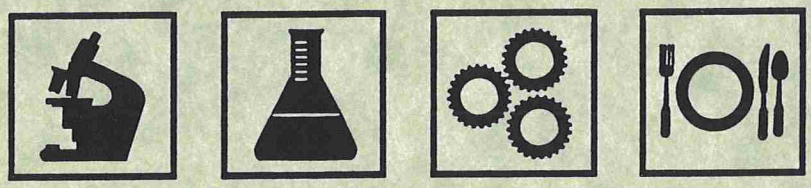
Supplement, 1996
Volume 59
Pages 1-70
CODEN: JFPRDR 59(Sup)1-70 (1996)
ISSN: 0362-028X

Journal of Food Protection



"The mission of IAMFES is to provide food safety professionals worldwide with a forum to exchange information on protecting the food supply."

Supplement to *Journal of Food Protection*

Published by the
International Association of Milk,
Food and Environmental
Sanitarians, Inc.
6200 Aurora Avenue
Suite 200W
Des Moines, Iowa 50322-2863 U.S.A

Journal of Food Protection (ISSN-0362028X) is published monthly beginning with the January number by the International Association of Milk, Food and Environmental Sanitarians, Inc., executive offices at 6200 Aurora Avenue, Suite 200W, Des Moines, IA 50322-2863, U.S.A. Each volume comprises 12 numbers.

Postmaster: Send address changes to *Journal of Food Protection*, IAMFES, 6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2863, U.S.A.

Manuscripts: Correspondence regarding manuscripts and other reading material should be addressed to Michelle L. Sproul, Publications Assistant, IAMFES, Inc.

Instructions for Authors for the use of contributors of papers can be obtained from Michelle L. Sproul, Publications Assistant, IAMFES, Inc.

Orders for Reprints: All orders for reprints should be sent to IAMFES, Inc. *Note:* **Single reprints are not available from the above address; address requests to principal author.**

Business Matters: Correspondence regarding business matters, advertising, subscriptions, orders for single copies, etc. should be addressed to David W. Tharp, Director of Finance, IAMFES, Inc.

Volumes on Microfilm are available from Xerox University Microfilms, 300 N. Zeeb Rd., Ann Arbor, MI 48106.

Membership Dues: Membership in the Association is available to individuals only. Direct dues are currently $120.00 per year and include subscriptions to the *JOURNAL OF FOOD PROTECTION* and *DAIRY FOOD AND ENVIRONMENTAL SANITATION.*

Student membership is $60.00 per year, which includes both journals. Verification of full-time student status is required. No cancellations accepted.

Sustaining Membership: A sustaining membership in IAMFES is available to companies at a rate of $485.00 per year, which includes $100.00 credit toward an ad in the "annual meeting issue" of the Journal. For more information, contact IAMFES, 6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2863; 515-276-3344.

Subscription Rates: $175.00 per year. Single copies $29.00 each. No cancellations accepted.

Postage: Outside U.S. add $22.50 per journal for surface delivery; add $95.00 per journal for air mail delivery. U.S. FUNDS ONLY – ON U.S. BANK. Single copies add $9.00 per issue.

Journal of Food Protection

ISSN:0362-028X
Official Publication
International Association of Milk, Food and Environmental Sanitarians, Inc.
Reg. U.S. Pat. Off.

Vol. 59 **1996** **Supplement**

Abstract Book

for the

83rd Annual Meeting of IAMFES

Supplement to *Journal of Food Protection*, Volume 59, 1996

This is a collection of abstracts from the 1996 IAMFES Annual Meeting held in *Seattle, Washington on June 30-July 3, 1996*

Risks, Realities, and Roulette

The Mission of IAMFES is to Provide Food Safety Professionals Worldwide with a Forum to Exchange Information on Protecting the Food Supply

IAMFES Author List

Goff, J. H., University of Arkansas (41)
Gottemoller, T., Archer Daniel Midland (93)
Gourama, H., Penn State (177, 240)
Graber, J. L., University of Nebraska (38)
Gravani, R., Cornell University (66, 161)
Guzewich, J., New York Dept. of Health (224)
Hackney, C. R., Virginia Polytechnic Institute & State University (108)
Harmon, K., Iowa State University (59)
Harris, L. J., University of California-Davis (163)
Harrison, J. A., University of Georgia (192)
Hathaway, S., Ministry of Agriculture & Fisheries Regulatory Authority (222)
Henyon, D., Elopak Inc., (158)
Heredia, N. L., Universidad A. De Nuevo Leon (65)
Himelbloom, B. H., University of Alaska-FITC (130)
Hitchins, T. D., Food and Drug Administration (87)
Howard, R., CDC (136)
Huang, Y., University of Georgia (131, 132)
Jamieson, C., Valley Agricultural Software (27)
Jaquette, C. B., University of Georgia (79)
Jassim, S., University of Guelph (77)
Jaykus, L. A., North Carolina State University (139, 205)
Jeong, D. K., Kosin University (37)
Johnson, E., University of Wisconsin (216)
Johnson, R., Agriculture Canada (97)
Karch, H., University of Würzburg (31)
Karumanchiri, A., Liquor Control Board of Ontario (207)
Kaspar, C. W., Food Research Institute (101)
Katta, S. K., University of Nebraska (176)
King, A. D., USDA (241)
Kochevar, S. L., Colorado State University (124)
Kome, H., Spartanburg (SC) Herald-Journal (138)
Lachica, R. V., U.S. Army Natick Lab (102)
Lammerding, A., Agriculture & Agri-Food Canada (220)
Langlois, B. E., University of Kentucky (103)
Larsson, A. J., University of Minnesota (84)
Lee, M. B., Ryerson Polytechnic University (71)
Lewandowski, V., University of Minnesota (72)
Limón, J. C., Universidade Autonoma de Nuevo Leon (64)
Lin, C. M., University of Florida (112)
Line, J. E., USDA-ARS-RRC-PMSRU (14)
Linton, R. H., Purdue University (19)
Lohman, J., Sustainable Agri-Services (25)
Lopez, E., Hospital de Ninos (32)
Mach, P. A., 3M Company (85)
Manger, R., USFDA (202)
Marshall, R., University of Missouri (93)
McCardell, A. J., DuPont Experimental Station (121)
McClure, P., Unilever Research (88)
McIntyre, L., University of Guelph (153)
McKellar, R. C., Agriculture & Agri-Food Canada (45)
McNamara, A. M., USDA-FSIS (140)
Meng, J., University of Georgia (188)
Meyer, J. D., University of Wisconsin-Madison (80)
Miller, A. J., USDA-ARS-ERRC (18)
Mills, V., Evergreen Packaging (157)
Morales, R. A., North Carolina State University (170)
Musgrove, M. T., USDA-ARS-RRC-PMSRU (13)
Nannapaneni, R., University of Arkansas (36)
Nazarowec-White, M., Health Canada (105)
Nedoluha, P. C., University of Maryland (126)
Neill, M., Brown University and Memorial Hospital of Rhode Island (28)
Nettles-Cutter, C., USDA-ARS U.S. Meat Animal Research Center (15)
Nickelson, R., National Cattleman's Beef Association (144)
Nisbet, D. J., USDA-ARS (232)
Notermans, S., National Institute of Public Health and Environmental Protection (197)
Nutsch, A. L., Kansas State University (9)
Odumeru, J. A., Ontario Ministry of Agriculture (76)
Ogden, J., NM Dept. of Agriculture (22)
Okagbue, R. N., Applied Biology & Biochemistry (63)
Olsen, E., Chateau Ste. Michelle (211)
Peters, D. L., University of Nebraska (168)
Pillai, S. D., Texas A&M University System (236)
Powell, D., University of Guelph (162)
Prince, G., The Kroger Company (247)
Quinlan, J. J., North Carolina State University (54)
Rajkowski, K. T., USDA-ARS-ERRC (119)
Rambo, S., Dean Foods Company (93)
Raybaudi, R. M., Universidad Central de Venezuela (42)
Reinhart, C., Waukesha Cherry Burrell (158)
Riemann, J., Excel Corporation (243)
Ricke, S. C., Texas A & M University (234)
Riordan, D. C. R., The National Food Centre Dunsinea (186)
Roberts, C., USDA/FDA Foodborne Illness Information Center (165)
Ross, T., University of Tasmania (100)
Sandberg, G. P., 3M Company (52)

Journal of Food Protection, Vol. 59, No. Supplement, Pages 5-70

Abstracts

(1) SENSE, NONSENSE, AND SCIENCE

J. Schwarcz, Vanier College, Saint-Laurent, Quebec H4L 3X9 Canada

A little nonsense, now and then, is relished by the best of men. These days, however, we seem to be dealing with more than just a little nonsense. Nutritional gurus with no scientific background are preaching to packed audiences and expert sounding quacks claim cures for virtually any disease using a variety of enemas, magnets or dietary supplements. Modern technology is often presented as the villain that has plundered our environment, tainted our food supply and pilfered our health. The salvation, it is claimed, is "to go back to nature!" It is important for members of the scientific community to understand how these notions arise and to explore ways of educating the public about the use of scientific method to evaluate concerns.

