the fecal paste resulted in less ($P < 0.05$) removal (1.8 to 3.9 after 2 h and 0.9 to 2.6 log CFU/cm^2 after 4 h) of bacteria. Numbers of bacteria removed decreased with time, regardless of the washing treatment. The most effective washing agent was 74°C water at all washing times. Therefore, time of exposure to fecal contamination, affected the attachment of bacteria to beef carcass fat.

(90) RADIATION RESISTANCE OF PATHOGENIC *ESCHERICHIA COLI* SEROTYPES

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Gamma radiation resistance (D-value in kGy) of nonpathogenic isolates (ATCC 11775 and 25922), two enterotoxic serotypes (O78:H11 and O78:K80:H12), two enteroinvasive serotypes (O29:NM and O124:NM), five isolates of enterohemorrhagic serotype O26:H11, two isolates of enteropathogenic serotype O111:NM, and four isolates of the enterohemorrhagic serotype O157:H7 of *Escherichia coli* in ground beef at 5°C were 0.23, 0.23, 0.31, 0.25, 0.37, and 0.26 kGy; and at -5°C 0.33, 0.30, 0.47, 0.41, 0.40, and 0.39 kGy, respectively. Though all values were in the same general range of gamma radiation resistance, many values differed significantly from those of the other serotypes. The gamma radiation resistance, at 5°C of *E. coli* O157:H7 grown on ground beef (0.23 kGy) did not differ significantly from that of cells grown in trypticase soy broth and added to sterile ground beef. All tested serotypes of *E. coli* would be completely eliminated from ground beef by gamma radiation doses of 3.0 kGy at 5°C.

(91) EFFICACY OF TRISODIUM PHOSPHATE FOR KILLING *SALMONELLA* ON TOMATOES

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A study was undertaken to determine the effectiveness of AvGARD™ trisodium phosphate (TSP) in wash water on inactivation of *Salmonella* on the surface and in core tissue of unwashed, mature green tomatoes. A *Salmonella montevideo* strain isolated from a patient involved in a 1993 salmonellosis outbreak implicating fresh ripe tomatoes was the test organism. Tomatoes (25°C) were submerged in a suspension of *S. montevideo* cells for 2 min, air dried and submerged (15 sec or 2 min) in solutions (37°C) containing 0, 1, 4, 8, 10, 12 or 15% TSP. The surface and core tissue populations were 5.18 $\log_{10}$ CFU/cm^2 and 5.58 $\log_{10}$ CFU/g, respectively, before dipping into control and TSP solutions. Complete inactivation of *S. montevideo* on the tomato surface was achieved by dipping tomatoes into a 15% TSP solution for 15 sec. Significant ($P < 0.05$) reductions were obtained by dipping tomatoes in a 1% solution for 15 sec. Populations were significantly reduced in core tissue of tomatoes dipped in 4-15% TSP. However, even at 15%, only about a 2 $\log_{10}$ reduction was achieved. Upon ripening, the hue and chroma of tomatoes, indices of color and brightness, respectively, were unaffected by treatment with TSP. The use of TSP as a sanitizing agent in wash water for mature green tomatoes appears to have good potential.

(92) MODELING THE EFFECT OF TEMPERATURE ON GROWTH RATE AND LAG TIME OF *BACILLUS STEAROTHERMOPHILUS* USING VARIANCE STABILIZING TRANSFORMATIONS

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Bacillus stearothermophilus is a thermophilic organism associated with flat sour spoilage of canned foods. The spores of this organism are heat resistant and survive most conventional thermal processes. If a canned food product containing viable *B. stearothermophilus* spores is stored at a temperature above 40°C, spoilage may result. The growth rate and lag time of *B. stearothermophilus* ATCC 12980 vegetative cells in tryptic soy broth (TSB) were determined over the temperature range 40-70°C. A Gompertz curve was fit to the data at

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each experimental temperature. The parameters from the curve were used to determine growth rate and lag time. There was less variation in growth rate at low growth rates compared to high growth rates. If a model was fit to the data without considering this variance trend, incorrect predictions would result. A transformation which corrects variance in the data set was needed. Firstly, standard transformations like natural logarithm and square root were used. Secondly, Box-Cox analysis was done to determine correct transformation λ for the data set. Two models (response surface and Davey) and three transformations (square root, log, and λ) were used with the data. The response surface model with natural logarithm ($r^2 = 0.97$) and Davey model with natural logarithm ($r^2 = 0.97$) transformations best describes the relationship between growth rate and temperature. Similarly a response surface model with square root ($r^2 = 0.92$) and Davey model with square root (r^2=0.92) transformations can be used for predicting lag time. These models can subsequently be expanded to include the effect of other factors like salt content or pH on predicted lag time and growth rate. These models would be useful in predicting the shelf life of thermostabilized food which are occasionally stored under elevated temperatures.

(93) REDUCTION OF FOODBORNE PATHOGENS ON BEEF CARCASS TISSUE USING SODIUM BICARBONATE AND HYDROGEN PEROXIDE

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In an attempt to control carcass contamination, a search for effective carcass washing treatments has become a major focus in the area of microbiological meat safety. Spray washing treatments utilizing 1% sodium bicarbonate and 3% hydrogen peroxide were performed to evaluate their effect on *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes*. Adipose and lean tissue samples, taken immediately off a processing line, were cut into 1.5 cm thick and 7.5 cm^2 sections and surface inoculated with 10 ml of the appropriate bacteria in sterile weigh boats. After inoculation, a spray wash treatment was executed with hand held spray bottles using 3.6 ml of 1% sodium bicarbonate followed by 3.6 ml of 3% hydrogen peroxide for a total treatment time of 30 min. Samples sprayed with equal volumes of water and samples receiving no spray treatment were included as controls. Half of the adipose and lean samples were immediately analyzed and the remaining samples were held at 4°C for 18 hours before analysis. Results indicated that *E. coli* O157:H7 and *S. typhimurium* were most sensitive to the sodium bicarbonate and hydrogen peroxide treatment with a one log reduction. There was no significant effect ($P < 0.05$) on *L. monocytogenes*. Reduction in bacterial counts indicates that a spray wash treatment consisting of the right combination of safe and acceptable solutions is effective with regard to actual microbial safety as well as cost and consumer acceptance.

(94) EFFECT OF CHLORINE DIOXIDE SPRAY WASHES FOR REDUCING FECAL CONTAMINATION ON BEEF

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Chlorine dioxide (ClO_2) was examined for reducing bacterial populations on fecally contaminated beef carcass tissue (BTC) in two separate experiments. In the first phase of the study, individual pieces of BCT with approximately 5.8 $\log_{10}$ CFU/cm^2 were spray treated (10 s; 16°C) with tank concentrations of chlorine dioxide (ClO_2) ranging from 0 to 20 ppm. Bacterial populations were reduced by no more than 0.93 $\log_{10}$ CFU/cm^2, regardless of concentration, and were not statistically different ($p < 0.05$) than water treated BCT. In the second phase of this study, tap water (16°C) and ClO_2 at a tank concentration of 20 ppm were sprayed for 15, 30, and 60 s onto BCT inoculated with 6.60 $\log_{10}$ CFU/cm^2 and compared. While spray treatments with ClO_2 or water did affect reductions of 1.53 to 2.07 $\log_{10}$ CFU/cm^2, spray treatments with either water or ClO_2 at 15, 30, and 60 s were not statistically different ($p \leq 0.05$). Similar reductions (1.61 $\log_{10}$ CFU/ cm^2) were observed when BCT was spray treated for 60 s with tap water followed by a 60 s spray wash with ClO_2 These results demonstrate that spray treatments with ClO_2 are no more effective than water for reducing fecal contamination on beef.

(95) ANTIMICROBIAL PROPERTIES OF VOLATILE HORSERADISH DISTILLATES

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Increased consumer interest in "natural foods" has led to renewed consideration of natural antimicrobial systems as food preservatives. Extracts of plants in the Cruciferae family are known to contain potent microbial inhibitors, mainly isothiocyanates. The volatility of these compounds has hindered practical applications in the past, but they could find a useful role as adjuncts in modern packaging systems that employ impermeable packaging materials and modified atmospheres. The effect of vaporized steam distillates of horseradish root (*Armoracia lapathifolia*) against several bacterial and fungal isolates inoculated onto agar disks was evaluated in a closed, impermeable model system. Germination of *Penicillium expansum*,

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Aspergillus flavus and *Botrytis cinerea* spores was completely inhibited by 200 µg/L air, and the distillates had sporicidal activity. Pseudomonads isolated from lettuce were slightly more resistant to the effects of the extract but could not grow in the presence of 300 µg/L air. Growth of *Listeria monocytogenes* Scott A and *Salmonella typhimurium* was inhibited by 1000 µg/L air but 2000 µg/L air were required to prevent growth of *Escherichia coli* O157:H7. Bactericidal activity was observed with all the experimental strains. Analysis by gas chromatography revealed that allyl isothiocyanate was the main component of the distillates, followed by ß-phenylethyl isothiocyanate.

(96) EFFECT OF CITRICIDAL, CHLORINE AND BENOMYL ON *VIBRIO CHOLERAE* GROWTH IN VITRO

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Since 1991, cholera has caused serious economic and public health problems in America. Because of the epidemic present in the continent, and the prevailing irrigation practices using wastewaters, it is important to evaluate products that reduce the possible risk of infection by *Vibrio cholerae*. In addition the treatment must be harmless for humans. The minimum inhibitory concentrations and the contact times of benomyl, chlorine and Citricidal were assessed on the survival of *Vibrio cholerae*. A population dynamic study was performed to attain a concentration of 10^8 CFU/ml (Maniatis et al. 1983). The quantitative suspension tests according to Russell (1982) and Erlich (1990) were used to evaluate these chemical compounds. Benomyl did not show bactericidal effect at the times 5-10 minutes and concentrations (200-10,000 ppm) tested; chlorine, however, was effective at 100ppm with a 10 minute exposure. On the other hand, Citricidal was effective at 50 ppm with a 5 minute exposure. With chloride and Citricidal, we observed that increasing concentrations led to shorter exposure times to attain the *V. cholerae* growth inhibition. Therefore, we recommend the use of Citricidal for the control of *Vibrio cholerae* in the cleaning of wastewaters, which are used for vegetable irrigation, as well as a postharvest disease control agent.

(97) EFFECT OF PROCESSING PROTOCOLS ON THE QUALITY OF AQUACULTURED FRESH CATFISH FILLETS

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Fresh aquacultured catfish fillets were obtained from 3 processors using different processing protocols. The fillets were evaluated for the microbial quality during four seasons (summer, autumn, winter and spring). Twenty freshly processed fillets were randomly picked and each fillet was placed in a sterile polyethylene bag. The fillets were transported on ice-pack by air within 24 h after processing. Five fillets were randomly selected for microbial analysis. Each fillet was weighed and an equal volume of sterile 0.1% peptone water at 0-1°C was added aseptically. The fillet was massaged for 120 s and the supernalent was used for indicative bacteriological quality. The aerobic (35°C for 48 h) and psychrotrophic (35°C for 96 h) plate counts were enumerated using 3M™ Petrifilm™ Aerobic Count plates. The *Escherichia coli* (35°C for 24-48 h) and total coliform (35°C for 24-48 h) counts were enumerated on 3M™ Petrifilm™ *E. coli* count plates. The *Staphylococcus aureus* counts were determined on Baird-Parker agar (35°C for 48 h). There were significant differences in aerobic, psychrotrophic, total coliform, *E. coli* and *S. aureus* counts due to temperature effect during production of aquacultured catfish and variations in processing protocols. The *E. coli* and *S. aureus* counts were significantly different during the four seasons. The *E. coli* and *S. aureus* counts were high during hot weather and low during cold weather. There was a significant difference in aerobic, psychrotrophic and total coliform counts among the three processors during the warm seasons, these differences were reduced during the cold seasons.

(98) INTERVENTION THROUGH THE USE OF HAND-TRIMMING, CHEMICAL SANITIZERS AND HOT WATER SPRAY-WASHING TO REMOVE FECAL AND MICROBIOLOGICAL CONTAMINATION FROM BEEF ADIPOSE TISSUE

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Several studies have found that spray-washing treatments involving water alone or in combination with organic acids reduce microbiological counts on carcasses. The objective of this study was to compare chemical solutions and hot water spray-washing interventions with hand-trimming/spray-washing for their ability to remove fecal material and to reduce bacterial contamination on beef adipose tissue. Beef brisket samples were contaminated with 2.5 cm^2 of a bovine fecal paste inoculated with *Escherichia coli* (ATCC 11370). After inoculation, the samples were treated by spray-washing at various water temperatures (16-74°C) and pressures (2.76-20.68 bar) followed or preceded by sanitiz-

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ing sprays of 5% hydrogen peroxide, 2% acetic acid, 12% trisodium phosphate, or 0.5% ozonated water at 20 psi; hand-trimming; or a combination of the two, followed by subsequent visual evaluation and microbiological analyses. Spray-washing with 74°C water caused reductions in bacterial counts exceeding 3.0 log_{10} CFU/cm^2 with or without chemical solutions. Hot (74°C) water was also effective in removing fecal material from beef adipose tissue. Among the chemicals, hydrogen peroxide (5%) and ozonated water (0.5%) were the most effective in reducing microbial contamination.

(99) A SURVEY OF COLLEGE STUDENTS' KNOWLEDGE OF FOOD SAFETY AND HOME FOOD PREPARATION PRACTICES

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A 48-question survey was administered to 650 college students at Cornell University to determine their knowledge of specific food safety terms and issues, as well as home food preparation practices. The results were compared to those of a national survey (Williamson et al, 1991). The food safety knowledge index score was cross-tabulated with gender, college class, interest in food safety, geographical location and food preparation involvement. Women scored slightly higher than men. A higher interest in food safety, being involved in food preparation, as well as higher level of education was associated with higher scores. Respondents from farms and rural areas also had the highest scores. Cross-tabulations between food safety knowledge and food preparation practices questions showed that knowledge is not always associated with the correct preparation practices. College students sometimes had better knowledge of food safety, but respondents of the national survey used better preparation practices.

(100) ECOLOGY AND CONTROL OF BREAD SPOILAGE BY ROPE-FORMING *BACILLUS*

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Rope spoilage of brown bread by *Bacillus subtilis* and *Bacillus licheniformis* causes economic losses to the local baking industry. *Bacillus* spores originate from the raw materials (flour, yeast and crumbs), and equipment surfaces contacting the dough before baking. Spores of these strains survive the baking process due to their high heat resistance compared to other *Bacillus* species. In ropey bread, the *Bacillus* cells form slimy extracellular polysaccharides and produce confluent biofilms. Extracellular amylolytic activity was demonstrated by scanning electron microscopy and plate diffusion assays. Preservative evaluation against rope by laboratory based accelerated methods proved unsuccessful. Evaluation of bread preservatives by the baking test, however, indicated combinations of calcium propionate and vinegar to be most effective in reducing rope spoilage while displaying only a slight inhibitory effect on yeast activity.

(101) HACCP IN PRACTICE

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A structural and philosophic modification of the HACCP System is proposed. Original seven principles are condensed into four, and two new principles are added. Definition of objectives is included as Principle 1, to establish clear and measurable parameters, which will serve to evaluate the impact and cost/benefit relation derived from an application of HACCP. Redesign of processes, new Principle 3, seeks to modify risky situations, prior to determinate CCP's. From the philosophic point of view, HACCP concept is expanded to be conceived as a System capable to integrate and rationalize all the activities oriented to guarantee the quality (not only safety) of food products, and to quantify and communicate the results of efforts devoted to quality. Changes are done to increase the acceptance of the System among the food industry, as well as to adapt it to the changing conditions affecting the management of food processing and control today.

(102) PRETREATMENT OF MEAT IN THE SLAUGHTER PROCESS

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Microbial contamination of animal carcasses is a result of the necessary procedures required to process live animals into retail meat. The contamination can be minimized by good manufacturing processes, but the total elimination of foodborne pathogenic microorganisms is difficult, if not impossible. A variety of intervention methods have been developed to reduce the levels of contaminating bacteria on carcasses, with carcass washing and sanitizing being one of the most common procedures. Sanitizing agents include hot water, chlorine and short chain organic acids. The effectiveness of these compounds varies by the concentration used, the

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temperature of the sanitizers and contact time, the sensitivity of the native microflora to the specific compound, and to a certain extent the design of the specific experiments. The consensus of the research is that carcass washing and sanitizing can reduce the initial levels of bacteria on the surface of carcasses.

(103) FOOD ADDITIVES IN PROCESSED MEATS

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Commercially prepared ready-to-eat meat products have a long and favorable history of safety in the U.S. Many of the products are perishable and require refrigerated storage. Others are shelf stable. The safety and quality of ready-to-eat meats are influenced by many factors, including the ingredients used during formulation. This presentation will discuss safety and quality concerns, approaches used by industry to address the concerns, and where improvements may be desirable. Time permitting, examples will be described to demonstrate how additives are used to enhance safety and quality.