(2) GENERAL FOOD SAFETY INFORMATION AND MEDICAL ADVICE FOR TRAVELLERS

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

In the 21st century, we can expect increased national and international travel. Many countries will continue to rely on tourism as a main source of income. Currently, contaminated food and drink are significant health hazards for 4 billion travellers each year, and 20 to 50% of travellers may suffer from diarrhea and other foodborne diseases. Hence, travellers and the tourism industry are very concerned about food and water safety. In spite of known foodborne disease outbreaks, United States residents are privileged to have some of the safest food and water supplies in the world. Hygienic standards of food preparation and safe water supplies do not always exist worldwide. Applying the principles of HACCP, the presentation offers a list of microbial pathogens found in food and water in many regions of the world. Pathogen controls are identified. Information is given that allows travellers to monitor, measure and judge standards of food and water safety in their own and other countries. Techniques to select and handle food and water to minimize the risk of illness are given. General information is provided concerning medical treatment of various foodborne illnesses, diseases, and remedies or medication that travellers can use.

(3) CRUISE SHIP SANITATION

D. W. Turner, Vessel Sanitation Program, Department of Health and Human Services, Centers for Disease Control, 1015 N. America Way, Rm. 107, Miami, FL 33132

The Vessel Sanitation Program is a cooperative activity between the cruise ship industry and the Centers for Disease Control and Prevention. The purpose and goals of the program are to achieve and maintain a level of sanitation that will minimize the risk of disease outbreaks and to provide a healthful environment for passengers and crew. To accomplish this, sanitation inspections are carried out on all passenger ships that arrive in the United States from foreign ports. Inspection coverage includes the potable water system, food safety aspects, and general cleanliness and maintenance. In addition, all vessels arriving in the U.S. are required to report the incidence of diarrheal illness and any other communicable disease.

Vessel Sanitation Program personnel also conduct training of shipboard employees on the sanitation standards. This is done on a quarterly basis with an average of 90 persons in attendance for each seminar.

A summary of sanitation inspection results is published on a bimonthly basis. Copies of the publication and individual inspection reports are available to the general public, travel agents, and other interested individuals on request.

Initial plan reviews are conducted for all new buildings and construction inspections are carried out at the shipyards prior to delivery. Currently there are approximately 18 new ships under contract for delivery through 1999.

(4) ENTERIC BACTERIAL CONTAMINATION OF PUBLIC RESTROOMS

C. P. Gerba,* D. I. Kennedy and C. E. Enriquez, Department of Soil, Water, and Environmental Science, University of Arizona, Tucson, AZ 85721

Although public restrooms are often thought of as places that harbor disease-causing microorganisms, no surveys appear to have been done to document the occurrence of enteric organisms in these facilities. A cotton swab was used to sample surfaces and placed into a test tube containing 10 ml of Coliert solution, which allows detection of both coliforms and *Escherichia coli*. Thirty-one different sites were sampled in 47 restrooms. Sinks, floors, toilet seats, and sanitary napkin disposal were found to be the most contaminated. Coliform bacteria could be isolated in more than 60% of the sinks, and *E. coli* in 20%. Surfaces in high traffic restrooms (bus terminals, educational institutions) were most likely to be contaminated, followed by fast food restaurants and public restrooms in hospitals. Female restrooms were more contaminated at all sites than male restrooms. Coliform bacteria were isolated twice as often in areas adjacent to the toilet in female restrooms vs. male restrooms. Restrooms with one stall were the most contaminated, followed by those with four stalls/urinals. *Salmonella* spp. were identified on a tap, sinks, floor, and the bottom of a toilet seat.

(6) ETHNIC FOOD SAFETY

G. I. Swick, Marion County Health Department, 98 McKinley Park Blvd., Marion, OH 43302

Ethnic restaurants have become popular in the past two decades. Almost every town has one, and in larger cities, the number could be several hundred.

Problems arise because the laws generally do not make special concessions for the cultural differences and traditions of people from foreign lands. In most cases the owners and food service workers do not intentionally violate the laws, but are simply preparing their recipes the way their ancestors did and do not understand how or why they should change. Along with their recipes, they also bring their culture, traditions, and families.

It is not unusual to find families working together from morning to night, and their children are in the place of business which takes on a homelike atmosphere. Problems arise when inspecting an ethnic restaurant due to the language barrier. It is difficult to inspect without asking questions and explaining how to correct violations. Teaching is important, and if the sanitarian cannot communicate with the food service workers, his or her effectiveness is being severely restricted. Other commonalities include the use of cloth towels for wiping hands, crowded work areas, covering food with cloth napkins, and improper food temperatures by our standards.

This presentation highlights the differences between ethnic and traditional restaurants, and includes slides taken in ethnic restaurant kitchens, discussion of inspection techniques for use in ethnic restaurants, and hand outs to help sanitarians communicate with food service workers.

(7) ETHNIC FOODS AND CROSS-CULTURAL COMMUNICATION

J. H. Gans, Santa Clara County Department of Environmental Health, 2220 Moopark Avenue, San Jose, CA 95128

Santa Clara County, California, has long been recognized as an outstanding example of the American "melting pot." Immigrants to the region have historically brought with them various customs that differ from established American practices.

When the influx of Southeast Asians occurred in the 1980s, many previously unknown food products and preparation methods were introduced. Environmental Health inspection personnel were not familiar with the mysterious products and methods they were encountering in Southeast Asian food establishments. Inspectors had received very little, if any, training in how these different foods should be handled.

When American food code requirements were applied to these ethnic foods by American inspectors, who were ill-prepared to understand the unique cultural differences of these new immigrants, a clash of cultures occurred. A task force was convened to study the situation and develop solutions, with an emphasis on food protection.

Working in cooperation with the University of California, Davis, Department of Food Science and Technology, the task force conducted food histories of 33 different Vietnamese prepared food products. Samples of the foods were tested for water activity and pH in an effort to determine the potential hazard of each food item.

Working in cooperation with several Vietnamese community organizations, the task force also developed methods for improvements in cross-cultural communication. It was essential that task force members adopted an open-minded attitude and practiced effective listening skills, especially during this aspect of the overall effort.

The presentation discusses the efforts and results of the task force (both successes and failures) and stresses the link between ethnic food safety and effective cross-cultural communication. It also endeavors to encourage listeners to accept a challenge to try something of a similar nature in their own retail food inspection programs in their regions of the country.

(8) ECOLOGY OF *SALMONELLA, CAMPYLOBACTER* AND *LISTERIA* IN CHICKEN PRODUCTION

S. Bailey,* N. Stern and N. Cox, USDA-ARS-RRC, Russell Research Center, Athens, GA, 30604-5677

Salmonella, *Campylobacter* and *Listeria* are human bacterial enteropathogens that have been associated with chicken products. Each has been shown to have unique sources and ecological niches. Sources of *Salmonella* include feed, the environment, rodents, insects, wild birds, and most importantly, breeder flocks and the hatchery. Young chicks are highly susceptible whereas older chickens are less susceptible to colonization. Colonization rates peak at about 3 weeks of age and decline thereafter. *Campylobacter* are not found in the eggs or hatchery, but are frequently found in the environment, rodents and wild birds. In the laboratory, young chicks are susceptible to colonization, but in nature *Campylobacter* are not usually seen until 2 to 3 weeks of age. The majority of the flock will frequently become colonized within a few days and will remain so throughout growout. Although widespread in the environment, *Listeria* does not readily colonize chickens and is infrequently found in chickens during growout. Epidemiological studies indicate that *Listeria* is a processing plant cross-contaminant on processed chickens.