(104) PACKAGING AND STORAGE CONDITIONS TO ENHANCE MEAT SAFETY

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The safety of ready-to-eat meats is affected by a variety of factors, including product composition, post-cooking hygiene, packaging material, package headspace, temperature of storage and distribution, and consumer handling. Packaging for ready-to-eat meats ranges widely in complexity; this range reflects the varying use of antimicrobial hurdles in product formulation and processing. The more sensitivity ready-to-eat meats are often packaged under vacuum or modified atmosphere. The general antibacterial effects of increased CO_2 decreased O_2 modified atmosphere packaging are well documented for raw meats and poultry. Less well understood are the effects of such atmospheres on the survival and growth of pathogenic bacteria that may contaminate cooked meat products. Several types of research protocols, including mathematical modeling of pathogen growth, have been explored and will be discussed.

This presentation will summarize pertinent studies on the survival and growth of pathogenic bacteria on ready-to-eat meats stored under modified atmosphere. It will also emphasize three key areas for guaranteeing safety of these products (scrupulous post-cool hygiene, appropriate packaging systems, and a consistently low temperature) and how these areas can be controlled, monitored, and verified under Hazard Analysis Critical Control Points (HACCP) system of food safety assurance.

(105) ELIMINATION OF PATHOGENS ON RED MEATS BY IRRADIATION

Donald W. Thayer, Research Leader Food Safety, USDA, ARS, NAA, Eastern Regional Research Center, Philadelphia, PA 19118

Foodborne pathogens on meat, such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* species and *Staphylococcus aureus* can be significantly reduced or eliminated by ionizing radiation doses of 3.0 kGy or less. Irradiation temperature and atmosphere may significantly alter resistance of pathogens to radiation on meats. For example, radiation resistance typically increases markedly below freezing and may be greater in the absence of oxygen. The radiation resistance of pathogens generally can be expected to be slightly greater on processed meats because of the presence of salt or the reduction of water content such as that due to cooking. Some bacteria are more sensitive to radiation on some types of meat than on others; for example, salmonellae are more sensitive to gamma radiation on pork than on beef or lamb. The applicability of food irradiation for the control of pathogens and the factors that influence the effectiveness of the treatment are the subject of the presentation.

(106) NOVEL APPROACHES IN HURDLES TECHNOLOGY

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Bacterial contamination on meat carcasses is an unavoidable consequence of converting live animals to meat for human consumption. Without considering irradiation, the total elimination of both pathogenic and spoilage bacteria from whole carcasses or fresh meat products does not appear to be attainable at the present time. Given that the microbiological stability and safety of many fresh and processed foods are not based only on one intervention, investigations and implementation of multiple hurdles to reduce bacterial populations associated with carcass surfaces are warranted. Hurdles to be reviewed include bacteriocins and various antimicrobial compounds, edible films, desiccation, competitive inhibition and other novel approaches.

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(107) **HURDLES IN GETTING HURDLE APPROVAL**

Dane Bernard, Vice President, Food Safety and Strategic Prog., National Food Processors Assn.

Most scientists recognize that even a "wonderful" new compound or treatment which enhances the safety or quality of a product cannot be automatically put to use. If the technological advance involves addition of a compound to a food, regulatory approvals must be obtained. What most scientists do not recognize is how arduous a task this can become and what types of data must be generated to support petitions for approval. Hurdles to improving safety and quality of foods involve not only technological and approval problems but can involve other problems as well. For example, certain foods may have a standard of identity which would prevent use of a certain additive or compound. In addition, environmental impact of certain treatments and/or compounds often must be considered in addition to whether the compound or treatment is effective. There is also the politicization of the scientific endeavor. There are many special groups who, for non-scientific reasons, may wish to block use of a particular compound or piece of technology. Thus, scientists need to be aware of these potential hurdles and how to avoid them if possible.

(108) **INFLUENCE OF pH AND INCUBATION TEMPERATURE ON VIRULENCE AND FATTY ACIDS OF *YERSINIA ENTEROCOLITICA***

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The effects of pH and storage temperature on changes in the fatty acid (FA) composition and virulence factors of *Yersinia enterocolitica* were investigated. FA extracts of three strains of *Y. enterocolitica* grown at pH 5, 7 and 9 and incubated at 4 and 20°C were examined using gas chromatography. When cultured at pH 5, *Y. enterocolitica* produced increased proportions of cyclopropane and saturated FAs and decreased amounts of unsaturated FAs as compared to levels produced during growth at pH 9. At pH 5, saturated FAs comprised 73-86% of the total FAs, whereas at pH 9, saturated FAs decreased to 47-72% of the total FAs. Generally, *Y. enterocolitica* strains exhibited a reduced proportion of cis-16:1^9 as pH was reduced accompanied by a significantly higher level of 17:0Δ with increasing pH. Temperature had a significant effect on the production of the unsaturated FAs, cis-16:1^9 and cis-18:1^9. Indicator tests for virulence demonstrated that strain YE321 (serotype 0:20) lost its virulence factor, while strains YE133 (serotype 0:8) and YE228 (serotype 0:3) retained their virulence markers following incubation at 4 and 20°C at pH 5, 7 and 9. These results suggest that adverse storage conditions may result in cellular alterations that enhance the ability of *Y. enterocolitica* to survive and maintain virulence.

(109) **GROWTH OF *LISTERIA MONOCYTOGENES* AND *YERSINIA ENTEROCOLITICA* ON COOKED POULTRY STORED UNDER MODIFIED ATMOSPHERE AT 3.5, 6.5 AND 10°C**

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Modified atmosphere packaged (MAP) ready-to-eat poultry was evaluated for its ability to support the growth of psychrotrophic pathogens stored at 3.5, 6.5, and 10°C over a 2 to 5 week period. Poultry cuts were injected with either a control brine (salts and flavors) or a test brine containing sodium lactate and a shelf-life extender ALTA™ 2341. Poultry cuts were oven roasted and collected immediately out of the oven (no background microbiota) or after passing through a cooling tunnel (background microbiota developed on controls). Cuts were inoculated (1000 CFU/ ca. 150 g piece) with five-strain composite mixtures of *Listeria monocytogenes* or *Yersinia enterocolitica* and packaged under 40:60 CO_2:N_2. Both *L. monocytogenes* and *Y. enterocolitica* grew under all test conditions. Temperature had the greatest effect on the growth of the pathogens with maximum populations (10^7 - 10^9 CFU/piece) achieved in 2 weeks (6.5 or 10°C) or 4 to 5 weeks (3.5°C). Addition of lactate and ALTA 2341 to the brine extended the lag phase of *L. monocytogenes*. The additives had less of an effect at the higher temperatures than at 3.5°C. In some treatments the additives extended the lag phase and decreased the growth rate of *Y. enterocolitica*. The background microbiota did not influence the growth of either pathogen. Presence of a competing microbiota and/or lactate and ALTA 2341 cannot replace minimization of contamination and strict temperature control for the safety of ready-to-eat MAP products.

(110) **NATURAL OCCURRENCE OF *LISTERIA MONOCYTOGENES* IN FRESH BLUE CRAB (*CALLINECTES SAPIDUS*) MEAT AND ITS GROWTH CHARACTERISTICS AT REFRIGERATION TEMPERATURES**

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The ability of Listeriae to grow over a wide temperature range, including refrigeration temperatures, is of great concern to the food industry. For seafood processors in particular, the zero tolerance for *L. monocytogenes* in ready-to-eat foods, makes it necessary to study the behavior and growth characteristics of this pathogen under normal commercial storage conditions. In this study, 126 samples of fresh blue

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crabmeat collected from different processing facilities were analyzed for the presence of *Listeria* spp. Thirteen samples (10%) were positive for *Listeria*, with 10 samples positive for *L. monocytogenes* and 3 samples positive for *L. innocua*. Fraser broth was used in a 5-tube most probable number (MPN) enumeration, in duplicate of 25 g samples incubated at 36°C for 24 hours and plated in Modified Oxford Agar and Blood Agar with API strip confirmation. The natural occurring levels of *Listeria* in fresh blue crabmeat, were always less than 100 MPN/g with only one exception in which the MPN index was 1100/g. A *L. monocytogenes* strain (168) isolated from fresh blue crabmeat was inoculated (less than 50 CFU/g) into pasteurized crabmeat and incubated at 1.1, 2.2 and 5°C during 21 days. Growth curves were obtained by analyzing, in duplicate, 25 g samples at intervals of 0, 8, 10, 12, 14, 16, 18 and 21 d. In the absence of competing microflora, *L. monocytogenes* had a faster growth rate as the storage temperature increased, with approximately a 7 log increase in population at 5°C and only a 2.5 log increase in population at 1.1°C after the 21 d of incubation.

(111) THE EFFECT OF IRON LEVELS ON GROWTH, TOXICITY AND ADHERENCE OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI*

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The importance of iron on the growth and adherence characteristics and on levels of verotoxin production of six strains of enterohemorrhagic *Escherichia coli* (EHEC) and *E. coli* K-12 was monitored. Trypticase Soy Broth, 0.3% (TSB) and freshly prepared beef extract (BE) with and without 200 μM α, α^1-dipyridyl were inoculated with 10^2-10^3 bacteria/ml and incubated at 9.5°C. *E. coli* were enumerated on 0.3% Trypticase Soy Broth plus 1.5% agar (TSBA) or beef extract plus 1.5% agar (BEA) at 30°C after 24 h. Growth was followed for 14 d. Siderophore production was monitored on Chrome Azurol S agar (CAS) (Schwyn and Neilands, 1986). A steady increase in numbers of EHEC was seen in TSB without iron chelator, reaching 10^7-10^8 CFU/ml in 8 d. In iron-depleted TSB, a slight increase (less than 0.5 logs) was seen after the first day of incubation followed by a steady decrease in numbers until no EHEC were found (14 d). Initially, no differences were noted in siderophore production between EHEC growing in the iron-containing or iron-depleted media indicating that siderphores were produced in both growth environments. Siderophore production began to decrease after day 4 in the iron-depleted media while no reduction was seen in the iron-containing media. The ability of iron-stressed and unstressed EHEC to adhere to HT-29 and/or T_{84} cultured intestinal cells was monitored using the Fluorescent Actin Stain (Knutton et al., 1989) and attaching/effacing (AE) lesions were confirmed by TEM. Levels of verotoxin produced by iron-stressed and unstressed EHEC was quantified against Vero cells using a cytotoxicity microtiter method (Konowalchuk et al., 1977).

(112) ACID ADAPTATION IN *LISTERIA MONOCYTOGENES* SCOTT A

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Studies have shown that *Salmonella typhimurium* and *Escherichia coli* grown at pH 5-6 (adaptation pH) are more resistant to lethal (challenge) pH (e.g., 3.0) than cells grown at neutral pH. This acid tolerance response was investigated in *Listeria monocytogenes* Scott A. Cells adapted at pH 5.2-5.6 showed the highest percent recovery when subsequently exposed to pH 3.0 for 45 minutes as compared to cells adapted at pH 5.0, 5.8, or 6.0. When the time of adaptation (i.e. growth at pH 5.5) was varied, followed by exposure to pH 3.0 for 45 minutes, there was an initial high recovery (30 minutes adaptation) that decreased to a minimum at 2 hours of adaptation and then increased again up to 4 hours adaptation. Variation of challenge pH between 2.0 and 4.0 showed the percent recovery was highest at pH 3.5. *L. monocytogenes* Scott A was grown at 35°C at either pH 7.0 or 5.5 in phosphate buffered trypticase soy broth containing 0.6% yeast extract for 3 hours and challenged at pH 3.5, with samples removed at t_0 and hourly for 6 hours. The experiment was replicated 6 times. There was a significant difference ($p < 0.01$) in recovery between cultures grown at pH 7 and those adapted at pH 5.5 after 2 hours exposure to pH 3.5. A similar adaptation response was shown to occur at 21°C. Initial experiments indicate that the presence of chloramphenicol (200 μg/ml) in the medium during adaptation does not prevent adaptation from occurring. This suggests that protein synthesis is not required for the response.

(113) STRESS PROTEIN AND FATTY ACID COMPOSITION EFFECTS ON HEAT RESISTANCE OF *ESCHERICHIA COLI* O157:H7

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Several proteins produced during heat shock as well as changes in fatty acid composition of cell membranes of bacteria are associated with thermal resistance. The role of these two phenomena in heat resis-

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tance was evaluated in *Escherichia coli* O157:H7. Five incubation treatments were evaluated; 35°C with and without heat shock (45°C), 35°C with stearic or oleic acid, and 45°C with and without subsequent incubation at 35°C. Heat resistance (D_{55c} values) was determined. Fatty acid profiles were analyzed by gas chromatography (GC). Presence of GroEl heat shock protein from cultures incubated at 45°C, with and without subsequent incubation at 35°C, was determined by Western blotting. Cells which were heat shocked, grown at 45°C, and subsequently incubated at 35°C showed higher D_{55c} values (13.53, 15.69, and 13.87 min, respectively) compared to cells grown at 35°C (7.83 min). Cells supplemented with stearic and oleic acids showed intermediate D_{55c} values (9.21 and 9.76 min, respectively). GroEL heat shock protein was produced and persisted on subsequent incubation at 35°C. GC analysis showed incorporation of the fatty acids into the cells. The production of stress proteins seems to be the major factor in thermal resistance of *E. coli* O157:H7.

(114) SURVIVAL CHARACTERISTICS AND INJURY OF *ESCHERICHIA COLI* O157:H7 DURING CONVENTIONAL AND MICROWAVE HEATING AT CONSTANT TEMPERATURES

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Microwave thermoprocessing is gaining popularity among consumers in spite of decreased interest of food manufacturers. The interactions between foods and microwaves are complex and result in uneven heating. Therefore, it is difficult to predict destruction parameters of bacteria in food by applying methods developed for conventional thermoprocessing. A microwave oven system working in the steady-state mode was designed by Welt et al. (Microwave World, 1992). Thus, reliable microbial destruction data may be determined and safe end-point cooking temperature guidelines established. In the research reported here inactivation rates for *Escherichia coli* O157:H7 during conventional and microwave heating at 57°C and 60°C temperatures were determined. The level of cell injury was evaluated using two media, Tryptic soy agar (TSA) and Plate count agar with 1% sodium pyruvate (PCA + 1% NaPyr). The D-values were obtained by heating samples at 57°C and 60°C in water bath (WB) and modified microwave open (MW). When the TSA was used, the D_{57C}-values were 11.8 min (WB) and 19.72 min (MW) and the D_{60C} were 1.00 min (WB) and 0.96 min (MW). Enumeration on PCA + 1% NaPyr gave the following values D_{57C} - 20.1 min (WB), 28.8 min (MW) and D_{60C} - 2.91 min (WB) and 1.81 min (MW). Calculated z-values were 2.8°C (WB) and 2.3°C (MW) on TSA media and 2.5°C (MW) and 3.6°C (WB) enumerated on PCA + 1% NaPyr. Statistical analysis was used to determine if significant differences were related to the mode of heating and/or recovery media. Lethalities delivered to duplicate samples during microwave and conventional heating were calculated and compared by applying General Method.

(115) COMPARISON OF D_{55C}-VALUES OF ANTIBIOTIC-RESISTANT AND ANTIBIOTIC-SENSITIVE STRAINS OF *SALMONELLA*

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An estimated two million cases of salmonellosis occur each year in the United States. The emergence of antibiotic-resistant (AR) strains of *Salmonella* may intensify this problem. Therefore, the objective of this study was to compare heat resistance of AR and non-antibiotic-resistant (Non-AR) strains of *Salmonella* at 55°C.

Strains of AR *Salmonella* used were *S. typhimurium* SA911477, *S. heidelberg* SA911320, *S. enteritidis* PU024 and *S. enteritidis* PU01. Cultures were obtained from Agriculture Canada and Purdue University. Corresponding non-AR strains were *S. typhimurium* ATCC 23564, *S. heidelberg* ATCC 8326 and *S. enteritidis* ATCC 13076. All cultures were incubated at 37°C for 24 h in Tryptic Soy Broth (TSB), washed using centrifugation and suspended in sterile 0.1% peptone. Heating studies were carried out by inoculating sterile 0.1% peptone (500 ml), preheated to 55°C, with ca. log 7.0 CFU/ml in a shaking water bath. At 5 minute intervals, a 1.0 ml sample of the inoculated heating medium was removed and placed in 0.1% peptone cooled to room temperature. Survivors were determined using the pour plate technique with Standard Methods Agar. The log CFU/ml of survivors was plotted versus time and the slope determined by linear regression. The D-value was the negative inverse of the slope.

The mean $D_{55°C}$ for *Salmonella typhimurium* was similar for each type being 6.44 min for the Non-AR and 6.61 min for the AR strain. For *Salmonella heidelberg*, the AR strain had a slightly higher $D_{55°C}$ of 6.43 min compared to the Non-AR strain $D_{55°C}$ of 5.51 min. A statistically significant difference was found between the *Salmonella enteritidis* strains. For AR strains PU024 and PU01 the $D_{55°C}$ values were 15.6 and 14.7 min, respectively while that of the Non-AR ATCC strain was 6.10 min. A higher D-value for AR *Salmonella enteritidis* may require reexamination of whether current time/temperature recommendations are adequate to kill these strains.