(9) EVALUATION OF A STEAM PASTEURIZATION PROCESS IN A COMMERCIAL BEEF PROCESSING FACILITY

A. L. Nutsch,* R. K. Phebus, D. E. Schafer, M. J. Riemann, R. C. Wilson and J. Leising, Kansas State University, Room 139, Call Hall, Manhattan, KS 66506

This research evaluated the effectiveness of a steam pasteurization process for reducing naturally occurring bacterial populations on freshly slaughtered beef sides in a large commercial facility. Over a period of 10 days, 140 randomly chosen beef sides were sampled for microbiological analyses immediately before, immediately after, and 24 h after steam treatment. Samples were analyzed for total aerobic bacteria, *E. coli* (biotype I), coliforms, and *Enterobacteriaceae* populations using Petrifilm™ plates and

for the qualitative presence of *Salmonella* using USDA-FSIS procedures. The process significantly ($P<0.01$) reduced mean APCs from 2.19 $\log_{10}$ CFU/cm^2 before treatment to 0.84 $\log_{10}$ CFU/cm^2 immediately after and to 0.94 $\log_{10}$ CFU/cm^2 24 h after treatment. Despite very low initial populations of *E. coli*, coliforms, and *Enterobacteriaceae*, significant reductions were observed immediately after and 24 h after treatment, with populations being virtually undetectable. One carcass (0.7%) was positive for *Salmonella* before treatment; all carcasses were negative after steam treatment. These data indicate that steam pasteurization is very effective at reducing overall bacterial populations on freshly slaughtered beef carcasses. The system effectively serves as an important critical control point for HACCP systems at the slaughter phase of beef processing. In conjunction with other microbial interventions, this process can play an important role in reducing the risk of pathogenic bacteria in meat and meat products.

(10) CHARACTERIZATION OF *LACTOCOCCUS* SPP. ISOLATED FROM COOKED, MODIFIED ATMOSPHERE PACKAGED POULTRY MEAT

R. K. Barakat* and L. J. Harris, University of Guelph, Department of Food Science, Guelph, Ontario N1G 2W1 Canada

A total of 206 strains of lactic acid bacteria were isolated from cooked, modified atmosphere packaged (MAP) poultry meat, and were tentatively identified as *Carnobacterium*, *Enterococcus*, and *Lactococcus* spp. based on morphology and biochemical characteristics. The isolates were further classified at the species level using carbohydrate fermentation patterns. More than 50% of the isolates were tentatively characterized as *Lactococcus raffinolactis*. In order to confirm the validity of the identification, representative species were selected from each genera. Two universal primers based on 16S rRNA were used to amplify an 880-bp region of the rRNA genes by the polymerase chain reaction. The amplified fragment was sequenced for each of the selected strains and the sequences were aligned and compared with published sequences of reference strains of lactic acid bacteria. While carnobacteria and enterococci are common in MAP meats, lactococci have seldom been isolated from such products.

(11) THE OPTIMIZATION OF A LACTIC ACID TREATMENT FOR THE IMPROVEMENT OF THE MICROBIOLOGICAL QUALITY AND SAFETY OF POULTRY CARCASSES

D. A. Bautista,* N. Sylvester, S. Barbut and M. W. Griffiths, University of Guelph, Guelph, Ontario N1G 2W1 Canada

Fecally contaminated poultry carcasses are a safety risk and contribute to financial losses. To improve the safety and economics of poultry processing, lactic acid may be used to decontaminate these carcasses. The optimum conditions for lactic acid treatment were determined by a response surface composite design. Turkey carcasses (n=144) were exposed to temperature and pH combinations outlined by the design. Lactic acid (4.25% w/w) significantly ($P<0.001$) lowered populations of total aerobic bacteria and coliforms with increasing pH and temperature. Optimal performance was achieved with a lactic acid (4.25% w/w) treatment at a temperature of 40°C and pH 3. Using a randomized complete block design, a pilot scale study was performed to compare a) condemned control carcasses, b) accepted control carcasses, c) condemned carcasses treated with lactic acid and d) accepted carcasses treated with a commercial inside/outside wash (i.e., water @ 10°C). The results indicated that carcasses treated with lactic acid had significantly ($P<0.001$) lower total aerobic bacteria and coliform counts than any of the other test groups. Although lactic acid treatments may be able to decontaminate meat surfaces, good statistical planning can effectively optimize treatments.

(12) LEVEL OF *CAMPYLOBACTER* ON THE FARM ASSOCIATED WITH LEVELS ON PROCESSED CARCASSES

N. J. Stern, USDA-ARS-RRC, P.O. Box 5677, Athens, GA 30604-5677

The relationship of *Campylobacter* spp. levels in broiler poultry production to levels in processed carcasses was studied. Fourteen flocks (20,000 birds each) were sampled. One day prior to slaughter, 50 fecal samples/flock were assayed. These were the first flocks to be processed the next morning and 50 carcass rinses were taken from the post-chill processing location. Levels from the droppings ranged from non-detectable to ~10^8 CFU/g, and carcass levels ranged from non-detectable to ~10^6 CFU. Levels in the fecal samples were not correlated ($r = .08$) to the levels on processed carcasses. We are continuing to assess this relationship. This study also suggests that 25-fold differences in carcass levels of *Campylobacter* spp. exist, and therefore as the microbiological quality is improved, consumer exposure will be reduced.

(13) AN EFFECTIVE PROCEDURE FOR THE DETECTION OF *CAMPYLOBACTER* SPP. ON BROILER CARCASSES BY RINSING DIRECTLY WITH ENRICHMENT BROTH

M. T. Musgrove,* N. J. Stern and R. Johnson, USDA-ARS-RRC-PMSRU, P.O. Box 5677, Athens, GA 30604-5677

An experiment was performed to determine the efficacy of a method to detect the presence of *Campylobacter* spp. on broiler carcasses by rinsing the entire carcass with enrichment broth. Carcasses were rinsed with Bolton enrichment broth, a medium that uses normal atmosphere and quiescent incubation, for one minute. The rinsate was then transferred to a zip-lock bag and incubated for 24 h before being plated onto Campy-Cefex agar plates. Each sample was also analyzed using an automated enzyme-linked fluorescent assay (ELFA) developed by BioMerieux, Inc. To determine the relative effectiveness of this method, each

carcass was first sampled by a modification of the Bacteriological Analytical Manual (BAM) procedure utilizing Hunt enrichment broth. After analyzing 70 carcasses, the following results were achieved: using the new method, 70/70 were positive both culturally and by ELFA with an average of 6.43 log CFU *Campy.*/ml enrichment broth. The BAM procedure also yielded 70/70 positives culturally but only 67/70 by ELFA, with an average of 6.29 log CFU *Campy.*/ml enrichment broth. Rinsing directly with Bolton enrichment broth is an effective, logistically simple method that yields results 24 hours faster than the BAM procedure.

(14) COMPARISON OF *IN OVO* TREATMENTS FOR REDUCTION OF *SALMONELLA* COLONIZATION IN BROILER CHICKENS

J. E. Line,* N. J. Stern, J. S. Bailey and N. A. Cox, USDA-ARS-RRC-PMSRU, P.O. Box 5677, Athens, GA 30604-5677

We compared the *in ovo* treatment of chicken eggs with the yeast *Saccharomyces boulardii* (SB) to *in ovo* treatment with enrofloxacin for reducing colonization of chicks with *Salmonella*. A combined SB and enrofloxacin treatment was also evaluated. Fertile broiler eggs (240) were treated on day 18 of incubation. A small hole was drilled in the blunt end of each egg and either the yeast, the antibiotic, or both were administered with a needle and syringe. Chicks were challenged at hatch by spraying about 8000 *Salmonella* cells into the hatching environment. After 12 h the groups of chicks were moved to isolation units. On day 7 post-hatch, the chicks were sacrificed and the ceca were aseptically removed and analyzed for *Salmonella* content. The mean results from duplicate treatment groups demonstrated that, after sample enrichment, 95% of the positive control chicks were colonized by *Salmonella*; in contrast, only 10% of the chicks from groups treated with SB alone were positive for *Salmonella*. A mean of 50% of the chicks in the enrofloxacin-treated groups were colonized and none of the chicks treated with both SB and enrofloxacin were colonized by *Salmonella*.

(15) IMMOBILIZATION OF NISIN IN AN EDIBLE GEL FOR REDUCING BACTERIA ON THE SURFACE OF BEEF AND IN GROUND BEEF

C. Nettles-Cutter* and G. R. Siragusa, USDA-ARS, U.S. Meat Animal Research Center, Box 166, Clay Center, NE 68933

Two experiments were performed to determine if immobilization of nisin in an edible gel was an effective delivery system for a bacteriocin to the carcass surfaces as well as into ground beef. In both experiments, post-rigor lean and adipose beef carcass tissues were inoculated with *Brochothrix thermosphacta (BT)*, left untreated (U), or treated with 100 µg/ml nisin (N), calcium alginate (A), or 100 µg/ml nisin immobilized in a calcium alginate gel (AN). In the first experiment, samples were refrigerated after treatments and bacterial populations and nisin activity were determined at 0, 1, 2, and 7 days. In the second experiment, tissues were made into ground beef (15% adipose, 85% lean) and refrigerated, and bacterial populations and nisin activity were determined at 0, 7, and 14 days. In both experiments, U, A, and N treatments of beef tissues did not suppress bacterial growth for the duration of the experiment. However, in experiment 1, bacterial populations treated with AN were suppressed to < 2.42 $\log_{10}$ CFU/cm² by day 7. In experiment 2, bacterial populations treated with AN were suppressed to <1.3 $\log_{10}$ CFU/cm² at day 0; however, bacterial populations of AN grew to similar levels of U, A, and N (>7 $\log_{10}$ CFU/cm²) by day 14. Bacteriocin titers taken from both experiments demonstrated that nisin activity was higher in AN than in N samples up to day 7. These studies indicate that nisin delivered in an edible gel resulted in greater antimicrobial activity than the addition of liquid nisin to surface tissue or ground beef.

(16) STATISTICAL EVALUATION OF A POULTRY PROCESS FOR THE DETERMINATION OF OVERALL QUALITY USING CONVENTIONAL MICROBIOLOGY AND ATP BIOLUMINESCENCE

D. A. Bautista,* S. Barbut, J. P. Vaillancourt, L. J. Harris and M. W. Griffiths, Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1 Canada

Because of the time involved, microbiological analysis is recognized to be of little value for monitoring CCP's in a HACCP program. Instead, microbiology should be used to verify that the HACCP program is operating correctly. However, to get a better interpretation of the effectiveness of the program, statistical designs should be used instead of complete randomized testing. At a poultry processing facility, several key areas were designated as observation points. Using a Latin square design, each area was sampled by a conventional swabbing technique and modified ATP bioluminescence swab assay for total aerobic bacteria and overall hygiene, respectively. The results indicated that two distinct graphical representation can be generated by either methods that can significantly ($P<0.001$) interpret the effectiveness of the program during processing. At the same time, poultry lots and individual days can be significantly ($P<0.001$) interpreted for overall hygiene. The ATP bioluminescence assay has the advantage of assessing the level of hygiene within 5-10 mins. of sampling. In conclusion, this approach of validation can help to determine that the HACCP program designed for a poultry operation is working successfully.

(17) ENVIRONMENTAL ANALYSIS METHODS UTILIZED TO DETERMINE THE CONTAMINATION SOURCE IN A SAUSAGE PROCESSING PLANT

S. Shumaker* and J. Feirtag, University of Minnesota, 225 Food Science & Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108

Lactobacilli contamination was identified as the cause of spoilage of vacuum-packed cooked sausage at the processing plant studied. Thirty-five *Lactoba-*

cillus strains were isolated throughout the processing and packaging areas, as well as from the water used to cool product in the steamers. Six *Lactobacillus* strains were isolated from spoiled, packaged product. The heat treatment used to cook the product was determined to effectively eliminate the *Lactobacillus* strains. The packaging environment and the steamer water used to cool the product were both identified as sources of contamination by matching *Lactobacillus* strains isolated in these areas with those found in the spoiled, packaged product by the API 50 CH method. Microbiological and ATP-bioluminescence analyses of cleansing/sanitation and visual assessment of process flow layout revealed lack of hygiene and process control, resulting in cross-contamination of cooked product.

(18) COMPARISON OF F+RNA COLIPHAGE AND COLIFORM LEVELS AS FECAL CONTAMINATION INDICATORS IN A PORK SLAUGHTERHOUSE ENVIRONMENT

A. J. Miller* and B. S. Eblen, USDA-ARS-ERRC, 600 East Mermaid Lane, Wyndmoor, PA 19038

A male-specific F+RNA coliphage plaque assay and the 3M Petrifilm assay for total coliforms/*Escherichia coli* biotype I were compared as fecal contamination indicators for aspects of the pork slaughter process. Over 400 samples were split and tested, including sponge swabs from animal hauling truck floors and dressed carcasses; fecal material; water from the scald tank, dehairer, gambrel table, and polishers; and water from each stage of water treatment. Numbers of phage plaque forming units per g or ml were usually 1.5 to 2.5 $\log_{10}$ lower than coliform or *E. coli* counts, indicating that the assay for the former is less sensitive. Correlations between virus and bacterial indicators were higher in water than in solid samples. Coliphage is more chlorine resistant than bacteria, and may be a more rigorous biomarker for the safety of treated water. While the relationship between indicator microorganisms and the presence of pathogens remains contentious, they may be used successfully in the application of HACCP in the food processing environment.

(19) QUANTITY AND DISTRIBUTION OF AIRBORNE MICROORGANISMS IN POULTRY PROCESSING ENVIRONMENTS

R. H. Linton,* K. R. Lutgring, M. W. Peugh, A. J. Heber and N. J. Zimmerman, Department of Food Science, 1160 Smith Hall, Purdue University, West Lafayette, IN 47907

Four poultry slaughter operations (2 turkey, 2 duck) were examined for airborne microorganisms by means of total aerobic plate count (TPC), yeast/mold count (Y&MC), and psychrotrophic plate count (PPC). For each plant, approximately 40 sites were sampled on 4 different visits (fall, winter, spring, and summer) using an Anderson Air Sampler containing either Tryptic Soy Agar (for TPC and PPC) or Rose Bengal Agar (for Y&MC). Sampling sites were categorized into 7 areas, including: an outside control, shackling, picking, evisceration, post chiller, cut-up, portion packaging, and whole bird packaging. Airborne microbial counts in each plant showed a typical pattern; they were highest in shackling and decreased as the process continued toward packaging. TPC ranged from 1.3×10^6 CFU/m^3 in shackling to 4.3×10^1 CFU/m^3 for the outside control. PPC usually represented >50% of the total microbial population and ranged from 1.3×10^6 CFU/m^3 to 7.0×10^1 CFU/m^3. Y&MC typically represented 10% of the total microbial population and ranged from 2.9×10^4 CFU/m^3 to 3.5×10^1 CFU/m^3. Airborne microbial counts for turkey and duck slaughter environments were similar and only small differences were noted for counts between seasons. This study helped to identify source and quantity of microbial airborne contaminants that may affect product safety and shelf life. This information will help in slaughter plant design, when developing ventilation strategies, and when performing worker response assessment.

(21) LARGE HERD MANAGEMENT: THE KEY TO FUTURE PROFITABILITY

T. Furman, 111 E. Secretariat Drive, Tempe, AZ 85284

Dairy producers are faced with major challenges to maintain profitability under current fiscal conditions. At the same time, this challenge promises to provide greater opportunity for aggressive dairy managers to become more productive and profitable in the future. Dairy industry professionals who serve dairymen can contribute significantly to the success of the progressive dairy producer.

Dairies, although they will remain basically family enterprises, will get larger. Economy of scale permits greater efficiency, higher productivity and increased profits. But as the dairy unit becomes larger, management philosophies will have to change to accommodate the increased number of farm staff necessary to run such operations. To be certain, hundreds of "cow-side" decisions must be made correctly, each day, by the farm staff in the absence of the owner or even middle managers. Systems never necessary before with small, owner-operator dairymen will need to be implemented to organize work and assure that these "cow-side" decisions are made correctly for profitability of the dairy and for the best welfare of animals.