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(116) DOSE-RESPONSE OF *SALMONELLA* IN CHEESE

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In September and October, 1994, 75 cases of *Salmonella berta* infection occurred in a rural community in southern Ontario. A specialty cooked cheese product, sold at a local farmers' market, and frequently used as a spread on toast, was identified as the vehicle. The source of the *Salmonella* was chicken carcasses soaked overnight in one of the buckets used for cheese ripening. An attempt was made to calculate the dose response through the amount of cheese eaten and the *Salmonella* levels in available samples. Practically all who ate the cheese were ill. Although 15-250 g was typically consumed before onset of symptoms, some cases continued to eat cheese after they were ill. Hydrophobic grid membrane filter enzyme-labeled antibody direct counts (10^1-10^5 CFU/g), and counts after enrichment in tetrathionate broth at 43°C (10^7-10^8 CFU/mL) were obtained on 17 positive cheese samples. From the known amount of cheese eaten by some of the cases, ill persons had ingested 10^{3-7} CFU *Salmonella*.

(117) BIOLOGICAL CHARACTERIZATION OF *ENTEROBACTER SAKAZAKII*

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Enterobacter sakazakii was designated as a unique species in 1980, based on differences from *Enterobacter cloacae* in DNA relatedness, pigment production and biochemical reactions. This organism has been implicated in a severe form of neonatal meningitis. Although studies have failed to find an environmental source for the organism, dried-infant formula has been implicated in both outbreaks and sporadic cases of *E. sakazakii* meningitis. The severity of the infection in infants, plus lack of information on the ecology and pathogenicity of this organism, led us to a study of the biological characterization of the organism. Strains of *E. sakazakii* were isolated from dried-infant formula available on the Canadian retail market. The prevalence varied from 0-12% in samples evaluated from 5 different companies. Clinical *E. sakazakii* strains were obtained from hospital culture collections. Minimum growth temperatures of around 7.0 to 8.5°C were observed by using a temperature gradient incubator. The potential growth of *E. sakazakii* was followed using cocktails of food and clinical isolates in three different formula incubated at 4, 10 and 23°C. Average generation times were 40 min at 23°C and 4.98 h at 10°C. *E. sakazakii* strains did not grow at 4°C and appeared to die-off during storage. Phenotypic typing methods used included biotyping and antibiograms. From the 16 strains examined, three different biotypes and 4 antibiogram patterns were observed. This study stresses the importance of using aseptic methods and proper temperature control in the preparation, use and storage of dried-infant formula.

(118) SPOILAGE ECOLOGY OF VACUUM-PACKAGED VIENNA SAUSAGES

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Microbiological spoilage of vacuum-packaged vienna sausages was due to psychrotrophic aciduric leuconostocs and homofermentative lactobacilli. These bacteria recontaminated the sausages after smoke-cooking which was confirmed by scanning electron microscopy. Comparison of plasmid profiles of spoilage strains to those of factory environmental isolates could, however, not pinpoint their origin. Representative spoilage strains were heat sensitive by *in vitro* heat resistance determinations. This was reflected by at least fourfold shelf life extensions as a result of in-package pasteurization. The effect of in-package pasteurization was synergistically enhanced by low temperature storage, but not by acid treatment of sausage surfaces. The shelf life extension brought about by in-package pasteurization was, at least in part, ascribed to an increased predominance of pediococci and non-lactic acid bacteria. Several leuconostocs associated with sausage spoilage produced heat and acid stable proteinaceous bacteriocins.

(119) "MEAT INDUSTRY PROGRAM" INDEPENDENT-STUDY CERTIFICATE COURSE DEVELOPED THROUGH GOVERNMENT/INDUSTRY PARTNERSHIP

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The "Meat Industry Program" (MIP), an independent-study certificate program consisting of: Meat Industry Basics I, Meat Industry Basics II and Meat Technology, is offered through Independent Study, University of Guelph. The MIP was developed through a partnership among the Ontario Independent Meat Packers and Processors Association (OIMPP), Meat Industry Inspection Branch and the Education and Re-

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search Division of the Ontario Ministry of Agriculture, Food and Rural Affairs. Successful completion of the MIP is required for the OIMPP's Quality Accreditation Program. The overall objective of the MIP is to equip plant operators and employees with the skills and knowledge necessary to successfully compete in the meat processing industry. The course videos (Legislation Affecting Meat Plants, Health & Hygiene in Meat Plants, Sanitation & Handling in Meat Plants, Occupational Health & Safety in Meat Plants, Quality Control in Meat Plants, Slaughter of Hogs, Slaughter of Cattle, Slaughter of Lambs, Fresh Meat Processing - Pork, Fresh Meat Processing - Lamb, Fresh Meat Processing - Beef) will be available for viewing and the course materials will be on hand.

(120) BOVINE SPONGIFORM ENCEPHALOPATHY: POTENTIAL RISK FROM FOODS?

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Bovine spongiform encephalopathy (BSE) is a fatal neurological disease of cattle. It has been recognized in Great Britain since 1986, where it has since affected more than 140,000 cattle. It has also occurred at a much lower incidence in other countries. Cases outside Great Britain have been attributed to cattle or meat and bone meal imported from Great Britain. Imports of British cattle and meat and bone meal into the U.S. are prohibited. The USDA has for several years, been conducting active surveillance for BSE among U.S. cattle. The disease has not been detected and apparently has not occurred in the U.S.

BSE is one of a group of diseases (other examples scrapie of sheep, Creutzfeld-Jacob disease of humans) characterized by a prolonged (years) incubation period, a progressive spongiform degeneration of brain and accumulation of an abnormal form of a family of cell membrane proteins (prions) in the brain. This group of diseases is referred to as the prion diseases or the spongiform encephalopathies. There is strong (but still emerging and controversial) evidence that these diseases are caused by abnormal forms of prion protein. They can be inherited as the result of mutations in the prion gene. Under some circumstances they can be transmitted by injection or by feeding infected (abnormal prion protein containing) tissue to susceptible hosts. Infectivity of these "agents" is remarkably resistant to chemical and physical treatments used to inactivate conventional infectious agents. BSE was disseminated by feeding ruminant derived meat and bone meal containing BSE agent which was not completely inactivated by rendering. BSE is hypothesized to have emerged from a strain of scrapie which become adapted to cattle via continuous recycling of rendered byproducts. An alternative hypothesis is that the BSE agent originally arose in cattle and was disseminated through feeding rendered byproducts.

Like other prion diseases, BSE can be transmitted experientially across some species lines by parenteral injection and in some instances by feeding some tissues from affected individuals. Bioassays in mice have demonstrated the agent in brain and intestine. Bioassays of other tissues and milk from affected cattle have given negative results. In addition to cattle, there is evidence of spontaneous foodborne transmission of BSE to wild ruminants in zoological parks and to domestic cats. There is not evidence of transmission to humans. It has been hypothesized that transmission of BSE to humans might have occurred, but not yet been recognized because of the prolonged incubation period for prion diseases in humans. Obviously this hypothesis can neither be refuted nor supported with existing evidence. However by comparison with scrapie, it seems unlikely. In spite of years of human exposure to scrapie, there is no evidence of transmission of scrapie to humans. If BSE is ultimately found to be transmissible to humans, the risk that the route of the transmission would be foodborne would appear to be low. The oral route of transmission is several orders of magnitude less sensitive than is the parenteral route for transmission for other prion diseases. Furthermore the BSE agent appears to be restricted to, or present at high titer only in tissues which are comparatively less frequently used for human food. In the U.S. the risk would be even less because the disease does not occur in the U.S.

It has been hypothesized that scrapie could be transmitted to U.S. cattle causing BSE in the U.S. The risk of this happening also appears to be low. The ration of sheep to cattle, prevalence of scrapie, and use of meat and bone meal to supplement cattle diets are all much less favorable for such transmission in the U.S., than those conditions as they existed in Great Britain at the time BSE emerged. Furthermore when U.S. scrapie agent was transmitted to cattle by parenteral injection, the cattle developed a disease distinctly different from BSE. Cattle fed raw brain or rendered carcasses from scrapie infected sheep remain normal more than 4 years after exposure.

(121) VIABILITY OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS IN BEVERAGES: CORRELATION OF IN VITRO EXCYSTATION WITH INCLUSION OR EXCLUSION OF FLUOROGENIC VITAL DYES

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Cryptosporidium parvum is an enteric coccidian protozoan which produces an environmentally stable oocyst that is excreted in the feces of infected individuals. There have been seven documented waterborne outbreaks in North America. Water systems provide water to the food and beverage industry for cleaning

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raw products and inclusion in the products themselves which increase the risk of contamination. No information is available on the survival of oocysts in any type of food or beverage.

The objective of this study is to evaluate the survival of *C. parvum* in beverages: carbonated drinks, including beer and soda pop, fruit juice, baby formula, and tap water. The products were stored at room temperature (22°C) and 4°C and seeded at a concentration of 10^6/ml. Subsamples were collected at times 0, 4, 8, and 24 hours and examined under differential interference contrast (DIC) for the enumeration of sporozoites, by fluorogenic vital dyes, DAPI and PI, after 24 hours to determine viability and by epifluorescence microscopy over seven days to determine concentrations.

Viable morphology of the oocysts decreased after 24 hours of exposure in the beers, sodas, and fruit juices; however, the baby formula had no delirious effects on the oocysts. Sporozoite counts showed at least a twofold decrease at 4°C and up to fourfold at 22°C. The beverages tested caused a slight decrease in fluorescence, using IFA, as compared to tap water controls. Using the vital stains, viability was assessed and resulted in a decrease of greater than 85% in the beers, sodas, and fruit juices at each temperature. The baby formula and the tap water showed only 11% and 30% reductions in viability after 24 hours. Ethanol and pH controls were compared to determine the inactivating factor in each of the beverages tested to date. Recent research has reevaluated the viability of the oocysts in each beverage using in vitro excystation as a second viability assay. These results will be compared and correlated.

(122) GROWING CONCERNS AND RECENT OUTBREAKS OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* NON-O157:H7 SEROTYPES

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Verocytotoxin-producing *E. coli* (VTEC) of serotype O157:H7 have been shown to be important agents of foodborne disease in humans worldwide. While the majority of research effort has been targeted on this serotype it is becoming more evident that other serotypes of VTEC can also be associated with human disease. An increasing number of these non-O157:H7 VTEC have been isolated from humans suffering from HUS and diarrhea. Recently a number of foodborne outbreaks in the USA, Australia and other countries have been attributed to non-O157:H7 VTEC serotypes. Surveys of animal populations in a variety of countries have shown that the cattle reservoir contains more than 100 serotypes of VTEC, many of which are similar to those isolated from humans. The diversity and complexity of the VTEC family requires that laboratories and public health surveillance systems have the ability to detect and monitor all serotypes of VTEC.

(123) STAPHYLOCOCCI - ARE ATYPICAL AND NON *S. AUREUS* TOXIGENIC SPECIES ON THE HORIZON?

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Historically, staphylococcal food poisoning is one of the more commonly reported foodborne illnesses and is caused by the ingestion of preformed enterotoxin in foods. With exceptions, enterotoxin production is thought to be associated with coagulase positive rather than coagulase negative staphylococcal species. Of the coagulase positive species (*aureus, intermedius, lypticus, delphi*), *Staphylococcus aureus* has been thought to be exclusively implicated in human foodborne illness. However, other coagulase positive species as well as coagulase negative staphylococci have been enterotoxigenic or shown to be etiologically associated with staphylococcal foodborne intoxication. Rather recently, *S. intermedius*, a common species in veterinary flora, was associated with human foodborne intoxication caused by the ingestion of margarine and butter blend. Of the conventional ancillary tests (typical reaction on Baird-Parker agar, coagulase expression, thermostable nuclease [TNase], production, lysostaphin sensitivity and anaerobic utilization of glucose and mannitol) coagulase activity has been used as an indication of an isolate to be a potential foodborne pathogen. More recently, TNase has been suggested as being a more reliable indicator of enterotoxigenicity. While all of the ancillary tests may be useful in the identification of the staphylococci, evidence suggests that total reliance of these ancillary metabolic expressions may not be as reliable as hoped for determining whether a staphylococcal isolate is a potential foodborne pathogen. In a study of 151 strains of staphylococci (100 enterotoxigenic and 51 nonenterotoxigenic), the false-negative rates in identifying the enterotoxigenic group as typical *S. aureus* were as follows: 11.0% for colonial morphology on Baird-Parker agar, 8.0% for coagulase activity, 7.0% for TNase production, 4.0% for lysostaphin sensitivity and 2.0% and 4.0%, respective, for anaerobic utilization of glucose and mannitol. A more significant approach for determining whether *Staphylococcus* is a potential pathogen is to test directly for enterotoxigenicity with one of the rapid methods available for demonstrating enterotoxin production. None of the conventional identification tests have been, conclusively, shown to be associated with enterotoxin synthesis. Furthermore, evidence exists that enterotoxin production is a characteristic of the *Staphylococcus* Genus rather than a species specific entity.

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(124) *ARCOBACTER* AND *HELICOBACTER*— RISKS FOR FOODS AND BEVERAGES

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The Family Campylobacteriaceae includes *Campylobacter*, *Helicobacter* and *Arcobacter*. *Campylobacter jejuni* and *Campylobacter coli* are the major causes of acute enteritis in humans. *Helicobacter pylori* causes human ulcers and has been linked to cancer. *H. pylori* organisms have been recovered from water and from vegetables. The genus *Arcobacter* was proposed in 1991 to include aerotolerant *Campylobacter*-like organisms recovered from cases of livestock abortion and human enteritis. *Arcobacter butzleri* organisms have been cultured from water, poultry and from ground pork products. The purpose of this presentation is to examine the evidence for considering *Helicobacter* species and *Arcobacter* species as emerging foodborne pathogens.

(125) DEALING WITH AN EXPANDING GLOBAL FOOD SUPPLY

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We have a competitive world economy and cannot exist in isolation. Migration, overseas education and travel have resulted in tremendous demands for foods from other parts of the world. With advances in communication systems, products can be moved rapidly throughout the world at competitive prices. There is a two way concern on safety and quality by importing and exporting countries: the microbiological risks from products/ingredients from countries which have varying microbiological standards and the dumping of substandard products in countries with insufficient monitoring systems.

HACCP based on hazards and risks specific to a particular product are being increasingly recommended as a quality assurance tool to meet the current and future safety demands of the world's food supply. The scope of these developments implies an urgent need for a thorough understanding of the dynamics of food production including the available infrastructure, trained human resources, cultural factors as well as food needs and wants. Quality assurance activities should not only depend on government's regulatory machinery, but also on self regulation by industry groups, exporters and importers. Enough has been said on these principles. It is left to countries and industries to exercise adherence to ensure safety and quality. Assistance from the developed world and international organizations to train resources in developing countries on the standard safety procedures is urgently needed. Government industry partnership and mutual recognition (of certification bodies) based on HACCP and designed after ISO guidelines are some of the means by which importing countries can monitor and address expanding global food supply. A network of these certification bodies worldwide should be considered. The basic provisions of GATT (General Agreement on Tariffs and Trade) on Sanitary and Phytosanitary Measures signed on 15 April 1994, when fully implemented, should be able to address these concerns.

(126) TRANSFORMATION OF BACTERIAL LUCIFERASE DNA INTO *ESCHERICHIA COLI* O157:H7 FOR USE AS A MARKER IN GROUND BEEF

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To monitor the activity of *E. coli* O157:H7 within a ground beef system, an appropriate marker is needed to distinguish it from the normal flora found in meat. The objective of this study was to determine if bacterial Luciferin DNA cloned into *E. coli* O157:H7 could be used as an accurate marker for this microorganism within a ground beef process. The plasmid $pyub_{261}$, which contains the Luciferin DNA, was transformed into *E. coli* DH 5 α. The luminescent activity was quantified in Relative Light Units (RLU's) on a luminometer. Transformation of the Luciferin DNA from DH 5 α into O157:H7 was accomplished by amplifying the plasmid with 20μg/ml chloramphenicol for isolation, purifying the DNA using a cesium-chloride-ethidium bromide gradient, and transforming competent cells by the calcium chloride method. Two replications of ground beef samples were inoculated with 1 ml of *E. coli* DH 5 α culture in the center of each 25g meatball for a target level of 50 CFU/g. Samples were serially diluted and plated on luria broth agar containing 50 mg/ml of kanamycin. The average luminescent reading for the samples was 121 RLU's +/- 9. The average plate count was 41 CFU/g +/- 14.6. Similar results were obtained with transformed *E. coli* O157:H7. Measurement of the luminescent activity takes only two hours compared to the traditional plating methods used for *E. coli* O157:H7 isolation, which can take up to 96 hours. Therefore, this marker would be more beneficial to monitor the presence of *E. coli* O157:H7 in a laboratory model of a ground beef processing environment.