Dairy producers will utilize management techniques borrowed from other industries to involve all of their staff to work for higher standards and greater productivity. Total Quality Management, as one example, can help dairy managers establish output goals, organize work and monitor results. It will be a challenge for agribusiness enterprises that historically have relied on "good people just doing the right thing" to take a proactive approach to people management.

Adopting technologies that result in greater efficiency of production is a second area of opportunity. These new innovations will have to be cost-effective. They may directly affect productivity (e.g., BST) or indirectly impact milk production through genetics, reproduction, feeding, or loss-control record management. The successful large dairy herd manager will be

innovative in adapting these technologies, yet shrewd in his/her selection of innovations that may apply to his/her enterprise.

Innovation and change will evolve in an atmosphere of food safety regulation. But rather than this regulation being from outside (e.g. governmental regulation) it will come from within the dairy industry in the form of quality and food safety programs and initiatives. Quality as well as food safety will become equally a producer and a marketing objective.

Producers will find both short-term survival and long-term opportunity by expansion, implementing innovative management philosophies and utilizing appropriate new technologies judiciously.

(23) THE DOUBLE 50 WITH HIGH COUNTS: AND OTHER SANITARIANS' NIGHTMARES

K. Sloan, Germania Dairy Automation, 923 Woodland Avenue, Centralia, WA 98531

The commercialization of the dairy industry is upon us with profitability and survival being dependent on one fact alone: "He who produces 100 pounds of high quality milk for the least money, wins!" To attain these efficiencies, maximum productivity of labor and capital resources must be achieved. The fantasy has always been to have robots and computers milk the cows while the dairyman golfs and lives the good life. Five things will prevent that from happening in our lifetime: 1) manure, 2) moisture, 3) chemicals, 4) cows, 5) economy of scale, the latter being the single factor that will determine the design of dairy equipment in the future. 20 years ago a double 4 herringbone parlor was all one man could handle. In 1996 the Offhause Dairy in Batavia, NY, operates a double 20 Germania Pro-Time I Herringbone Parlor with one man at a rate of 160 cows per hour. The herd average is 24,000# and the SCC is 128,000. Large parlors (now Double 50's and growing) challenge existing CIP system concepts and require us to create ways to make sure that the "Big Three" are accomplished: 1) temperature/hot water, 2) chemical concentration and 3) contact time.

Easy, you say, just throw more soap and hot water at increasing velocity into the system and hope that the receiver doesn't trap out. Several new concepts in CIP are being refined such as High/Low Vacuum Systems with no milk pumps in the parlor, Vacuumized Vertical Wash/Transfer Vats that eliminate the need of receivers in the parlor, Turbo Wash where the different components in the system are washed sequentially, and Frequency Controllers on the milk pumps that change the speed of the milk pumps to enhance cooling efficiencies. Simplification and standardization of CIP systems is an evolving process driven by larger parlors and more stringent quality standards. Sit back, relax, and we'll review them all. The systems will be different from farm to farm. The facts of the "Big Three" and common sense will allow us to clean 'em as big as they can make 'em.

(24) WESTERN MILK HAULING CONCEPTS

A. Saylor, Food and Drug Administration, 200 C Street S.W., HF5-626, Washington, D.C.

An examination of the hauling practices for milk in the Western States identified practices and tanker construction modifications that need to be examined for food safety risks.

(25) ECO-AGRICULTURE/SUSTAINING THE DAIRY COW

J. Lohman, Sustainable Agri-Services, 817 Rita Street, Sedro Woolley, WA 98284

ECO is not only a prefix for ECO-logical, but also for ECO-nomical. According to the National Dairy Herd Improvement Association the average age of last calving for the American dairy cow is less than 46 months. This means she produces milk for somewhat less than two full lactations and is then culled for one or more symptoms. Unfortunately these symptoms are seen as the cause for her being culled, with the principal causes of these symptoms are seldom pursued. The status quo has been to treat the symptom of the disease rather than address the cause. Industry trade journals frequently publish articles regarding these symptoms. Their suggestions have been progressively addressed in the Northwest for the last twenty years. Yet numerous herd health issues continue to haunt us and bring about unprofitably high levels of involuntary culling. Frequently these cull cows have multiple symptoms and dairymen feel that 70-90% of these animals have livers that do not pass USDA inspection. Multiple disease symptoms are frequently caused by issues of toxicity. Sources of toxicity are numerous on the dairy farm but rarely discussed with the dairymen by the self-serving factions that sell the products or promote various practices.

As an industry we can throw all the money we have at the 'symptoms' but until we get to the root of the problems the economic return will be marginal at best. There are dots that need to be connected and lines drawn that will illustrate the direct link between today's health issues and the imbalances in the soils. The Bible says that the man is made of the dust of the Earth. The same chemical elements found in the soil make up our bodies. The only difference between the two is that the human molecular structure is more complex. Human bodies require nutrition found in the form of plants, meat, milk, and eggs. All animals get their food directly or indirectly from plants, and all plants get their food from the soil. Therefore, mineral-deficient soil may be one of the greatest original sources of disease in the world today. Conversely, remineralizing the soils and bringing back the natural balance required is likely to be the most ECO-effective exercise to eradicate disease.

(26) ELECTRONIC COMMUNICATION ON THE DAIRY FARM

R. A. Cady, Washington State University, 7612 Pioneer Way E., Puyallup, WA 98371

A decade ago, it was assumed that 90% of all dairy farms would be using computers for management. However, current computer adoption has only reached 30% to 40%. Advent of electronic information, however, is an area of ever-increasing interest. Current information is readily available to producers from 3 main sources: electronic mail, CD-ROM and the WorldWide Web (WWW). E-mail provides unprecedented access and communication abilities with industry experts and fellow producers through personal mail, bulletin boards and list server discussion groups. Dairy-L is one of the oldest list servers in agriculture, started in 1989, and is often cited in current dairy industry discussions. Dairy-L has over 1,800 subscribers from 39 countries and all 50 states and Canadian provinces. Subscribers consist mainly of university extension and research faculty, veterinarians, industry representatives and consultants, government employees and farmers. The fastest growing subscriber group is dairy producers. The National Dairy Database (NDD) is available on CD-ROM and contains over 3,000 pages of written text compiled from dairy-related extension publications written during the past 10 years. In addition, the NDD has computer worksheets that can be downloaded, a graphics, and an industry expert directory. The NDD is updated every18 months. The WWW is the newest form of electronic communication, giving Web surfers access to large amounts of graphic, video, audio and textual information. Reports may be generated from remote sites using farm data and 4 dairy farmers have set up their own Web homepage.

(27) FARM USES OF COMPUTER TECHNOLOGY

C. Jameson, Valley Agricultural Software, 2861 South K Street, Tulare, CA 93274

Computer uses on dairies include programs for dairy management, nutrition balancing, feed commodity management and accounting. This talk will be on dairy management and feed commodity management programs.

Dairy management programs are used to keep track of individual cows. As such they replace individual hand paper records such as cards or entry books. Records are kept for the following reasons (in decreasing order of importance): 1. Record information about individual cows; 2. Make work lists for jobs that need to be done; 3. Find cows that don't follow normal trends for action; 4. Follow herd level trends of the dairy; 5. Make predictive projections for the future; 6. Run trials to determine the effectiveness of various management actions; 7. Provide research data.

To satisfy these needs a system must:

1. Have fast and easy data entry capabilities
2. Be flexible to meet the individual needs of a dairy
3. Be secure, that is, able to backup data fast and easily

Food, roughage and grain, is 60% of a western dairy's expense. Commodity feed management programs provide control for the biggest single expenditure on the dairy. As such they provide loading instructions for mixers, calculate intake both "as is" and on a dry matter basis and keep inventory control. Some are integrated with the mixer truck scales to provide easy and accurate data of daily usage. Accurate knowledge of what is being fed can make substantial savings in a dairy's budget.