(127) A NEW RAPID METHOD FOR THE DETECTION OF *E. COLI* O157:H7 IN RAW MEAT

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A new method, the EZ COLI Rapid Detection System, consisting of a selective enrichment medium with a dye indicator and a rapid detection kit, allows for enrichment and presumptive identification of

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E. coli O157:H7 in ≤ 24 hours. The detection kit is based upon an immunological microchannel assay constructed within a standard pipette tip. One to 12 tips can be tested simultaneously in less than 30 minutes. A total of 97 raw meat samples including beef, pork, turkey and chicken were spiked with low numbers of organisms (10-100 CFU/25 g of meat) and were tested using the EZ COLI Rapid Detection System. *E. coli* O157 (42 strains), *E. coli* non-O157 (12 strains) and other organisms (11 strains) were tested. After a minimum 15 hour incubation, all 42 strains of *E. coli* O157 changed the color of the broth and were positive with the detector kit. All 12 strains of *E. coli* non-O157 and a few closely related organisms (i.e., *Citrobacter*, *Klebsiella* and *Hafnia*) changed the color of the broth, however were negative with the detector kit. Organisms cited in the literature as cross-reacting with commercial O157 antisera (i.e., *Salmonella* 0:N and *Yersinia* 0:9) were also tested in the system. Although the *Salmonella* did not change the medium color indicator, the two strains tested were positive in the detector. The *Yersinia* did not grow in the selective medium and, as a result, was not tested with the detector. These preliminary results indicate that the EZ COLI Rapid Detection System provides a rapid and specific means of detecting *E. coli* O157 in raw meat samples.

(128) DETECTION OF *ESCHERICHIA COLI* O157:H7 IN FOODS BY MULTIPLEX PCR

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A sensitive and specific multiplex polymerase chain reaction (PCR) assay was developed for detection of Shiga-like toxin-producing *E. coli* O157 in ground beef and raw milk. DNA primers specific for the enterohemorrhagic *E. coli* (EHEC) *eaeA* gene, conserved sequences of Shiga-like toxins I and II and for the large plasmid (ca. 60 MDa) of EHEC were employed in the multiplex PCR to amplify the DNA targets simultaneously in one reaction. Ground beef and raw milk samples were inoculated with *E. coli* O157:H7 and incubated at 37°C for various time periods in modified EC broth containing novobiocin. After incubation, aliquots of the cultures were removed for DNA extraction followed by multiplex PCR. Initial sample inocula of < 2 CFU/g for ground beef and <1 CFU/ml for raw milk could be detected in the PCR. The multiplex PCR can be used for specific detection of EHEC serotype O157 in foods.

(129) EVALUATION OF A RAPID SCREENING KIT FOR THE DETECTION OF *ESCHERICHIA COLI* O157 IN FOODS

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The TECRA *E. coli* O157 Visual Immunoassay (VIA) is an enzyme-linked immunosorbent assay (ELISA) for the detection of *E. coli* O157 in food, following an overnight enrichment step. Both motile and non-motile strains of *E. coli* O157 are detected, within 20 hours. In the first part of the study, ten foods, including raw meats and dairy products, were artificially inoculated with various strains of *E. coli* O157 including H7 positive and H negative serotypes. Two inoculation levels were used: low level (1-5 cells/25 g) and high level (10-50 cells/25 g), with five replicates of each. In addition, each food was tested uninoculated, in triplicate. The TECRA VIA was compared to the USDA-recommended enrichment methods for raw meats and to published enrichment and plating methods for dairy products. In the second part of the study, over two hundred dairy products purchased from local supermarkets were screened for *E. coli* O157 using the TECRA VIA. The dairy products were selected as those which would not undergo further heat treatment prior to consumption, as these are considered to be most at risk of contamination with this organism. None of the samples was found to contain *E. coli* O157 using this method. Results showed the TECRA *E. coli* O157 immunoassay to be simple to use, fast, and appears to be sensitive and specific.

(130) COMPARISON OF FIVE MEDIA FOR ENUMERATION OF *ESCHERICHIA COLI* O157:H7

Alicia Orta-Ramirez,* Graduate Student, J. F. Price, and J. S. Cherry-Merritt, Dept. of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824-1224

Five media Petrifilm™ Coliform Plate Counts (CPC), Petrifilm™ *E. coli* Plate Counts (EPC), McConkey-Plate Count agar (50:50) (MPC), Phenol Red Sorbitol agar supplemented with 1% Pyruvate (PRSP), and McConkey Sorbitol agar (MCS), were compared in two studies for their ability to enumerate *Escherichia coli* O157:H7 (EC O157:H7). Both 58°C-heat-shocked (HS) and nonheat-shocked (NHS) cultures were used. In the first study, CPC, EPC and MPC (pour plate method) were inoculated in triplicate for each culture. Mean log CFU/mL were 8.9 and 4.7 (CPC), 8.9 and 4.7 (ECP), and 8.8 and 4.6 (MCP), for NHS and HS cultures, respectively. No significant differences were found among the three media for both HS and NHS cultures. In the second study, CPC, PRSP and MCS (pour plate method) were inoculated in triplicate for each culture. Mean log CFU/mL were 9.1 and 4.3 (CPC), 9.0 and 4.9 (PRSP), and 8.8 and 4.3 (MCS) for NHS and HS cultures, respectively. There were no significant differences in log CFU/mL between CPC and PRSP for the NHS culture and both had higher mean counts than MCS. For the HS culture PRSP had higher mean counts than CPC and MCS. Results suggest that PRSP was more effective for enumeration of both HS and NHS EC O157:H7 cells than any of the other media tested.

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(131) E*COLITE, THE NEW STANDARD IN MONITORING COLIFORMS AND *ESCHERICHIA COLI* CONTAMINATION IN WATER

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A one step method has been developed for rapid enumeration of coliforms and *E. coli* at the level of 1 to 100 bacteria per 100 ml. The procedure uses selective inducers and substrates for specific detection of: 1) coliforms due to activity of beta-galactosidase (color); and 2) *E. coli* due to additional activity of beta-glucuronidase (luminescence). Lauryl sulphate is used to limit growth of gram positive bacteria. Bacteria belonging to *Escherichia*, *Enterobacter*, *Klebsiella* and *Citrobacter* all were detected with better than 95% confidence. In an independent study it was reported that over 60 strains of coliforms were detected with 100% efficiency. Of those 60 strains, 38 were identified as *Escherichia coli* with 95% efficiency (38/40). *Hafnia*, *Serratia* and *Shigella* spps. were also detected with lower efficiency (50-80%). Close to 200 other strains of gram negative bacteria were not detected.

The incubation is carried out for 16 hours. Using a rich media allows for faster growth and is less affected by disinfectants or oxidizers that are commonly applied in water treatment. Identification is easily accomplished by color change and luminescence measurement.

The test can be used for monitoring drinking water, processing water or surface water. Also, the test is being evaluated as an economical first step broad screening method for pathogenic *E. coli* in food. The final identification is accomplished by more elaborate methods such as immunological or DNA probes. The media also supports good growth of *Shigella* and *Salmonella* species which could be detected by turbidity in the microwell and further identified with specific tests for these species.

(132) EVALUATION OF UNIVERSAL PREENRICHMENT VERSUS LACTOSE BROTH PLUS VARIOUS PLATING MEDIA FOR ISOLATING SALMONELLAE FROM NATURALLY CONTAMINATED FRESH CHICKEN AND PORK SAUSAGE

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Two preenrichment broths, Universal (UP) and Lactose (LB), two selective enrichment broths, Tetrathionate Brilliant Green (TBG) and Selenite Cystine (SC), and 5 plating media: Xylose Lysine Desoxycholate with 5 mg/liter of novobiocin (XLD-N), Bismuth Sulfite (BIS), Hektoen Enteric with 80 mg/liter of novobiocin (HE-N), Double Modified Lysine Iron Agar (DMLIA), and Brilliant Green Sulfa with 15 mg/liter of novobiocin (BGS-N), were evaluated to determine their efficacies for the recovery of salmonellae from 200 samples of naturally contaminated fresh chicken and pork sausage. UP has been investigated by Bailey and Cox for the simultaneous recovery of *Listeria* and *Salmonella* in food products to minimize the amount of sample, time and media needed as compared to using separate preenrichment broths. In this study, salmonellae were detected in significantly ($P < 0.05$) fewer chicken and sausage samples after preenrichment for 24 hours at 35°C in UP compared to preenrichment in LB. Following current USDA procedures for the recovery of Salmonellae, samples were enriched in both TBG for 24 hours at 42°C and SC for 24 hours at 35°C. Comparing the two enrichment media, significantly ($P < 0.05$) more samples were positive for *Salmonella* when enriched in TBG. The plating medium DMLIA has been recommended by the USDA as an alternative to XLD. Comparing the five plating media, salmonellae were most frequently detected using XLD-N, HE-N, and BGS-N, whereas DMLIA and BIS showed the lowest recovery rates. Of the methods tested, the best procedure for the recovery of salmonellae in fresh chicken and pork sausage includes preenriching in lactose broth, enriching in TBG at 42°C and streaking onto XLD-N, HE-N and BGS-N plates.

(133) DETECTION OF *SALMONELLA* IN FOODS BY TRANSDUCTION OF ICE NUCLEATION GENES

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The Bacterial Ice Nucleation Detection (BIND) assay, which detects *Salmonella* in foods in less than 1 day, is based on transduction of bacterial ice nucleation genes by recombinant phage. Detection limits as low as 200 CFU/ml, low background signal, and insensitivity to non-*Salmonella* enteric bacteria are seen with real food samples. Assays utilize 1 ml subsamples of ISO 6579:1990 (Buffered Peptone Water) non-selective preenrichments. Detection is signaled by freezing of 50 µl samples in microtiter plate wells that also contain the "freezing indicator" dye carboxyfluorescein. The method requires no wash steps. A preliminary validation of BIND (5 meat and 7 non-meat foods prespiked with low and high levels of 20 *Salmonella* serotypes) agreed completely with the ISO 6579:1990 microbiological reference method. In conclusion, the BIND method is capable of providing accurate detection of *Salmonella* in foods in less than 24 hours.

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(134) EVALUATION OF AN AUTOMATED ASSAY FOR THE DETECTION OF *L. MONOCYTOGENES* IN FOOD PRODUCTS

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An automated assay for the detection of *L. monocytogenes* in food products has been developed for the VIDAS® system. The test is an enzyme linked immunofluorescent assay (ELFA). Performance of the assay was evaluated in a series of studies with pure cultures and food products. For 27 *L. monocytogenes* isolates tested, the limit of detection was 10^6 cells per ml. Forty-seven non-*monocytogenes Listeria* species and 37 non-*Listeria* species were tested in the cross reactivity study. None of these isolates were positive. In the food study 30 naturally contaminated food products were tested in triplicate by standard methods and the ELFA method. Twenty-three sample replicates were positive with at least one method, and agreement between methods was 87%. Eleven replicates were negative with standard methods and positive with the automated assay. Five of these 11 replicates were confirmed positive with subsequent culture of the ELFA enrichment broths. One sample was negative with the ELFA method and positive with standard methods.

(135) A NEW RAPID METHOD FOR DETECTION AND ENUMERATION OF *LISTERIA MONOCYTOGENES* IN FOOD SAMPLES

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Levels of *Listeria monocytogenes* in broth and foods were determined using a new instrument for rapid detection and enumeration of microorganisms. The instrument (BioSys-128®) is based on optical detection of metabolic processes during microbial growth. Changes in color of indicators are recorded at short time intervals by an optical sensor and are expressed as optical units. Measurements of these changes are conduced in a zone separated from the sample but in equilibrium with it, and hence are not masked by debris in the samples. Several pH (e.g., bromcresol purple) and redox (e.g., resazurin) indicators were tested for the examination of *L. monocytogenes* strains in non-selective media. Selective enrichment media containing the indicators were employed for the detection of Listeriae, the presence of *Salmonella typhimurium* and *Escherichia coli*, and in naturally contaminated milk, cheese and meat samples. The measured detection times were inversely proportional to the logarithm of the initial Listeriae numbers in the samples. Presence of *L. monocytogenes* in artificially contaminated pork sausage and effect of antimicrobials on the detection time correlated with plate counts.

(136) GENOMIC FINGERPRINTING OF *BIFIDOBACTERIUM* SPP. FROM AN INFANT

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Bifidobacterium comprise the dominant flora of portions of the human intestine and several have found applications in foods. To screen for additional isolates with food application potential, a newborn was screened for bifidobacteria from birth to 6 months. Of 104 samples tested, 83 were presumptive positive for *Bifidobacterium* by morphology on a selective medium. Contour clamped homogenous electric fields (CHEF) electrophoresis analysis of a single colony from each of 43 of the 83 positive samples identified 8 and 12 restriction endonuclease digestion profiles (REDP) with *DraI* and *XbaI*, respectively. The REDP of isolates from the infant were identical to the REDP of several isolates from the mother (birth-6 mo.) and select isolates from a sibling (1-6 mo.), but not isolates from the father. The data indicated the infant was inoculated with bifidobacteria from the mother during delivery. Also, the REDP of several isolates was similar to the REDP of well-characterized isolates of *Bifidobacterium*, while others displayed unique REDP. Studies are underway to further chronicle the diversity and succession of all strains via CHEF and to screen these strains and others recovered for desirable traits to improve food quality and safety.

(137) OPTIMIZATION OF POLYMERASE CHAIN REACTION PARAMETERS UTILIZING AN EXPERIMENTAL DESIGN APPROACH

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The sensitivity and specificity of a PCR assay is a function of numerous reaction variables encompassing concentrations of reagents as well as those parameters associated with temperature cycling. Optimization procedures traditionally involve varying a single parameter while holding others constant and repeating the process for all variables. The number of experimental runs required for this approach increases exponentially with the number of system variables. Therefore, it would be desirable to have a method which provides virtually equivalent information with a

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significant reduction in experimentation. Experimental design techniques provide such a method and have been used to optimize a PCR assay. Essentially, a screening design based on multiple-linear regression was used to identify the most significant parameters. This subset was then further analyzed by performing additional runs and fitting the data to a quadratic model containing linear, interaction, and squared components. The nonlinear terms made possible the generation of response surfaces sufficient to optimize the sensitivity and selectivity of a *Salmonella* detection assay. There were 12 initial variables which were screened down to the following set: units of enzyme, temperature and time of the annealing/elongation step. Optimization was then based on the response surfaces derived from data using this set. HPLC was used to separate and quantitate the PCR products. This study clearly demonstrated a methodical and pragmatic approach of investigating and optimizing multi-variable systems through the use of experimental design techniques.

(138) ANTIBIOTICS AND SULFONAMIDES IN MEAT SAMPLES DESTINED TO HUMAN CONSUMPTION

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Antibiotics and sulfonamides are administered to livestock both as a veterinary treatment and as a feed additive for prophylactic purposes. Residual antibiotics in meat are undesirable. The possibility of bacterial resistance and physical persistence of residues are the two major concerns surrounding the controversy of drug use in animals. The Mexican Government has proposed Official Norms in order to limit the risk of contaminants for the consumer. Our laboratory analyzed meat samples of livestock and poultry for export and domestic market. The samples were analyzed for antibiotics and sulfonamides using a biological receptor assay (Charm Test II). The survey included 41 samples of beef, 43 of swine and 12 for poultry, collected during 1993 and 1994. In addition, a system for data quality control was developed to confirm the results. Sulfonamides were the most frequently detected residues, followed by streptomycin, tetracycline, chloramphenicol and penicillin. Erythromycin was not detected in any sample. The data did not show violative levels for any of the antibiotics or sulfonamides detected and those found are not considered potentially hazardous for public health.

(139) BIODEGRADATION OF AFLATOXINS BY *FLAVOBACTERIUM AURANTIACUM* IN CULTURE MEDIA

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Aflatoxins are undesirable in food products because of their toxic, carcinogenic, mutagenic, and teratogenic properties. Degradation of aflatoxins by *Flavobacterium aurantiacum* (*Exiguobacterium aurantiacum*) was studied. Aflatoxins were spiked (4 ppm) in Tryptic Soy Broth (TSB) inoculated with a ca. 10^5 CFU/ml of the test culture. Residual toxin concentrations and microbial counts were evaluated at 0, 6, 12, 24, 48, and 72 h. Residual toxins from media extracts were quantified by high performance liquid chromatography (HPLC). Aflatoxin B_1 concentration decreased by 69.5% by the end of 72 h at 32°C. Aflatoxin G_1 concentration was reduced by 99% in 48 h. Simultaneous spiking of B_1 and G_1 indicated that the organism preferentially removes G_1 in the presence of B_1. By the end of 48 h, the concentration of G_1 decreased by 92% while aflatoxin B_1 only decreased by 26%. Aflatoxin B_2, which was initially present as a contaminant, showed an increase in concentration by 50%. This could imply biotransformation of B_1 to B_2. Enhancement of toxin degrading ability of the bacterium was seen after preenriching it with broth containing aflatoxin B_1 (4 ppm) for 48 h at 32°C. Residual toxin levels were 4% and 73% in broth inoculated with induced and non-induced bacterial cultures, respectively, after 72 h at 32°C.

(140) LIGHTNING™: INTRODUCTION OF A MACHINE-SIDE RAPID HYGIENE MONITORING SYSTEM

Elizabeth E. Ehrenfeld,* Ph.D., Research Scientist, S. A. Miller, K. L. Barber, and C. R. Carpenter, IDEXX Labs, One IDEXX Drive; Westbrook, ME 04092

Food plant hygiene has classically been monitored by swabbing production lines and running total plate count assays to determine the extent of microbial contamination. This is a long (2-5 days for results) procedure that does not allow for real-time decisions on whether a site is clean or dirty. In contrast, use of ATP-bioluminescence to assess hygiene allows instantaneous evaluation. In this system the amount of biological residue (food material as well as microbial contamination) present on a given surface is quantitated by measuring the amount of ATP using the firefly enzyme luciferase.

This poster presents data collected with a new highly sensitive hand-held luminometer developed for both field and laboratory use. All reagents are premeasured in a disposable device which is swabbed over a surface, activated by squeezing, and then inserted into the luminometer. Correlations to level of cleanliness in field studies (measured by standard plate count) and the level of sensitivity measured in moles of ATP are shown. In addition, the test's insensitivity to inactivation by a number of substances normally present in a food processing environment (sanitizers, low and high pH foods) is demonstrated.

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(141) EVALUATION OF MICROBIAL SWABS FOR RELEASING HCMC AND THEIR VIABILITY ON ICE USING 3M™ PETRIFILM™

Custy F. Fernandes,* G. J. Flick Jr., J. L. Silva, T. A. McCaskey and A. F. Hood, Department of Food Science & Technology, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061

Both low and high levels of heterogeneous catfish microbial cells (HCMC) were deposited on commonly used microbial swabs (viz, Ca-Alginate {CA}, Cotton {CO}, Dacron {DA} and Rayon {RA}). The efficacy of releasing the deposited HCMC and viability of released HCMC were compared. The HCMC was rinsed from fresh catfish fillets with Butterfield's phosphate buffer (BPB) pH 7.2. The HCMC cells were stored at -80°C in dimethyl sulfoxide (5% v/v) and used as an inoculum source. Microbial swabs were aseptically inoculated with 100 µL of the HCMC. Three microbial swabs (CO, DA and RA) were suspended in 9.9 ml of BPB pH 7.2 to release the cells. The HCMC from CA swab was released in 9.9 ml BPB containing sodium citrate (1.0% w/v & pH 7.2). A 100 µL inoculum of the HCMC was added to 9.9 ml of BPB and used as the control. The HCMC was deposited at low (10^3) and high (10^5) levels on the microbial swabs. Following deposition and release of bacterial cells the five treatments for each load level were held on ice (0.1°C) and microbial analyses were performed after 5.0 min. The experiments were repeated 3 times using 3M™ Petrifilm™ Aerobic and Total Coliform Count plates in 0.1% peptone water (PW) as diluant. For the low deposition level of HCMC, the aerobic count was enumerated at 25°C following 48 h of incubation. For the high level, both aerobic and total coliform (incubation at 35°C for 24-48 h) counts were determined. Additionally, the viability of the released HCMC was evaluated following their release from two microbial swabs (DA and RA). The HCMC at low and high levels was released in either PW or BPB and held on ice. The aerobic count was enumerated at low level and aerobic and total coliform counts were enumerated at the high level after 0, 4, 8 and 24 h. There was no significant difference between the HCMC released from the four swabs and the control in the aerobic plate count at the low level as well as the aerobic and total coliform counts at the high level of deposited cells. There was no significant difference in aerobic plate count released among the four microbial swabs (CA, CO, DA and RA) for the aerobic plate count at the low level as well as the aerobic and total coliform counts released at the high level. There was no significant difference in the viability of aerobic plate counts at the low level as well as aerobic and total coliform counts at the high level released in either PW or BPB from the two microbial swabs (DA and RA) and held on ice during the 24 h incubation study.

(142) THE USE OF A SINGLE TABLET FOR DELIVERY OF CRITICAL REAGENTS TO A POLYMERASE CHAIN REACTION

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The polymerase chain reaction (PCR) is a technique used for the amplification of a specific target DNA. To successfully perform PCR requires pipetting small volumes of critical reagents including specific primers and DNA polymerase. To minimize the inherent reaction to reaction variability associated with pipetting small volumes, a PCR reagent tablet has been developed for use in a *Salmonella* detection assay. The tablet has been formulated to delivery the correct quantity of primer, deoxynucleotides and DNA polymerase to perform a 50 µl amplification reaction. The tablet is hydrated directly with bacterial lysate. The tablets are produced using spray-freezing technology, followed by freeze-drying and tableting. The tablets have an exceptionally high content uniformity (A_{260} % C.V. = 2.3), fast dissolution times (< 30 seconds) and an extremely low moisture content (< 1%). The PCR reagent tablet has been shown to have a sensitivity of 10 CFUs/reaction and a shelf-life of at least 12 months when stored at 4°C. The development of this tablet has greatly improved the reproducibility and the ease of performing PCR. Further development of this assay system may lead to a user friendly PCR based method for the detection of *Salmonella*.

(143) DETERMINATION OF IN MUSCLE, LIVER AND KIDNEY FROM PORK PRODUCED IN SONORA, MEXICO

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Pork is one of the major meat sources in the human diet. Considerable information is available about its nutritional characteristics. The presence of toxic residues in food has recently caused public concern. This includes residues that may accumulate in specific organs, like heavy metal and cause severe damage. The purpose of this work was to provide information about the concentration of metals (Cd, Pb, Cu, As, Hg) in meat produced by the Sonoran pork industry. Ninety-nine samples were obtained from local wholesale meat markets. The concentration of the elements was determined by atomic absorption spectrometry after microwave digestion. Analytical accuracy was confirmed by standard addition. Mercury was not detected in any sample, all other analyzed metals were found under the maximum permitted values specified by the Regulatory Agencies of Mexico and the United States. The pork meat produced in Sonora has a good quality and does not represent any risk to human health.

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(144) CHEMICAL AND MINERAL ANALYSIS OF SURIMI-BASED SEAFOOD PRODUCTS

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Twenty brands of surimi-based products including fish balls, shrimp balls, squid balls, fish cakes, kamaboko, and imitation crabmeats were collected from domestic Oriental markets and analyzed for proximate composition, 5 macro (Ca, Mg, P, K, and Na) and 14 micro elements (Zn, Cu, Fe, Si, Co, Cr, Mn, Al, Mo, Ni, Pb, Sr, Ba, and B). Proximate composition and mineral content of samples were varied. Protein ranged from 8.59% (fish cake) to 12.62% (fish ball). Fat ranged from 0.03% (fish cake) to 4.84% (cuttlefish ball). Carbohydrates ranged from 7.29% (cuttlefish ball) to 29.42% (kamaboko). Sodium ranged from 316.61 mg/100 g (fish cake) to 994.34 mg/100 g (fish ball). However, kamaboko had high levels of Al (15.05-26.25 ppm) and B (2.87-8.11ppm). Fish ball made from milk fish had high levels of Ni (2.32 ppm), Pb (2.35 ppm), Ba (3.72 ppm), Cu (4.16 ppm), Zn (7.23 ppm), Co (1.50 ppm) and Sr (5.02 ppm).

(145) COMPARISON OF ISO-GRID™, DRBC, PETRIFILM™, AND PDA POUR PLATE METHODS FOR ENUMERATING YEASTS AND MOLDS ON SHREDDED CHEESE

Steven C. Ingham,* Food Safety Extension Specialist, and Jee-Hoon Ryu, University of Wisconsin-Madison, Dept. of Food Science, 1605 Linden Drive, Madison, WI 53706-1565

This study compared three alternative methods with the traditional Potato Dextrose agar + chlortetracycline pour plate (PDA) method for enumerating yeasts and molds in 35 shredded cheese samples stored at 7°C or 25°C. The ISO-GRID™ hydrophobic grid membrane filtration uses YM-11agar medium, can sample the equivalent of three sample dilutions per plate, inhibits spreading of mold colonies, and requires only a two day incubation as compared to the five day incubation used in the PDA method. The Dichloran Rose-Bengal Chloramphenicol (DRBC) spread plate method is reportedly advantageous because the medium inhibits overgrowth by mold colonies. The Petrifilm™ method is convenient because no medium preparation is necessary. Results of the ISO-GRID™HGMF, DRBC, Petrifilm™, and PDA methods were all highly correlated ($r^2 \geq 0.96$). None of the alternative methods was significantly different ($p < 0.05$, student t test) from the PDA method or from the other alternative methods. The results of this study show that price, speed, and convenience can be primary considerations in choosing among these methods for enumerating yeasts and molds in shredded cheeses.

(146) USE OF BLUE LAKE AS AN INDICATOR OF BACTERIAL PENETRATION INTO EGGS

Jeong-Weon Kim,* Research Associate, Michael F. Slavik and Joel T. Walker, Dept. of Poultry Science, Univ. of Arkansas, Fayetteville, AR 72701

Blue Lake, a water-insoluble dye was evaluated as an indicator of bacterial penetration into eggs. Because the diameter of Blue Lake particle (~0.6 µm) is slightly smaller than the sizes of most bacteria and its blue color allows easy visualization, Blue Lake has been used as an excellent simulator of bacteria in meat studies. In this study, various groups of eggs (fresh-laid; commercial; water-washed) were dipped in 0.5% (w/v) Blue Lake in 1% Triton X-100 solution for 2 min and incubated at room temperature up to 24 h. Penetration was detected by counting the blue dots on shell membranes after breaking the eggs. Fresh-laid eggs did not allow any penetration up to 24 h. Commercial eggs allowed the easiest penetration where 100% eggs showed blue dots (> 300/egg) even at 2 min incubation. Water-washed eggs allowed much less penetration than commercial eggs in which the number of blue dots were < 10/egg at 2 min and 20-80 at 20 min. Above results matched very well with the results of microbiological test where eggs were inoculated with *Salmonella enteritidis* and cells were recovered by conventional method, and also with the morphological study of eggshell surfaces using electron microscopy where fresh-laid eggs had intact cuticle layers, but commercial eggs did not.

(147) RAPID ESTIMATION OF RAW MILK QUALITY

W.M. Lachowsky,* Scientist, M. Griffiths, L. Harris, J. Odumeru and L. Szijarto, O.M. A.F.R.A., A.F.L.S.C., 95 Stone Rd. W., Guelph, Ont. N1H 8J7

Bulk tank milk samples were analyzed for bacterial levels, colony forming units (CFU/ml), using pour/spread plates under different incubation time/temperature conditions. Samples were incubated at 32°C/48 h; 30°C/72 h; 21°C/25 h; 7°C/7 days. These samples were then analyzed using two rapid techniques: Biotrace (ATP) and Malthus (30°C). There was a high degree of correlation between 32°C/48 h and 30°C/72 h ($R^2 = 0.99$). There was also a high degree of correlation between the 21°C/25 h. and the 7°C/7 d ($R^2 = 0.82$). The Biotrace had a higher degree of agreement (kappa = 0.49) than the Malthus (kappa = 0.33), for samples with bacterial count < log 5.00 CFU/ml.

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(148) EVALUATION OF MINIATURIZED MICROBIAL INHIBITION ASSAY FOR SCREENING OF ANTIMICROBIAL RESIDUES IN ANIMAL TISSUES

Mark Mitchell,* Antimicrobial Residue Screening Scientist, Jerzy Samoluk and Arlene Yee, Ontario Ministry of Agriculture, Food and Rural Affairs, Agricultural and Food Laboratory Services Branch, Agriculture and Food Laboratory Services Centre, P.O. Box 3650, 95 Stone Rd. W., Guelph, Ontario, Canada N1H 8J7

Residues of veterinary drugs in milk and meat marketed for human consumption are unacceptable. Residues are of concern due to their possible adverse effects on people allergic to antibiotics, the potential increase of antibiotic-resistant pathogens in humans, and their effects on food processing such as inhibition of starter cultures. Current screening assays employed for regulatory purposes employ inhibition of growth of an indicator organism for detection of residues. Indicator organisms used in the Ontario provincial meat residue monitoring program include both *Bacillus subtilis* and *Sarcina lutea*. Such tests have been shown to have poor sensitivities to certain classes of antibiotics, require 24 hours for a test result and are technically demanding. A test based on the Brilliant Black Reduction Test (BR test) was developed at our laboratory for the screening of raw milk samples using the indicator organism *Bacillus stearothermophilus*. This test has been adapted to a 96 well microtitre plate format and test results can be obtained after 4 hours incubation. Results can be read using a microtitre plate reader at 595 nm. Such a test could show promise for screening large numbers of tissue samples, prior to confirmatory testing. This study was initiated to evaluate the specificity and sensitivity of this test compared to the currently used meat test for detection of the most commonly used classes of antibiotics. Members of the beta-lactam, tetracycline, aminoglycoside, macrolide, quinolone and sulfonamide families were spiked at various concentrations in phosphate buffer and tested using both assay methods. Our new test detected all classes of antimicrobial drugs at levels 2-3 times lower than the currently used test method, in addition to detecting members of the sulfonamide class that the current test is insensitive to. This enhanced sensitivity meets or exceeds all currently published safe levels for residues in animal tissue in Canada. It would appear that this test will offer several advantages as a screening test. Such advantages include a higher sensitivity and broader specificity to various antibiotic classes, detection of sulfonamides, ease of automation and shorter incubation time. This test could be used as a prescreen at the abattoirs since it is simple to perform. Current work is ongoing at our lab in evaluating its performance in animal tissues.

(149) THE CHARM ALKALINE PHOSPHATASE TEST: RAPID BIOLUMINESCENCE METHOD FOR THE DETERMINATION OF ALKALINE PHOSPHATASE IN PASTEURIZED MILK AND OTHER DAIRY PRODUCTS - COLLABORATIVE STUDY

Gerard Ruth,* V.P. Marketing, and E. Zomer, Charm Sciences Inc., 36 Franklin St., Malden, MA 02148

An interlaboratory performance evaluation of the Charm Alkaline Phosphatase test was conducted in 14 labs. Three levels of positive references were tested. Six replicates gave a coefficient of variation (CV) of 12%. Participating laboratories ran six assays simultaneously without training. Test results indicated good ruggedness and repeatability of the method. Selectivity: Overall, 112 coded negatives were tested and all were reported as negative at the 3 screening levels of 0.1%, 0.05% and 0.025% raw milk. Results indicated selectivity of better than 95% confidence. Sensitivity: A probit analysis was performed using the pos./neg. results reported by the participating labs. At the 0.1% raw milk screening level, all positives containing 0.1% and 0.2% raw milk in all matrices were detected as positive (224/224). 65% (72/112) of the samples with 0.05% raw milk were detected as positive. Although noncalibrated equipment was used, by using a positive standard reference for control point determination, results can be normalized, and the detection level for positives with 95% confidence set, at any desired level, down to and including 0.01% raw milk. Limit of detection is approximately 0.005% raw milk.

Quantitation of results was done using a regression analysis. For example, 0.05% and 0.1% raw milk samples gave averages of 130 and 279 mU for all laboratories, with % CV of 12 and 14 (SD = 15 and 40), respectively.

For comparison, four participating laboratories used the Fluorophos method for unflavored milk, and two participating laboratories used the Fluorophos method for chocolate milk and heavy cream. Using the Fluorophos test with 350 mU as cut off for positive, samples fortified with 0.2% raw milk were all detected as positive, while samples with 0.1% raw milk were detected 65% as positive.

(150) CHARM CLOXACILLIN ANTIBODY PERFORMANCE VALIDATED FOR BULK TANK MILK

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The Charm II and Charm I/Cowside II cloxacillin antibody test was validated under C.V.M. data requirements for milk screening tests labeled for testing milk at the bulk tank/tanker truck for drug residues.

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Selectivity Sensitivity and Dose Response - In a blind study by an independent lab, selectivity was 0 positives of 60 negative samples and sensitivity was 30 positives of thirty 10 ppb cloxacillin. The dose response data (% positive at each concentration) is demonstrated in Table 1. Thirty replicates were run at each concentration.

TABLE 1. Sensitivity, Selectivity and Dose Response (% positive at each concentration) of cloxacillin antibody tests

Cloxacillin Concentration	No. of Samples	Percent Positive Charm I/Cowside II	Percent Positive Charm II
0	60	0	0
3	30	23	0
5	30	70	3
7	30	77	60
9	30	93	100
10	30	100	100

Interferences - Independent lab evaluation for somatic cell interference demonstrated none at the 1.1 million/ml level. Bacterial interferences, both G+ and G-, were evaluated with blind samples; positive and negative samples were successfully distinguished in samples containing as much as 1 million bacteria/ml. No interferences were found from 28 animal drugs at 100 ppb and no interferences were found from the other five betalactam drugs (at 100 ppb) used in lactating cattle. Incurred residue from label dosing of a cow demonstrated naturally incurred concentrations behave similarly to spiked concentrations. Cloxacillin was cleared from the animal by 96 hours. The test worked appropriately with frozen milk samples. All test results met the specifications of the C.V.M. requirements.

(151) DETECTION BY PCR OF *CAMPYLOBACTER JEJUNI* IN CONTAMINATED CHICKEN PRODUCTS

Dr. Debra K. Winters,* Awilda E. O'Leary, XiaoLi Wang and M. F. Slavik, University of Arkansas, Biomass Bldg., Fayetteville, AR 72701

C. jejuni is a major cause of human acute gastroenteritis. Specific PCR primers could be used for identification of this bacterium, thus aiding in the diagnosis of the disease. We have developed a PCR assay for detection of *C. jejuni* and are evaluating its use with three different methods of clarifying chicken washes. These procedures include culturing prior to PCR as well as a method using direct washes with no prior culturing. We detected *C. jejuni* in 14 out of 15 chickens using all three methods. The cells have been confirmed as such by microbiological methods and by Southern blots with an internal probe specific for this organism. Using the direct wash procedure, we obtained several products in addition to that corresponding to *C. jejuni*. We are currently trying to determine the origin of these products and to optimize the wash procedure.