(29) *E. COLI* O111 — THE CAUSE OF AN OUTBREAK OF HUS AND ITS SURVIVAL DURING THE PROCESSING OF FERMENTED MEAT PRODUCTS

P. Desmarchelier* and F. Grau, Commonwealth Scientific and Industrial Research Organization, P.O. Box 3312, Tingalpa D.C., Queensland 4173 Australia

An outbreak of HUS in 1994/5 in South Australia was traced to the consumption of mettwurst containing Shiga-like toxin producing *Escherichia coli* (SLTEC). The survival of an O111 SLTEC isolated from a patient during this outbreak was compared in laboratory models with those of other *E. coli* strains.

The resistance of the O111 strain to lactic acid in the presence or absence of salt (a_w 0.90) in broth was within the the range shown by 3 *E. coli* O157 strains and a non-toxigenic strain. The ability of this O111 strain to survive during the production of a variety of fermented meats was also measured. The physiological state of the inoculum and the physicochemical parameters during fermentation and maturation influence the extent of this survival.

(30) CANADIAN PERSPECTIVES ON VTEC INFECTION

J. Wilson, Laboratory Centre for Disease Control, Health Canada, and University of Guelph, Guelph, Ontario N1G 2W1 Canada

Infection with verocytotoxigenic *E. coli* continues to be a significant cause of disease in Canada, despite a gradual decline in the reported animal incidence of this condition in recent years. The majority of reported infections are caused by *E. coli* O157:H7, although most VTEC isolated from cattle and ground beef are of other serotypes. Studies of the pathogenicity of non-O157 VTEC continue to be limited by the absence of an effective surveillance system for these organisms.

Recent outbreak investigations have identified consumption of water from shallow rural wells and direct contact with cattle as risk factors for infections with *E. coli* O157:H7. In addition, a five year retrospective spatial analysis of VTEC infections reported in Ontario has identified rural areas having high cattle densities as the highest risk areas for human VTEC

infection in the Province. Furthermore, serologic studies have determined that rural residents in Ontario have higher levels of antibody to VT1, an O157 antigen, than their urban counterparts, implying a higher degree of exposure to VTEC organisms. In prospective studies, asymptomatic fecal carriage of VTEC, including *E. coli* O157:H7 has been identified in farm family members and beef abattoir workers. This, along with the results of case-control studies, has led to an increasing emphasis on the importance of person-to-person spread in the epidemiology of VTEC infection.

In a serologic study of 1436 dairy cattle, the prevalence of antibodies to O157 antigen was 89% in cows and 49% in calves, suggesting that studies of *E. coli* O157:H7 culture status in cattle may significantly underestimate the true level of carriage of these organisms in the bovine population. Longitudinal studies of Ontario dairy cattle have demonstrated that bovine *E. coli* O157:H7 carriage is transient at the farm and animal levels and that cattle, not the farm environment, constitute the major reservoir for these organisms on dairy farms. Small farms having traditional management practices continue to be at highest risk of harboring VTEC organisms.

(31) SHIGA TOXIN-PRODUCING *E. COLI* INFECTIONS IN CONTINENTAL EUROPE

H. Karch, Institute of Hygiene and Microbiology of the University of Würzburg, Josef Schneider Street 2, D-97080, Würzburg, Germany

During the last decade there have been many reports from Europe describing the severity, and also the extreme variability, of the illness caused by Shiga toxin-producing *Escherichia coli* (STEC). The number of people affected in outbreaks in Europe has ranged from less than 10 to more than 100. The magnitude of the public health problem posed by STEC in Europe can only be roughly estimated because the reporting regulations for illnesses vary widely from country to country. In a recent prospective study from Germany in hospitalized children with diarrhea, STEC infections were the second most common bacterial causes of diarrheal diseases. Moreover, multicentre studies from France, Italy, Belgium, and Germany showed that both O157 and non-O157 STEC are a frequent cause of diarrhea and hemolytic uremic syndrome (HUS). There appear to be geographical differences in serotype prevalence. Among the non-O157 STEC associated with human disease, the serotypes O26:H11, O26:H$^-$, O103:H2, and O111:H$^-$ are most frequent. In addition, sorbitol fermenting O157:H7 strains have been described in Germany as an important cause of disease. Between 1985 and 1994 most STEC infections occurred during summer but in 1995 in Germany there was a high peak in winter. In terms of geographical distributions it is notable that since 1987 several areas of Germany have had significant increases in classical HUS.

Farm animals, especially cattle, have been identified as a reservoir for STEC O157:H7, O26:H11, O111:H$^-$, and O22:H8. These serotypes frequently carry classical stx1 and/or stx2 together with eaeA and EHEC-hly genes. Infections of humans with these serotypes could be linked to consumption of contaminated food. However, comprehensive surveys of the incidence of these serotypes in meat and dairy foodstuffs using conventional methods have consistently shown a very low incidence of these organisms. The majority of STEC isolated from animals and foodstuffs belong to other serotypes and usually express variants of stx2. These strains lack eaeA and other putative virulence genes.

The extensive movement of people and foodstuffs within and between different European countries makes STEC infection a cross-border problem. Unfortunately, in continental Europe efforts to survey and control STEC are not yet as intensive as those in North America, although a number of initiatives have recently been undertaken to more accurately describe the epidemiology of infection in Europe and to standardize methods for surveillance and laboratory investigations. It is already evident that with the immunomagnetic separation (IMS) method a more sensitive procedure for the isolation of O157 is available, and this method is expected to improve the level of correct diagnosis. This technical advance coupled with extensive case-control studies by a European study group should result in more adequate epidemiologic data. These findings in turn can be used to improve food safety and other hygienic measures that may be used to slow down or prevent the further spread of this pathogen.

(32) PERSPECTIVE ON SHIGA-LIKE TOXIN INFECTIONS IN ARGENTINA

E. L. Lopez, Hospital de Ninos "Ricardo Gutierrez," Joaquin V. Gonzalez 1649, 1407 Buenos Aires, Argentina

Argentina has the highest frequency of hemolytic uremic syndrome (HUS) in the world (a conservative estimated incidence of 300 cases per year). The risk of HUS in children from 6 to 48 months old, on the basis of census data, is approximately 22/100,000 in Buenos Aires. In Argentina, HUS is the most frequent cause of acute renal damage and one of the most important causes of chronic renal injury in children.

In our prospective studies, we have show during the spring-summer season that the incidence of Shiga-like toxin (SLT)-associated bloody diarrhea in children less than 5 years old is 30-39%. This means that Argentina also has one of the highest incidences of SLT-bloody diarrhea, which probably at least partially explains the high incidence of HUS in the country. We also have recently described the risk of HUS in SLT-associated bloody diarrhea (about 4-5%): 14% of children with SLT-diarrhea developed incomplete HUS. Our previous studies also showed that household

contacts of children with HUS are commonly colonized with SLT-producing *E. coli* (72%) and seroconversion occurs in 42% of them. No evidence of SLT was observed in feces of healthy children.

Although *E. coli* serotype O157:H7 is the predominant pathogen of the enterohemorrhagic *E. coli* (EHEC) group in the United States and other areas, in Argentina it has been associated with only 2-18% of HUS patients and 4.5-7% of children with bloody diarrhea. Other serotypes such as O11:NM, O15:H$^-$, O2:NM, O25:H$^-$, O75:NM, and O1:NM were also recognized.

In Argentina, meat is consumed from early in life: about 20% of Argentine children start to eat meat at 5 months old, and 80% of them have meat in their diets at least three times a week. Eighty percent of the meat is undercooked.

Few data about the incidence of SLT-producing *E. coli* in cows in our country are available. *E. coli* O157:H7 was isolated in only 7.7% of calves aged 1-3 weeks with bacillosis from different farms in Argentina; other strains of different serotypes were also isolated from the calves, but none were toxigenic. Preliminary data show that SLT-producing *E. coli* were present in fresh retail ground beef, determined by polymerase chain reaction (PCR). Further studies are ongoing in order to obtain more data.

(33) THE INVESTIGATION AND CONTROL OF VTEC IN THE UNITED KINGDOM: AN OVERVIEW

N. A. Simmons, Keats House, Guy's Hospital, 24-26 St. Thomas Street, London SE1 9RT United Kingdom

In the United Kingdom, the Advisory Committee for the Microbiological Safety of Food set up a working group in 1992 to assess the significance of verotoxigenic *E. coli* (VTEC) as a foodborne pathogen and to advise on any action that could be taken to reduce the foodborne disease associated with it.