(152) RAPID DETECTION AND BIOTYPING SCHEME FOR *CAMPYLOBACTER* STRAINS

Jorge B. Velazquez,*[2] Ana Jimenez,[2] Joaquin Rodriguez,[2] Benilda Chomon[3] and Tomas G. Villa[4], [2]Laboratory of Food Technology, Escuela Politecnica Superior, University of Santiago at Lugo; [1]Laboratory of Microbiology, Hospital Juan Canalejo, Coruna; [3]Hospital de Conxo, Santiago de Compostela and; [4]Department of Microbiology, University of Santiago, Spain

Biotyping of *Campylobacter* strains is sometimes complicated by the isolation of hippurate-negative *C. jejuni* or both nalidixic acid-resistant *C. jejuni* and *C. coli*. One hundred and two *Campylobacter* strains were isolated during a one-year period from 1561 patients from a rural region and affected by a food-borne enteric disease. Identification of the 102 strains as members of the genus *Campylobacter* was carried out by means of Gram stain, oxidase production, catalase production, latex agglutination and hippurate hydrolysis, allowing us the grouping of strains as: hippurate-positive *Campylobacter jejuni* (n = 90), *Campylobacter coli* (n = 10) and hippurate-negative *C. jejuni* (n = 2). Seventy-seven of the hippurate-positive and both hippurate-negative *C. jejuni* isolates were biotyped as *C. jejuni* subsp. *jejuni* biotype 1, nine as *C. jejuni* subsp. *jejuni* biotype 2 and four as *C. jejuni* subsp. *doylei* by using a commercial API-CAMPY system. Hippurate-hydrolysis was correlated to a simple scheme based on L-arginine arylamidase production, propionate assimilation and malate assimilation which yielded sensitivity and specificity values of 0.90 and 1.00, respectively. Positive and negative predictive values of 1.00 and 0.53 were achieved using this rapid biotyping scheme. Effective grouping of nalidixic acid-resistant *C. jejuni* and *C. coli* isolates (38.5% of the total) was also achieved. A rapid grouping of *C. jejuni* into biotypes could be achieved by considering both gamma glutamyl transferase production and nitrate reduction. The scheme presented in this work could be useful in the rapid identification and biotyping of food-borne *Campylobacter* strains.

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(153) EQUIVALENCY OF INSPECTION - PRACTICAL REALITIES IN THE REAL WORLD

Ian Kirk, Associate Director, Policy Development and Organization, Meat & Poultry Products, 59 Camelot Dr., Nepean, ON K1A 0Y9

Equivalency of Inspection has been a fundamental principle governing the trade in meat and meat products between countries for many years. It is a recognition of the fact that the means to provide a given result may differ from country to country due to geography, agricultural practices, prevalence of particular diseases and many other factors. The end result in terms of food product safety for both human and animal health, will be equivalent. In practical terms, however, the many factors that influence the recognition of equivalency can also impede trade, provide problems of possible non-tariff trade barriers and restrict innovative changes to inspection systems by an individual country. The signing of the NAFTA and GATT agreements are a step in the right direction but have not yet resolved some of the practical realities.

(154) THE EUROPEAN PERSPECTIVE ON MODERNIZING MEAT AND POULTRY INSPECTION

L. Pedroso,* Quality Director, J. Snyders and H. Shouwenburg, Fricarnes, SA E.N. 249 Km 14, 2725 Mem Martins - Portugal

Protection of public health has been considered one of the main objectives of ante- and post-mortem inspection. Meat inspection is basically directed towards controlling the end product, and very rarely utilized data from earlier stages. Its scientific validity, efficiency, and cost effectiveness are doubtful. With the growing concern among consumers about the safety of meat, approaches whereby the whole production chain are involved in the quality of the final product, and the international standardization of such procedures are of particular importance to provide better guarantees of quality and wholesomeness. Integrated quality control systems containing elements of HACCP (Hazard Analysis/Critical Control Point) and ISO (International Organization for Standardization) standards, as are being developed in Europe, can be important tools to improve farm production management, animal health and welfare, as well as modernizing meat inspection.

(155) HARMONIZATION OF REGULATION IN A GLOBAL ECONOMY

Jerome J. Kozak, MPH, International Dairy Foods Association, Washington, DC

All around the world, the dairy and food industries are positioning themselves to survive the competitive challenges of a global economy. At the same time, government agencies are struggling with the challenge of determining equivalency of health and sanitation requirements among trading partners. As a result, codes, ordinances, regulations, and inspectional systems will come under scrutiny to eliminate unnecessary trade barriers. How will equivalency be determined? What mechanisms will be used to determine the benchmarks? What role will Codex and IDF standards play in this process? One method may be to use HACCP as the yardstick by which equivalency is measured. The need to establish common ground in order to achieve international harmonization is vitally important to the economic well-being of the dairy and food industries.

(156) USDA AGENDA FOR EMERGING FOODBORNE PATHOGENS

M. Taylor, USDA, Washington, DC

(157) FOOD CODE — A PRACTICAL APPROACH

Ernest H. Julian, Ph.D. Rhode Island Dept. of Health (401 277-2750)

On August 14, 1994, Rhode Island became the first state in the nation to adopt the 1993 Food and Drug Administration's Model Food Code. The major change made to this Code was to prohibit the sale of undercooked hamburgers to children 12 years of age and younger. In addition, the "Demonstrations of Knowledge" section of the Code was modified to reflect Rhode Island's mandatory food manager training and certification program. This presentation will address why the Code was adopted; the role of the Code in an effective enforcement program; how industry support was obtained; and experiences in implementation. Specifically addressed will be the implementation of the most controversial segments of the Code including the consumer advisory requirements; the demonstration of knowledge section; the required 41°F refrigeration; and the double handwash/nailbrush requirements.

(158) FOODSERVICE PLAN REVIEW STANDARDIZATION FOR EFFICIENCY

Frederick (Rick) Petersen, R.S., Program Supervisor, Stamford Health Department, Stamford, CT

Foodservice plan review is a basic requirement of many federal, state and local sanitation codes. The U.S. Public Health Service in the 1962 model foodservice code set the first national requirement for facility plan review. The FDA 1976, 1982, and 1993 model codes contain wording requiring the submission of plans and specifications before a foodservice facility is constructed, extensively remodeled or converted.

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Uniform procedures for plan review would benefit the regulatory community, the affected industry and the consumer. The Northeast Region Plan Review Development Committee (NRPRDC) in 1989 and the Conference for Food Protection in 1990 set out to design guidance documents for facility plan review. In June 1994 a HACCP based menu driven plan review guide was distributed to health agencies in all 50 states for review and field testing.

The complexity of plan and specification review calls for establishment of training and credentialing of plan reviewers. The Conference for Food Protection and the FDA State Training Branch have developed a model training course and guide for plan review personnel which will be trial tested at two training courses in April and Aug. 95.

It is far better to design problems out before new facilities are put into service. Inspection time should more readily be spent on the causes identified by the Centers for Disease Control that make people ill.

(159) INTEGRATED PEST MANAGEMENT (IPM) IN FOOD FACILITIES

Ronald D. Gardner, Senior Extension Associate, Cornell University, Pesticide Management Education Program, 5123 Comstock Hall, Ithaca, NY 14853-0901

The management of pests in food facilities (food manufacturing, processing, warehousing and service) requires a high degree of professionalism combined with experience and knowledge. IPM is a systematic process that balances the use of cultural, biological and chemical procedures to reduce pests to tolerable levels. It is a holistic approach dedicated to removing causes rather than just treating symptoms.

Lasting success can be accomplished only when the reasons for the infestation are controlled. There is no magic dust; no single, simple remedy to solve pest problems in a lasting way. Several interventions, when well coordinated, can usually be more effective than only one. Combined, they can have a synergistic effect. An IPM program will identify ways to prevent pest entry, deny them access to food, water and harborage, monitor the facility regularly, identify the pest and determine the best options for control. Cost effectiveness is another major consideration that knits together an IPM program and must be assessed on a long term, as well as a short term basis.

There are differences between a traditional pest management program and an IPM program. Traditional programs often rely heavily on routine insurance pesticide applications. IPM programs rely heavily on monitoring and prescribe the use of pesticides based on need. Routine pesticide applications made in any structural program, have at least two negative impacts. First, pesticides applied when pests are not present are usually wasted, while contributing a risk to employees, the consumer and the environment. Also, the constant application of pesticides is one factor leading to pesticide resistance, thus threatening the future success of the program.

As a result of growing concerns about health and environmental problems associated with pesticide, the industry is facing increased demands from many fronts. One of these effects is fewer pesticides for use in the production of raw food commodities, and for use in structural pest management programs. The government has also begun mandating the adoption of IPM in many areas. Could this happen to the food industry? What would be needed to comply with IPM requirement? These and other aspects of IPM in Food Facilities will be discussed in this presentation.

(160) EQUIPMENT CLEANING AND SANITATION

Carlton Parker, Ecolab Inc.

Proper cleaning and sanitizing of equipment requires more than soap and water. In the food service industry, proper cleaning and sanitizing of equipment is crucial in preventing cross-contamination and spread of foodborne illnesses. Because of this, an integrated approach to cleaning and sanitizing equipment is important for the safety of employees and patrons. This approach should include proper selection of cleaning products and procedures, tailored to the cleaning specifications of each individual piece of equipment and food contact surface. In determining the proper products and procedures, every food service worker should have an understanding of detergents and sanitizers. Surfaces may appear clean and may have had a sanitizer applied, but may not be hygienically clean. By demonstrating the Bio-Trace system on a surface that "appears clean," symposium attendees will better understand how the proper combination of products and procedures can ensure a surface is hygienically clean.

(161) OVERCOMING THE "ALL OR NOTHING APPROACH" TO HACCP IMPLEMENTATION AT THE RETAIL LEVEL

John A. Marcello, R. S., Manager of Technical Education, The Educational Foundation of the National Restaurant Association, 250 S. Wacker Drive, Suite 1400, Chicago, IL 60606-5834

While there appears to be consensus on the seven principles that serve as the foundation for HACCP-based food safety systems, there are many interpretations as to their "practical" applications, especially within the foodservice industry. HACCP is as much a thought process as it is a management system. It incorporates principles of total quality management and employee empowerment into the development of a food safety program. Unfortunately, increasing emphasis and resources are being directed toward apply-

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ing HACCP throughout the industry, rather than facilitating the thought process behind this "management system". Attempts to standardize the approach result in defining HACCP in terms of flow charts and recordkeeping rather than encouraging behavior that controls and monitors the critical factors that contribute to foodborne illness. Diversity within the industry; varieties in types of operations, menus, culture, language, food safety knowledge and resources interact synergistically to create a complex environment that will totally frustrate a standardized approach to HACCP. HACCP is an industry-driven management system which establishes "deliverable" food safety objectives and provides flexibility for meeting specific operational needs.

(162) FRESH PRODUCE PROCESSING — A GLOBAL INDUSTRY PERSPECTIVE

Karl E. Olson, Ph.D., Principal Food Scientist, Dole Food Co., NA, 2102 Commerce Dr., San Jose, CA 95131

Food service, restaurant and supermarket sales have created a growing demand for fresh-cut produce. Convenience, health awareness and emphasis on freshness have contributed to the rise of the fresh-cut produce business both in North America and abroad. Over a billion pounds of fresh-cut produce was marketed in 1990 and this figure has more than doubled in 1994. Similar trends for the fresh-cut produce market are evident in Europe. In the U.S., fresh-cut produce processors operate both on the national and regional level. Refrigeration and rapid transit during distribution are essential for delivering high-quality fresh product to the consumer. Although there are many advantages to marketing fresh-cut produce over bulk produce, there are also considerable demands on the processor to ensure overall product safety. Despite the need to continually respond to competitive pressures, demand for new products and regulatory concerns, the future is indeed bright for this segment of the produce industry.

(163) THE EFFECT OF FARM MANAGEMENT PRACTICES ON THE MICROBIAL CONDITION OF FRESH-CUT VEGETABLES

John Tamagni, Partner, European Vegetable Specialties Farms, Inc., President, Tamagni Farms South, Inc.

European Vegetable Specialties Farms (EVS), a grower/shipper of specialty vegetables for the fresh-cut industry with an emphasis on radicchio production, has developed a farming plan based on use of good manufacturing practices and HACCP principles throughout all growing and shipping operations. As a supplier to the fresh-cut industry, EVS Farms has made a company decision to adapt these principles for use in the field in order to ensure the safest product possible.

EVS Farms is one of few growers following a written "Field HACCP" program. Safe growing practices include careful crop site selection, monitoring irrigation water quality, judicious use and selective placement of fertilizers and organic supplements. Crops are rotated with attention paid to field and border cleanliness. Integrated pest management principles are followed, pesticides are used only as a last resort and all produce is laboratory screened for pesticide residues.

Good manufacturing practices during harvesting include training of crew members in hygiene, food safety, and use of rubber gloves and hairnets. Sanitizing the gloves and harvesting knives plus a wash down of the packing machines prior to starting work are standard procedures. Product contact with the ground is minimized by machine boxing and packaging materials are protected until ready for use. Boxed product is vacuum cooled, thus eliminating water use and possible cross contamination. Timely transfer of product to monitored cold storage establishes the first step in maintenance of the necessary cold temperature chain.

(164) FRESH PRODUCE PROCESSING — RETAIL INDUSTRY PERSPECTIVE

Robert F. Stovicek, Ph.D., Primus Laboratories, Inc.

During the past few decades the retail produce departments have been an American win-win success story. The department has been one of the grocer's most successful financially while meeting or exceeding the expectations of an ever more demanding American consumer. During the average month in 1975 the typical produce department carried 65 items. In 1992 average number of items falling between artichokes and zucchini had risen to 262-295. With better than 50% of the retailers offering over 300 items during the summer months. The recent growth of fresh cut produce (93% in 1993) is providing consumers with even greater dietary variations and efficiencies of time. As is often true of growth it does not come without its pains. Staff training, consumer education and new equipment demands are growing. Finding floor or shelf space for 300 items is difficult. Assuring that each of those items is stored at its optimum storage temperature is nearly impossible. Making an already onerous task more burdensome along comes fresh cut produce in modified atmosphere packages with recommended display periods exceeding the shelf life of raw products. What happens in those packages is the question. Microbial horror theories abound and the probabilities of a catastrophe run the gambit. In a business in which profits are measured at low percentages or fractions of a percent retrofitting

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retail refrigerator space at $1,000 per foot is carefully considered. The retailers must not only balance the safety of their customers but the twenty-twenty hindsight review of the courts, the press and regulatory authorities with the cold realities of a low margin ultra competitive industry.

(165) WHAT IS NEW IN MODIFIED ATMOSPHERE PACKAGING OF FRESH-CUT PACKAGED VEGETABLES?

Gurmail S. Mudahar, Ph.D., Research Manager Food Science, DNA Plant Tech. Corp., 6701 San Pablo Ave., Oakland, CA 94608

Fresh-cut fresh vegetable category is expected to increase over $1 billion/year in retail sales by 1998. High demand by health conscious consumer is the driving force for the explosive growth of this category. Since the vegetables are only minimally processed, high quality raw materials, proper post harvest handling and use of optimum packaging system are the key factors to extend shelf life of pre-cut vegetables. Optimum packaging system is designed in response to vegetable respiration pattern and other biochemical reactions that take place in vegetable tissues after packaging. Packaging film, bag size, bag headspace volume and initial oxygen in the bag are the key components of the packaging system. The latest in packaging system for fresh-cut vegetables will be discussed in this part of the symposium.

(166) PRESENCE AND PUBLIC HEALTH IMPLICATIONS OF FOODBORNE PATHOGENS ON FRESH-CUT PACKAGED VEGETABLES

J. M. Farber, Research Scientist, Microbiology Research Division, Food Directorate, Health Canada, Ottawa, ON K1A 0L2

Whole produce items have traditionally been considered as a safe group of products from a microbiological standpoint. With the further processing of these products by cutting, chopping or peeling and placement into a sealed environment, various microbial hazards can be introduced. These hazards can lead to the survival and/or growth of foodborne pathogens and possibly to serious foodborne illness, especially if the products are temperature-abused. Although mesophilic microorganisms initially predominate on these products, psychrotrophic bacteria will gradually be selected for, and are the major organisms of concern because of the refrigerated storage of the product. However, fresh-cut vegetables can serve as vehicles for almost any foodborne pathogenic microorganism, including viruses and parasites. General microbiological principles dealing with the fresh-cut industry will be presented, along with recent research dealing with the incidence, survival and/or growth of foodborne pathogens on these products. The major organisms of concern in fresh-cut products will be discussed, along with an overview of some of the foodborne outbreaks that have occurred. Microbiological challenges to be addressed in the future will also be examined.

(167) PRESENT AND EMERGING CONTROL MEASURES FOR FRESH-CUT PACKAGED VEGETABLES

Larry R. Beuchat, Center for Food Safety and Quality Enhancement, University of Georgia, Griffin, GA 30223-1797

The presence of several genera and species of bacteria on raw vegetables that are capable of causing human diseases is well documented. Numerous outbreaks of gastroenteritis have been confirmed or epidemiologically linked to the consumption of a wide range of fresh vegetables. The realization that some of these pathogens are capable of growing at refrigeration temperatures routinely used to handle vegetables from the point of harvest to consumption has triggered an increased interest in evaluating and developing methods to eliminate or at least control their growth on minimally-processed packaged vegetables.

Opportunities to control pathogens on fresh vegetables begin in the field and exist at numerous critical points in post-harvest handling systems. However, one of the most critical points in terms of controlling the presence and numbers of pathogens on vegetables at the point of consumption occurs just before packaging. Washing whole or cut vegetables in chlorinated water will reduce populations of microorganisms by 10- to 100-fold but should not be relied upon to eliminate bacterial pathogens. Innovative approaches being explored as alternatives for controlling the growth of pathogens on fresh vegetables include the use of lactic acid bacteria and their bacteriocins, washing with plant juices, e.g. carrot juice, known to have antibacterial properties, incorporation of antimicrobials into edible films and washing in solutions of trisodium phosphate. Control of pathogens on minimally-processed vegetables can also be greatly enhanced through the use of appropriately designed and administered Hazard Analysis Critical Control Point (HACCP) programs. The effectiveness of procedures for sanitizing fresh vegetables and the potential value of HACCP programs for reducing health risks associated with consumption of minimally-processed packaged vegetables will be reviewed.

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(168) RADURIZATION—THE PASTEURIZATION OF FOODS BY IONIZING RADIATION

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Radiation pasteurization has been described as a cold pasteurization process. Foods which have been pasteurized by low dose ionizing radiation do not differ significantly in physical appearance from non-pasteurized foods and, in the case of meats, still appear fresh. Because of this, low dose irradiation has been suggested as a pasteurization process for fresh meats, analogous to thermal pasteurization for dairy products. Most bacteria of public health concern, especially salmonellae and *Escherichia coli* O157:H7, are relatively sensitive to radiation. Processing doses of 1.5 to 3.0 kGy are sufficient to reduce the levels of these bacteria, as they naturally occur in foods, to levels which are below the limits of detection. In addition, since irradiation can penetrate packaging material, foods can be prepackaged and then irradiated, eliminating the potential for post processing contamination.

(169) HIGH PRESSURE PROCESSING AS AN INTERVENTION STRATEGY FOR FOOD SAFETY

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High hydrostatic pressure has recently gained attention in the U.S. as a possible method of food preservation. This process is well-established in some countries, with processing of fruit spreads and beverages by high hydrostatic pressure being practiced in Japan. The technology was first introduced by the Meidi-Ya company, producing jams, fruit yogurts, and other semi-solid foods since 1990. Some of the recent interest in this technology stems from the reported improvements in quality and decontamination effect in specific products subjected to high pressure after packaging. High pressure has been used to treat pork slurries inoculated with *Campylobacter jejuni*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Yersinia enterocolitica*, and others, with treatment at 3,000 to 6,000 atm (44.1 to 88.2 psi) resulting in total destruction of all vegetative cells (10^6 cells/g), rendering the product free of these organisms. In whole muscle foods such as chicken, treatment at 13.7 M Pa (1,987 psi) for 2 h has been shown to reduce the population by 95%. Studies in our laboratory show that a 6-log reduction in the number of *Salmonella* inoculated into whole-muscle pork was achieved after a 12 min treatment at 60,000 psi, with total elimination being achieved at 80,000 psi and higher. Sensory evaluation studies have revealed that, although differences between treated and untreated products were detected by panelists, whole muscle foods treated by high pressure were perceived as more tender than untreated controls. Thus, high pressure processing offers the benefit of reducing/eliminating microbial contaminants while not affecting, and sometimes improving, the quality of foods. The information presented here should help food processors to better ascertain how this technology can be used to improve food safety.

(170) CHEMICAL TREATMENTS FOR DECONTAMINATION OF POULTRY

Amy L. Waldroup, Professor, Dept. Poultry Sci., Univ. of Arkansas, Fayetteville, AR 72701

Intervention strategies aimed at control of human pathogens during poultry processing include the use of modified equipment, increased use of water, improved sanitation, and the use of antimicrobial treatments including hot water, phosphates, chlorine, chlorine dioxide, organic acids, ozone, and irradiation. Not all of these agents are approved for use on poultry and the efficacy of some has been questioned.

Even with the use of one or all of the numerous strategies mentioned, raw meat and poultry will still harbor microorganisms, some of which will be potentially dangerous to humans. Thus, raw food products, including meat and poultry, will always have to be handled with care. Thus, education of the consumer regarding safe food handling practices is undoubtedly the most vital element in controlling the incidence of food-related illness. In addition, education of the foodservice industry, the home consumer, and others who handle food is by far the most cost effective method for protecting the health of today's consumer.

(171) ELECTRICAL PROPERTIES OF FOODS AND THE APPLICATION OF HIGH VOLTAGE PULSED ELECTRIC FIELDS

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High voltage pulsed electric fields, as a potential nonthermal process, inactivates microbial cells. Intensive pulsed electric fields also introduce energy to foods as ohmic heating which is a function of electric field strength, pulse duration, and electrical conductivity of foods. Treating foods with high electrical conductivity requires reduced electric field strength or pulse duration to maintain a nonthermal process. Also, bacterial survival rate increases with increased electrical conductivity. Critical electric field increases with decreased pulse duration and increased electrical conductivity. Therefore, electrical properties of foods pose a practical challenge to the application of high voltage pulsed electric fields technology.

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At high conductivity, the charge of dipole water molecules are quickly discharged locally, thus the effect of narrow pulses disappear rapidly. Electrical conductivity was found to correlate linearly with ionic strength and fluid temperature. Dielectric constant was found a function of solid concentration. A charging and discharging model predicted the burst pulse repetition frequency such that microbial cells experience a prolonged pulse duration. High frequency (1 to 2 kHz) moderate electric field strength (20 to 25 kV/cm) monopolar pulsed electric fields were found effective in inactivating microorganisms and in reducing the energy level of operation. *E. coli* and *Salmonella dublin* were selected as indicator bacteria. Viability of bacteria before and after treatment was assayed by colony-counting method.

(172) OSCILLATING MAGNETIC FIELD STABILIZATION OF FOODS

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The increase in demand for foods with fresh-like and improved quality with little thermal degradation of nutrients or sensory properties is promoting development of nonthermal processing technologies for the preservation of food. Static and oscillating magnetic fields alter the orientation of biomolecules and biomembranes to a direction parallel or perpendicular to the applied magnetic field, resulting in inactivation and/or altered growth rates depending on microorganism susceptibility, and extension of the shelf-life of foods. The applied magnetic field intensity depends on electrical resistivity and thickness of the food. Oscillating magnetic field treatment of food in flexible film packages is an advantage; reproducible effectiveness and uniform inactivation of microorganisms requires additional research.

(173) PRODUCT DEVELOPMENT CONSIDERATIONS FOR OHMIC PROCESSING

Margaret (Peggy) Swearingen,* Scientist, and P. T. Zoltai, Land O'Lakes, 4001 Lexington Ave., Arden Hills, MN 55126

OHMIC heating occurs when electric current is passed through an electrically conducting food product. The passage of current generates heat which sterilizes the food. A continuous flow OHMIC system can process particulates up to one inch cubed. Particulates and carrier medium can be heated virtually simultaneously, over processing of the carrier medium and temperature graduates within the particulates is then avoided. Temperature gradients within the particulates and mechanical damage to the particulates are minimized. OHMIC product quality exceeds traditional processing methods due to higher retention of flavor, texture, color and nutrients.

Thorough consideration of product and process specific variables will result in the development of high quality OHMIC products. Key concerns include pre-process assessment and treatments, process variables, particulates, carrier medium, and raw material compatibility.

(174) AN UPDATE ON THE *CAMPYLOBACTER* FAMILY: *CAMPYLOBACTER, HELICOBACTER* AND *ARCOBACTER*

Robert B. Gravani,* Ph.D., Professor of Food Science, and K. B. Shapiro, Cornell University, Department of Food Science, 11 Stocking Hall, Ithaca, NY 14853

In the last several years, three microorganisms, *Campylobacter*, *Helicobacter* and *Arcobacter* have emerged as important causes of human illnesses. *Campylobacter* is commonly found in the intestinal tracts of birds, cattle, sheep, goats and swine and has been incriminated in several foodborne outbreaks and sporadic illnesses throughout the U.S. Members of the genus *Helicobacter* colonize the mucus layer overlying gastric epithelial cells and produce gastric pathologies in their hosts. *H. pylori* is recognized as the primary cause of gastritis, a major contributor to peptic ulcer disease, and a significant risk factor in gastric cancer. Two *Arcobacter* species, *A. butzleri* and *A. cryaerophilus*, have been associated with human disease. At this time, knowledge of the clinical importance of these two species of *Arcobacters* is very limited. The most frequent risk factor, to date, is exposure to contaminated water and sewage. An update and review of these three important microorganisms will be presented.

(175) THE MYCOBACTERIA GROUP *MYCOBACTERIUM TUBERCULOSIS* AND *M. PARATUBERCULOSIS* REVISITED

C. Anne Muckle,* DVM, Ph.D., A. M. Lammerding and R. J. Irwin, Food Safety Risk Assessment Unit, Agriculture & Agri-Food Canada, Health of Animals Laboratory, 110 Stone Rd., W., Guelph, ON Canada N1G 3W4

The re-emergence of *Mycobacterium tuberculosis* as a worldwide public health threat and the emergence of potentially pathogenic environmental mycobacteria as opportunistic human pathogens demand immediate action by health care workers and scientists. Are these also food safety issues? *M. tuberculosis* is transmitted by aerosol and can spread rapidly in certain populations. Do tuberculosis-infected food handlers pose a risk through contamination of food and equipment? *M. paratuberculosis*, a pathogen of ruminants, has been detected in tissues of some humans with Crohn's disease. This organism has been reported to survive standard milk pasteurization procedures and has been detected in retail milk samples in the United Kingdom. Are cattle a source of *M. paratuberculosis* infection for humans through the consumption of milk

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and meat? This paper reviews the status of the mycobacteria group as human pathogens and the information required to determine their significance as foodborne disease agents.

(176) NEW ISSUES IN FOOD AND ENVIRONMENTAL VIROLOGY

Dean O. Cliver, Professor, Food Research Institute, University of Wisconsin-Madison, 1925 Willow Dr., Madison, WI 53706

In terms of numbers of illnesses, viruses were the 5th (Norwalk), 6th (hepatitis A), and 10th (other viruses) leading causes of foodborne disease in the U.S. from 1983 to 1987, yet viral gastroenteritides do not appear in the new, 1988 to 1992 CDC compilation, despite major advances in diagnostic methods. Occurrence of foodborne hepatitis A continues, but might be reduced if a vaccine were licensed and administered to food handlers. Rotaviruses also cause foodborne disease, but relatively rarely. Hepatitis E causes water-associated outbreaks and would therefore be expected to be transmitted via food as well. Other viruses may be transmitted sporadically via food or water, but are seldom implicated in outbreaks. Because viruses are still extremely difficult to detect in foods and water, appropriate indicators of viral contamination (perhaps bacteriophages) are still being sought.

(177) FOOD AND WATERBORNE PARASITES IN THE 90'S

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Parasitic diseases, often considered as health problems only in developing countries, are being recognized with increasing frequency in the United States and other developed countries. People are likely to encounter them while engaging in the basics of everyday living, e.g. taking a drink of water, eating a good meal, changing a child's diaper, swimming in a public pool or recreating on an amusement park water slide. While the classic foodborne parasites, such as *Trichinella*, beef and fish tapeworms, and *Toxoplasma* still occur in the U.S., they are greatly overshadowed by two protozoa (*Giardia* and *Cryptosporidium*) that are often waterborne. The largest waterborne outbreak in to history of the United Sates was caused by *Cryptosporidium* in 1993 when it infected more than 430,000 people in Milwaukee, Wisconsin. Since then, *Cryptosporidium* has been found in 97% of lakes, streams and rivers tested. More importantly small numbers of *Cryptosporidium* have been found in tap water in 54% of communities who use these water sources. The Milwaukee outbreak and several others since then have raised many questions about the ability of existing water treatment methods to prevent transmission of *Cryptosporidium* and other chlorine resistant organisms. While newer technology for killing or removing *Giardia* and *Cryptosporidium* is becoming available and may be affordable for persons in large cities, few treatment options are available for people in small communities.

(178) WHAT'S NEW IN FOODBORNE DISEASE AROUND THE WORLD

Ewen C. D. Todd and Morris E. Potter

This presentation will highlight areas of concern for potential foodborne illness in North America and other countries. Some of these have been documented for years but have received little attention. Others appear to be new issues. Topics to be addressed include widespread *Salmonella* infections from vegetable products in North America; *E. coli* O157:H7 infections from salami in Australia and the United States and from pasteurized milk in Scotland; campylobacteriosis from farm workers eating sandwiches in Ontario; yeast and mold causing mild acute illnesses in Canada; new fish parasite in Quebec; the spread of *Vibrio cholerae* through plankton in topical countries; *Cyclospora* infections in Nepal and Peru; changes in climate causing seafood poisonings in New Zealand and elsewhere; tetrodotoxin intoxications from marine snails in Taiwan; and the global threat of toxic bluegreen algae.

(179) *ESCHERICHIA COLI* O157:H7 — A CURRENT REVIEW

John P. Schrade, Regional Foodservice Specialist, FDA, Brooklyn, NY

The appearance of *E. coli* O157:H7, a rare but particularly virulent strain of bacteria, continues to be prevalent following the multistate outbreak of foodborne illness in 1993 in the western United States. *E. coli* is the most prominent member of the fecal coliform group and is a natural inhabitant of the intestinal tract of humans and warm blooded animals. The Food and Drug Administration (FDA) considers the presence of *E. coli* in a food product as an indicator of direct fecal contamination. Currently, there are five recognized classes of enterovirulent *E. coli* (collectively referred to as the EEC group) that cause gastroenteritis in humans. They include Enterotoxigenic *E. coli*; Enteropathogenic *E. coli*; Enteroinvasive *E. coli*; Enteroaggregative *E. coli*; and Enterohemorrhagic *E. coli*. Enterohemorrhagic *E. coli* O157:H7 produces potent toxins that cause severe damage to the lining of the intestine and if left untreated can develop into Hemolytic Uremic Syndrome or Thrombotic Thrombocytopenic Purpura. The Centers for Disease Control (CDC) estimate that the annual death toll from *E. coli* O157:H7 could be as many as 500.

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According to data supplied by CDC's National Center for Infectious Diseases, O157:H7 infections have involved ground beef; hot dogs; apple cider; raw milk; person-to-person transmission; mayonnaise; and shredded cheese. Documentation and reports of outbreaks of disease caused by *E. coli* O157:H7 are not consistent throughout the United States but as more states adopt the reporting requirement, more cases are certain to be detected. FDA has reacted to the need for greater safety measures at the retail segment of the industry by raising the cooking temperatures of food as specified in the Food Code. USDA's Food Safety and Inspection Service has developed a Pathogen Reduction Program to strengthen efforts to keep harmful pathogens out of the food supply.

(180) BARE HAND CONTACT WITH FOOD, WHY ISN'T HANDWASHING GOOD ENOUGH?

John J. Guzewich, Food Protection Section, New York State Department of Health, Albany, NY

Many people define this issue as judging the merits of requiring food workers to wear gloves when they prepare food. The issue is better described as a realization that food workers do work while they are ill with diseases that can be transmitted through food; therefore, prohibiting bare hand contact with ready-to-eat food provides an additional barrier to preventing foodborne disease transmission. Tongs, spatulas, deli paper, napkins, etc., are examples of such barriers. The evolution of this approach in New York State will be described, including a review of the foodborne disease data, that justify the need for a new approach. Existing regulations attempt to mitigate or prevent food worker spread diseases by prohibiting ill workers from working, requiring workers to wash their hands, requiring that food be prepared with the least possible manual contact, requiring cold food to be held at temperatures that inhibit bacterial multiplication and requiring cooling and reheating to temperatures that destroy pathogens. Regulations that prohibit bare hand contact with ready-to-eat food are not the answer to the problem of food worker contamination, but they do provide another tool or barrier that can readily be monitored as a critical control point.

(181) MICROBIOLOGICAL CONCERNS WITH VACUUM PACKAGING

E. Jeffery Rhodehamel* and LeAnne Jackson, Food & Drug Administration, 200 C St., S.W., HFS-615, Washington, DC 20204

Vacuum packaging as a food preservation technique has a long history of safe usage; however, the process presents a potential for food safety hazards. Vacuum packaging prevents the growth of aerobic spoilage organisms, oxidative processes, color deterioration, water vapor loss, and shrinkage of foods. By inhibiting chemical processes, it delays quality deterioration; it also inhibits the growth of aerobic spoilage organisms, which can cause unsightly slime, off-odors, and off-flavors, thereby extending the shelf life of foods. However, vacuum packaging may increase the risk of foodborne illnesses by providing an anaerobic environment that may permit the growth of pathogens while inhibiting the growth of normal spoilage organisms. The use of mild heat treatments (e.g., pasteurization) in combination with vacuum packaging may actually enhance the growth of *Clostridium botulinum* as a result of endospore survival. Reliance on refrigeration also presents a potential safety hazard because vacuum packaging does not prevent the growth of psychrotrophic pathogens such as *C. botulinum* or *Listeria monocytogenes*. In addition, by extending the shelf life of foods, vacuum packaging may provide the additional time necessary, even under proper refrigeration temperatures, for these foodborne pathogens to increase to illness-causing levels.