The group reported in 1995. Its report includes chapters on the clinical spectrum of VTEC disease, its epidemiology in the United Kingdom and elsewhere, laboratory methods, and VTEC in foods. It made 20 recommendations for the management of outbreaks, support for research, surveillance studies in man and animals, and food control measures. These recommendations have been largely accepted and will be reviewed in the presentation.

(34) OVERVIEW OF VTEC IN THE UNITED STATES

P. I. Tarr, Division of Gastroenterology, Children's Hospital and Medical Center and Department of Pediatrics, University of Washington School of Medicine, 4800 Sand Point Way, N. E., Seattle, WA 98105

Escherichia coli O157:H7 remains the most important Shiga-toxigenic *E. coli* in the United States from a medical standpoint. This organism is the leading cause of the postdiarrheal hemolytic uremic sydrome, and is relatively easy to detect using standard microbiologic techniques. However, there are clinical and epidemiological data to suggest that a selected few of the many non-O157:H7 Shiga-toxigenic *E. coli* found in food and animals may be pathogenic for humans. Unfortunately, available methods to detect these organisms are not easily adaptable to community clinical microbiology laboratories. Moreover, it is not certain which auxiliary traits render such common toxigenic organisms virulent for humans. Therefore, study of virulent non-O157:H7 Shiga-toxigenic *E. coli* in food and animals must await an improved definition of pathogencity in well designed, prospective, controlled, clinical, and epidemiologic studies.

(35) NUMERICAL METHODS TO DETERMINE SUITABILITY OF *LISTERIA MONOCYTOGENES* RIBOTYPE PATTERNS FOR NORMALIZATION AND MATCHING

B. J. Tenge,* K. C. Jinneman, N. L. Dang, F. S. Fry, W. E. Hill and M. M. Wekell, Seafood Products Research Center, USFDA, P.O. Box 3012, 22201 23 Drive SE, Bothell, WA 98041-3012

The accuracy of pattern matching results between ribotype patterns will be enhanced by using those patterns meeting suitability requirements for accurate and precise migration position normalization of component bands. Numerical methods are investigated to determine methods that assure properly normalized patterns. Peak-position/peak-area reports are generated from densitograms of *L. monocytogenes* ribotype gel banding patterns. The actual digitally measured banding positions of fragments are used for the numerical operations. *L. monocytogenes* strain patterns tested have mostly shown reproducible linear ranging behavior for the gel conditions used (that is, one pattern must be successfully linearly expanded or contracted to compare with other patterns). Relative migration position ratio measurements search for and identify naturally occurring internal reference bands across strain patterns. Secondary transformation base pair lengths indicate that cross-strain reference bands at approximately 3.8, 2.2 and 2.0 kb are most prevalent. Further suitability testing uses least squares linear regression methods to compare new test patterns' actual band positions from peak reports against *L. monocytogenes* reference positions from test replicates or reference strains. The residuals about the regressions and successful pattern matching results coincide to recommend suitability thresholds based on regression performance statistics.

(36) DIFFERENCES IN ELISA REACTIONS OF MONOCLONAL ANTIBODIES EM-6E11 (GENUS-SPECIFIC) AND EM-7G1 (SPECIES-SPECIFIC) AGAINST LIVE AND HEAT KILLED CELLS OF *LISTERIA* AND *LISTERIA MONOCYTOGENES*

R. Nannapaneni,* R. Story, A. K. Bhunia and M. G. Johnson, University of Arkansas, Fayetteville, AR 72704

Listeria genus-specific MAb EM-6E11 recognizing an epitope on both 43 and 94-97 kDa cell surface antigens, and *L. monocytogenes* species-specific MAb EM-7G1 recognizing an epitope on 66 kDa cell

surface antigen, were examined for their variability in reactions against all serotypes of *Listeria monocytogenes* (*L.m.*) and against *L. ivanovii, L. innocua (L. inn), L. seelgeri (L.s), L. welshimeri, L. grayi (L.g)* and *L. murrayi.* The bacterial cells, after being enriched in brain heart infusion (BHI) broth for 24 h at 37°C, were examined as live or heat killed cells (100°C for 20 min) by antigen-coated ELISA. MAb EM-6E11 recognized all *Listeria* species exhibiting greater reactions against *L.m.* 3b, 3c, 4c and 7, *L.inn.* 4ab, *L.s* and *L.g.* but with lower reactions against *L.m.* 3a and 4d compared with other *Listeria* spp. MAb EM-6E11 reactions decreased by 27-74% against heat killed cells of most *Listeria* species versus live cells, and there was a complete loss of detection of heat killed *L.m.* 4d. MAb EM-7G1 detected 10 of the 13 serotypes of *L. monocytogenes* without recognizing other *Listeria* species. MAb EM-7G1 exhibited greater reactions against *L.m.* 3a, 3c and 7 compared with other *L.m.* serotypes, whereas it did not detect *L.m.* 3b, 4a and 4c. MAb EM-7G1 reactions were decreased by 53-79% with heat killed cells of all *L.m.* serotypes versus live cells, but its species specificity was retained without exhibiting any cross-reactions against heat-killed cells of other *Listeria* spp.

(37) EVALUATION OF FIVE METHODS FOR DETECTION OF *LISTERIA* SPECIES IN MARKET MUSSELS

D. K. Jeong,* C. I. Chung, D. W. Gu and E. S. Nam, Department of Nutrition, Kosin University, Pusan 606-701 Korea

The presence of *Listeria* species on the outside surface of mussels was evaluated by using the USDA, FDA, modified FDA with Fraser broth (MFDA), Malthus and modified cold enrichment (MCE) methods. Mussel samples (n=420) were collected from local markets in Seoul, Korea. A total of one hundred and thirty-six (20.7%) *Listeria* spp. were isolated from the 420 samples. *L. innocua* was the most abundant *Listeria* spp., numbering 87 of the 136 isolates. The numbers of isolated *L. monocytogenes, L. murrayi* and *L. welshimeri* were 25 (6.0%), 20 (4.5%) and 4 (1.0%), respectively. The number of isolated *Listeria* spp. by the Malthus method was 32, which gave the highest recovery among the 5 detection methods. The isolated numbers of *Listeria* spp. by USDA, FDA with LPM agar, FDA with Oxford agar, MFDA with LPM agar, MFDA with Oxford agar, and MCE method were 24, 9, 14, 15, 19, and 12, respectively. The MFDA method with Fraser broth showed better recovery of *Listeria* spp. than the FDA method. The results of this study suggest that the Malthus and USDA methods are the most feasible methods for the detection of *Listeria* spp. in market mussels.

(38) ANTIMICROBIAL AGENTS INCORPORATED IN EDIBLE FILMS TO CONTROL MICROBIAL GROWTH

J. Graber,* M. Schnepf, S. Sumner, S. Cuppett and C. Weller, University of Nebraska, 202 Ruth Leverton Hall, Lincoln, NE 68583

A starch-based edible film in combination with antimicrobial agents was examined for inhibition of *Listeria monocytogenes* and effects on shelf life of fresh round steak. Fresh round steak was aseptically cut into 5 × 5 cm pieces. Meat samples were inoculated with 1.0×10^5 CFU/cm^2 *L. monocytogenes* and subjected to a filming treatment. Treatments included no film (I); control film (CF); 2% acetic acid spray followed by a 3% hydrogen peroxide spray (AA); control film with nisin at 150 μg/ml (F150); and treatment AA in combination with the nisin film (AF). The control film was prepared by dispersing sodium alginate (1%) into sterile water, adding a modified starch (2.5%), and heating to a boil. Nisin, if used, was incorporated prior to the sodium alginate. The film was set in an 8% calcium chloride solution. The meat samples were stored at 5°C for 1, 2 or 3 days. Samples were enumerated on modified Oxford media. For the shelf-life study, uninoculated meat samples received the same film treatments and were stored at 4°C and 12°C. Samples were analyzed for total plate count on days 0, 1, 2 and 3, and on 0, 3, 6, 9 and 12, for 4°C and 12°C samples, respectively. For inoculated samples, treatments I and CF had 0.3 and 0.2 log reductions on day 3, whereas F150, AA and AF had 3.2 log reductions for day 3. Shelf-life results for 4°C samples indicate AF exhibited the lowest log increase over time. Treatments I and CF had 3.7 and 2.9 log increases by day 12 whereas AF had a .7 log increase. For the 12°C samples, F150 had a log increase of 1.15 and AA .9 but AF had a 1.25 log decrease on day 3.