(182) OSHA IN THE FOODSERVICE INDUSTRY

Robert E. Harrington, Director Technical Service, Public Health & Safety, National Restaurant Association, 1200 Seventeenth Street, N.W., Washington, DC 20036-3097

Restaurant operators are being confronted with an increased number of complex federal regulations. In addition to numerous labor, tax, environmental, and public health regulations, foodservice operators must also comply with a growing number of Occupational Safety and Health Administration (OSHA) standards. The original intent of many of these OSHA regulations was to protect workers in specific high hazard industries. Today however, some OSHA standards are being applied to a wide range of industries far beyond those originally targeted. Although the foodservice industry is generally not considered a high hazard industry, compliance with OSHA standards has increasingly become more difficult and time consuming. Many current OSHA standards are performance-based and provide only vague guidelines and subjective performance levels of compliance. The operator who attempts to comply with these complex OSHA requirements may find compliance nearly impossible due to a pervasive lack of measurable objectives and performance levels in the standards. Although flexibility and site specificity in regulatory standards is desirable, operators must be given more definitive guidance on the scope of application and acceptable levels of compliance.

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(183) ECONOMIC FRAUD IN SEAFOOD: WHAT TO LOOK FOR AND HOW TO DEAL WITH IT

Mary Snyder, Chief, Policy Guidance Branch, Office of Seafood, U.S. FDA, Washington, DC

Economic fraud is a persistent problem in the seafood industry from harvester to the retailer. The vast number of species and product forms contribute to the problem. Knowing how to identify valuable species and differentiate them from those commonly substituted, as well as how to recognize common processed product cheats, will help eliminate the problem.

(184) HACCP TRAINING FOR SEAFOOD PROCESSORS

George J. Flick,* Professor, and Cameron R. Hackney, Food Science & Technology, Virginia Tech., Blacksburg, VA 24061-0418

The Seafood HACCP Alliance is a cooperative effort between federal and state seafood regulatory agencies, professional organizations of health regulatory officials, seafood trade associations, and state Sea Grant Marine Advisory Service programs. The objectives of the Alliance is to develop HACCP training materials that will ensure that regulators, educators, and the seafood industry have the same expectations of a mandatory quality assurance program based on the HACCP concept. This goal will be achieved by developing a HACCP training manual for use by educators in the public and private sector. The manual contains basic HACCP concepts as well as specific statutory requirements of the HACCP legislation. A mechanism for certification of attendees will also be considered by the Alliance.

(185) MICROBIOLOGICAL PROBLEMS ASSOCIATED WITH SEAFOOD

Cameron B. Hackney,* Professor and Department Head, and Sean Altekruse, Virginia Tech., Dept. of Food Science & Technology, Blacksburg, VA 24061-0418

Microbiological problems associated with seafoods can be divided into those that are naturally occurring and those associated with fecal contamination. Naturally occurring problems include members of the family Vibrionacea, *Clostridium botulinum*, *Bacillus cereus*, and histamine production by mesophilic microorganisms on dark fleshed finfish. Marine vibrios tend to show a seasonal variation and many, but not all, are moderately balophilic. There are many species of pathogenic vibrios, but the ones causing the most problems are *V. cholerae* (both 01 and non-01), *V. vulnificus*, and *V. parahaemolyticus*. Other members of the family such as Plesiomonas, have caused illness after consuming raw or undercooked shellfish. *C. botulinium* continues to be a problem. *B. cereus* has caused a limited number of illnesses associated with temperature abuse of seafoods. Parasites associated with some finfish species occasionally cause illness. Enteric viruses, associated with human fecal contamination, include Hepatitis A and E, Norwalk virus, and many of the unclassified viruses. Enteric bacterial pathogens, which may be of either human or animal origin, cause relatively few illnesses. Raw molluscan shellfish, are the vehicles most often associated with viral and bacterial pathogens; however, food handlers can contaminate any seafood product during preparation. Natural toxins will not be discussed in this presentation. Adequate thermal processing will eliminate most potential microbiological problems. Treatment with radiation will eliminate most vegetative pathogens, but will not destroy spores and viruses.

(186) THE SEAFOOD HOTLINE: WHAT QUESTIONS DO CONSUMERS ASK?

Ruth A. Welch, M.S., R.D. Public Affairs Specialist, Food and Drug Administration HFS-555, 200 C Street, Washington, DC 20204

The FDA Seafood Hotline was established in October 1992 to provide accurate and timely information to consumers on important seafood issues, including information for medically at risk individuals on the consumption of raw molluscan shellfish. During the first two years of operation, the hotline fielded nearly 50,000 calls, mailed 35,000 and "faxed" 3600 publications, provided 38,000 messages to callers, and provided over 13,000 units of information on raw shellfish. Although targeted at the consumer, the hotline's callers include physicians, harvesters, home economists, retailers, and many others. Questions range from serious to frivolous. Last year, the hotline provided information on the proposed seafood HACCP regulation, Seafood "Town Meetings" and The FDA Food Code via its prerecorded messages and fax on demand systems.

(187) THE SAFETY OF MAIL ORDER SMOKED SEAFOOD

Mary Losikoff, Consumer Safety Officer, Off. of Seafood, Cntr., for Food Safety & Appl. Nutr., FDA, 200 C St., SW, Washington, DC 20204

Some firms ship non-retorted, vacuum-packed, smoked fish at ambient temperatures or with inadequate temperature control. These products present a special hazard since vacuum packaging will retard the growth of spoilage organisms while creating an ideal environment for the growth of *Clostridium botulinum*. Only proper retorting in accordance with a scheduled process will make vacuum-packed smoked fish safe to store and ship unrefrigerated.

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(188) USE OF CARROT JUICE TO CONTROL *LISTERIA MONOCYTOGENES*

Larry R. Beuchat,* Professor, Robert E. Brackett and Michael P. Doyle, University of Georgia, Center for Food Safety and Quality Enhancement, Griffin, GA 30223-1797

The influence of pH and NaCl on survival and growth of *Listeria monocytogenes* in carrot juice was determined. Lethal and inhibitory effects were greatest at pH 5.0-6.4 and increased as the temperature was reduced from 20 to 12°C, and from 12 to 5°C. At concentrations up to 5%, NaCl protected against inactivation. The influence of dipping shredded lettuce in carrot juice or adding carrot juice to Brie cheese and frankfurter homogenates on survival and growth of *L. monocytogenes* was also studied. *L. monocytogenes* grew at 5, 12 and 20°C on lettuce not treated with carrot juice. Treatment with 20 or 50% juice resulted in significantly ($P \leq 0.05$) lower populations throughout a 14-day incubation period at 5°C. Treatment retarded growth on lettuce incubated at 12°C but was without affect at 20°C. Increased amounts of carrot juice, from 0 to 2, 5 or 10%, resulted in significant reductions in viable cells of *L. monocytogenes* in Brie cheese incubated at 5°C but not at 12 or 20°C. Carrot juice, when added at levels up to 10%, was ineffective in controlling the growth of *L. monocytogenes* in frankfurter homogenate. Treatment with carrot juice failed to influence growth of aerobic mesophilic microorganisms in lettuce, cheese and frankfurter, indicating that microflora naturally present in these foods or in carrot juice used to supplement them are resistant to antiListerial components in the juice.

(189) A REDUCED-TIME PROCEDURE FOR DETECTING HEAT-INJURED *LISTERIA MONOCYTOGENES* IN FOOD

M. P. Doyle,* Professor, J. R. Patel, C. -A. Hwang, L. R. Beuchat, and R. E. Brackett, Center for Food Safety and Quality Enhancement, University of Georgia, Griffin, GA 30223-1797

An immunoblot technique in conjunction with enrichment in Fraser broth (FB) containing either 400 µg of catalase per ml or 0.01 unit of Oxyrase® per ml was developed to detect both healthy and heat-injured *Listeria monocytogenes* in foods within 34 h. Studies evaluating recovery of heat-injured listeriae in the presence of oxygen scavengers revealed that sodium pyruvate (2.5 mg/ml), catalase (400 µg/ml) or Oxyrase® (0.01 unit/ml) greatly increased cell recovery. Enrichment in FB containing oxygen scavengers enabled recovery of heat-injured listeriae within 6 h compared to 24 h required for conventional methods. Combining this shortened enrichment procedure with culturing listeriae on selective agar (modified Oxford agar; 24 h) and performing a microcolony immunoblot assay using monoclonal antibodies to *L. monocytogenes* (ca. 4 h), listeriae can be detected within 34 h. This procedure was evaluated with *L. monocytogenes*-inoculated milk, cabbage slurry and beef slurry, and was successful in detecting heat-injured listeriae in all three foods.

(190) ESTABLISHMENT OF A BOVINE SURVEILLANCE PROGRAM FOR *ESCHERICHIA COLI* O157:H7 IN WASHINGTON STATE

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Monthly fecal sampling for verotoxic (VT) *E. coli* O157 was conducted in 13 dairy herds and one contract dairy heifer raising operation over a period of one year. Five of the dairy herds had been previously identified as positive and 8 as negative by sampling of 60 animals (or the entire herd if less than 60) on a single sampling date. In the follow-up sampling, the prevalence of VT *E. coli* O157 was significantly higher in the initially positive herds (median, 1.9%) than in initially negative ones (median, 0.2%); however the agent was eventually found in 4 of the 8 initially negative herds. The highest prevalence was detected in post-weaning heifers as had been previously observed. For all age groups, prevalence was highest during warm months (April-October) corroborating previous observations of seasonality. Detectable fecal shedding of VT *E. coli* O157 was strongly clustered temporally such that most positive fecals occurred on a minority of sample dates. Longitudinal fecal sampling was done in 1,091 identified cattle of which 56 were positive for VT *E. coli* O157 on one or more occasions. Long term carriage, beyond 3 consecutive samplings, was not observed; most cattle in which the agent was detected were observed to be positive on only one occasion. Subtyping of VT *E. coli* O157 isolates was performed using pulsed field gel electrophoresis (PFGE). Up to 5 different PFGE subtypes were found per herd with as many as 4 different subtypes found in a herd on a single sample date. In 5 herds, indistinguishable PFGE subtypes were found on multiple sample dates as much as 2 years apart. Five of the PFGE subtypes were found in more than one herd, with up to 600 km separating herds with indistinguishable subtypes.

(191) LIPID COMPOUNDS AS NOVEL BARRIERS FOR CONTROL OF *LISTERIA MONOCYTOGENES*

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Fatty acids and monoglycerides (MAGs) including C_{12}, $C_{18:2}$, $C_{18:3}$, MC_{10}, MC_{12} and coconut MAGs were tested against *Listeria monocytogenes* strain Scott A in culture media and in several foods. Fatty acids (50-

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200 μg/g) were antibacterial in culture media against *L. monocytogenes* but were not inhibitory at concentrations equal or less than 1,000 μg/g when tested in foods. In contrast, certain MAGs were inhibitory when tested in certain foods including Camembert cheese, seafood salad, imitation crabmeat, cooked shrimp, beef franks and turkey franks. The MAGs were most effective at 5°C compared to 12°C. MAGs differed in the inhibitory or bactericidal activity depending on the food. MC_{12} at concentrations of 500 to 1,000 μg/g, MC_{10} at concentrations ≥ 1,000 μg/g, the combination of MC_{10} (250 to 500 μg/g) and MC_{12} (250 to 500 μg/g), or coconut MAGs (500 to 1,000 μg/g) were inhibitory against *L. monocytogenes* in beef and turkey franks. MC_{12} (500 to 1,000 μg/g) showed the strongest inhibitory activity in Camembert cheese, imitation crabmeat, cooked shrimp and seafood salad stored at 5°C. The combination of the MAGs, particularly MC_{10} and MC_{12} showed synergistic activity in the various foods. Lowering the pH of foods by HCL or acidulants such as lactic acid enhanced the effectiveness of MAGs against *L. monocytogenes*. Antioxidants also enhanced the activity of MAGs by a mechanism possibly related to change in redox potential since the pH was unaffected. Certain potentiators enable utilization of lower concentrations of MAGs in foods. The results suggest that MAGs could serve as an inhibitor of *L. monocytogenes* in certain minimally-processed refrigerated foods.

(192) APPLICATION OF NOVEL BACTERIOCINS AS BIOCONTROL AGENTS TOWARDS *LISTERIA MONOCYTOGENES* IN FOODS: PROPERTIES AND INHIBITORY EFFECTIVENESS

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In recent years, numerous bacteriocins produced by lactic acid bacteria have been identified which are inhibitory to foodborne pathogens and spoilage microorganisms. Nisin, a bacteriocin commercially accepted as a direct food additive for low-sodium high-moisture pasteurized processed cheese spread in the U.S., has also been approved for limited use in pasteurized liquid egg product by the USDA for shelf life extension (pending ultimate FDA approval). In the food ingredient industry, other lactic culture fermentates have also been used as shelf life extenders in foods undoubtedly due to participation of bacteriocinogenic activity. *Lactobacillus cuvatus* FS47 and *Lactococcus lactis* FS56 previously isolated from food, produce prodigious amounts of bacteriocins (curvaticin FS47, lacticin FS56) inhibitory to *Listeria monocytogenes* and other pathogenic spoilage microorganisms. The objective of our study was to examine curvaticin FS47 and lacticin FS56 in comparison with nisin and pediocin for inhibitory effectiveness against *L. monocytogenes* either by use of the bacteriocinogenic cultures in challenge experiments or by use of the bacteriocins themselves. The occurrence of bacteriocin-resistant *Listeria* will also be discussed.

(193) EVALUATION OF PENICILLIN-BINDING PROTEINS FOR SUBTYPING *LISTERIA MONOCYTOGENES* AND EXAMINATION OF CURRENT TRENDS IN ANTIMICROBIAL RESISTANCE IN CLINICAL AND FOOD ISOLATES

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Patterns of penicillin-binding proteins (PBPs) were examined in clinical and food isolates of *Listeria monocytogenes* to attempt to differentiate outbreak strains from sporadic and environmental strains. Analysis for PBPs proved to be a slow and labor-intensive method that did not have the level of differentiation that could be obtained with multilocus enzyme electrophoresis (MLEE). In addition, some non-specific binding was noted between radio-labeled penicillin and proteins which were used as molecular weight standards. As a part of this study, 136 clinical and food isolates were screened for penicillin resistance by the disk diffusion method in an attempt to find penicillin-resistant strains which could, in turn, have an altered pattern of PBPs. No penicillin-resistant strains were found, and PBP patterns among strains of the same species and serotype were generally alike. PBP patterns among strains of different species showed some species-related variations. The examination of antimicrobial resistance by disk diffusion was expanded to include a total of 72 antimicrobial compounds. In general, all strains of *L. monocytogenes* tested were susceptible to the antimicrobials that are of clinical significance. A wide range of resistance and susceptibility among the strains was noted with methicillin and cefotaxime. The level of this resistance was not related to isolation source, serotype, MLEE type, or species. MIC values for these antimicrobials were determined by the E test, and it was found that variations in resistance to these compounds could differentiate among strains that had the same serotype and MLEE type. Duplicate isolates from the same patient or mother/baby paired isolates always had the same resistance patterns if the serotypes and MLEE types were the same; however, strains from different sources that had the same serotype and MLEE type did not always have the same resistance patterns. Thus resistance patterns to these two antimicrobials provided a crude but rapid subtyping method that can be used prior to serotyping and MLEE subtyping. Generally, strains of different *Listeria* species had similar patterns of resistance or susceptibility to the antimicrobials tested in this study; however, strains of *L. grayi* were significantly more resistant to rifampin than were strains of *monocytogenes, seeligeri, innocua, ivanovii*, or *welshimeri*. Resistance to rifampin may be an additional means of identifying strains of *L. grayi*.

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(194) INSERTION SEQUENCE FINGERPRINTING: A NEW SUBTYPING SYSTEM FOR *ESCHERICHIA COLI* O157:H7 STRAINS

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Insertion sequences (IS) are small segments of the chromosomes of bacterial cells that have the ability to copy and transpose from one place on the chromosome to another. In populations of *Escherichia coli*, ES exist in 5-50 copies per cell. Previous studies have shown that *E. coli* strains that are indistinguishable by many biochemical tests differ in both the number and positions of different IS elements. This means that molecular methods that detect differences in copy number and chromosomal locations of IS elements can reveal distinctive patterns or "IS fingerprints" for *E. coli* strains. We tested different methods and discovered two elements (IS30 and IS3) that were variable, and thus gave different fingerprints among O157:H7 strains that were otherwise similar in biochemical tests. With these IS fingerprints, we have been able to determine that certain IS subtypes have spread into several countries, and some occur both in humans and cows, an observation supporting the hypothesis that bovine herds are reservoirs for O157:H7 strains. Further study of *E. coli* O157:H7 strains with IS fingerprinting may be useful for elucidating how these bacteria spread from one place to another and the routes by which they are transmitted from animals to humans.

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