(39) INFLUENCE OF TEMPERATURE AND PREINCUBATION TEMPERATURE ON SURVIVAL OF *LISTERIA MONOCYTOGENES* AT PH 4.8

M. Gay,* K. R. Davey and O. Cerf, ASEPT, B. P. 49, 53020 Laval Cedex, France

Listeria monocytogenes is a foodborne pathogenic bacterium. This microorganism can be found in soft cheese. At the beginning of ripening, pH is about 4.85-4.90. The aim of this work was to study the influence of temperature and preincubation temperature (temperature at which the inoculum was cultivated) on survival of *L. monocytogenes* (strain Scott A) at pH 4.8. Survival studies of *L. monocytogenes* were made in a laboratory broth. Four incubation temperatures were studied (2°, 6°, 10° and 14°C) and two preincubation temperatures were tested (30°C or the studied temperature). Decimal reduction time was about 11 days

at 2°C with a preincubation at 2°C; 9 days at 6°C with a preincubation at 6°C; 4 days at 6°C with a preincubation at 30°C; and 1 day at 14°C with a preincubation at 14°C. Results obtained show that survival of *L. monocytogenes* (strain Scott A) at pH 4.8 depended on both temperature and preincubation temperature.

(40) SIGNIFICANCE OF PREINCUBATION TEMPERATURE AND INOCULUM SIZE ON GROWTH OF *LISTERIA MONOCYTOGENES*

M. Gay,* K. R. Davey and O. Cerf, ASEPT, B. P. 49, 53020 Laval Cedex, France

Listeria monocytogenes is able to grow in soft cheese. Two strains of *L. monocytogenes* (Scott A and V7) and one strain of *L. innocua* (Lin 11) were cultivated in a laboratory broth at 14°C and at pH 5.9. These conditions are those found during ripening of soft cheese. This study deals with the effect of the initial bacterial concentration (10 or 10^3 CFU/ml) and the preincubation temperature on subsequent growth. Preincubation treatments were 14°C for 96 h or 30°C for 48 h. Both regimes resulted in approximately the same level of inoculum and phase of growth. Samples containing about 10^3 CFU/ml were enumerated in duplicate by a pour plate method. Samples containing about 10 cells/ml were enumerated by the most probable number (MPN) technique. The MPN technique with 96 replicates was found to give statistical results as good as the direct plating technique when bacterial concentrations were sufficiently high to permit direct comparison. The detection threshold is 1 CFU in 5 ml with MPN. Results obtained show there is an influence of both studied factors on growth of *Listeria*. When inoculum was preincubated at 14°C, duration of the lag phase was about 1 day with the low initial bacterial concentration (10 cells/ml) and less than 23 h with the high initial bacterial concentration (10^3 CFU/ml). An increase in the duration of the lag phase with the low initial concentration was observed if the inoculum was cultivated at 30°C, in which case duration of the lag was 8 days. Growth of *Listeria* is affected by size, growth temperature, and stress of inoculum.

(41) THERMAL DESTRUCTION OF *LISTERIA INNOCUA* IN SOLID MUSCLE BEEF OR CHICKEN

J. H. Goff,* M. Christie, R. Story and M. G. Johnson, Departments of Food Science, Biological & Agricultural Engineering, and Center for Food Processing and Engineering, University of Arkansas, 272 Young Avenue, Fayetteville, AR 72704

Listeria innocua cells at 10^4 CFU gelled in calcium alginate beads were placed in the geometric center lines of peeled beef knuckle (2.5 cm thick) or on the surface of one side of a split chicken breast and covered by folding over the second side. Thermocouple probes were inserted in the beef or placed between the breast halves such that probe tips were perpendicular to the lines of inocula beads. After heating with dry or moist heat, beads were removed from meat pieces just at oven exit. *L. innocua* survivors in excised beads were determined by mashing the beads, decimally diluting, plating on TSA with 0.6% yeast extract, then incubating at 30°C for 48 h before counting. Using a minimum temperature of about 50°C (122°F) as being needed to cause cell killing, the areas under heating curves formed by plots of muscle temperature X time in min were calculated as total °C- min or F- min and correlated with the amounts of cell kill (Zaika et al., 1990, *J. Food Prot.* 53:18–21). In beef, a total kill of the *L. innocua* was achieved after exposure of muscle to 991.1°C- min (1857°F- min) when the rate of temperature increase was 3.21°C/min. There were survivors for center pieces experiencing °F- min values of 1550-1852. In chicken, a total kill of *L. innocua* was achieved after exposure of muscle to 815.6°C- min (1500°F- min.) when the rate of temperature increase was 4.15°C/min. The results reported here are consistent with those of Zaika et al. for *L. monocytogenes*-inoculated frankfurter emulsions in which 1,500°F- min was sufficient to kill only about 3 log cycles of such cells.

(42) EFFECT OF SOME ADDITIVES USED IN MEAT PRODUCTS ON BEHAVIOR OF *LISTERIA MONOCYTOGENES*

R. M. Raybaudi* and A. J. Martínez, Instituto de Ciencia y Tecnología de Alimentos, Universidad Central de Venezuela, P.O. Box 47.097, Caracas 1041 A, Venezuela

The effect of different levels of NaCl, $NaNO_2$, dextrose, skim milk, paprika, black pepper, nutmeg and garlic upon *L. monocytogenes* was studied *in vitro* using trypticase soy broth with yeast extract. Additionally, the fate of *L. monocytogenes* on hard salami inoculated with 10^6 CFU/g of *L. monocytogenes* was studied. The results suggest that increased dextrose, skim milk, black pepper and paprika levels did not have a significant effect upon *L. monocytogenes*. Levels of 2% garlic, 0.5% nutmeg and 100 ppm $NaNO_2$ completely inhibited the growth of *L. monocytogenes*. The levels of *L. monocytogenes* decreased 2 $\log_{10}$ units during aging of hard salami. Lactobacillus levels and pH value did not change significantly during aging of salami, suggesting that a_w and mixture of spices and curing salts could play an important role in reduction of *L. monocytogenes*.

(43) EVALUATION OF RAPID DNA EXTRACTION METHODS FOR DETECTION OF *LISTERIA MONOCYTOGENES* IN DAIRY PRODUCTS USING THE TAQMAN SEQUENCE DETECTION SYSTEM

T. Cox,* R. Behari, S. J. A. Flood, C. T. Yamashiro, C. Paszko-Kolva and R. J. Cano, California Polytechnic State University, Department of Biological Sciences, San Luis Obispo, CA 93407

DNA extraction procedures were compared as to their ease of use and compatibility with PCR methodologies for the detection of *Listeria monocytogenes* in dairy products, including whole and raw milk and various cheeses. The DNA extraction meth-

ods evaluated utilized Chelex® 100, guanidinium isothiocyanate/silica, or EnviroAmp® Sample Preparation Kit. These methods were specifically modified for high throughput sample analysis compatibility, as well as effective cell lysis and PCR inhibitor removal. The two PCR primers and a doubly labeled fluorescent probe, whose sequence was located between the two primers, were specific for the listeriolysin O (*hlyA*) gene of *L. monocytogenes*. The probe in the PCR allows for increased specificity and the direct detection of PCR product by the TaqMan™ Sequence Detection System and is amenable to high throughput analysis. The procedures were evaluated in a controlled study using artificially contaminated as well as uninoculated food samples. Sensitivity values for each method were established by gel electrophoresis and the TaqMan™ Sequence Detection System. The results obtained with this gel-free fluorescent assay compared favorably with culture methods and indicated that the efficiency of cell lysis and inhibitory removal for each extraction was dependent upon the type of dairy product analyzed. When used in conjunction with the appropriate DNA extraction procedure, the fluorescent detection of PCR products with the TaqMan™ Sequence Detection System allows for the rapid and simple screening of *L. monocytogenes* in dairy products.

(44) SURVEY ON *LISTERIA* SPP. CONTAMINATION OF KOREAN MARKET PORK

C. I. Chung,* D. W. Gu and D. K. Jeong, Konkuk University, Dairy Science Department, 93-1 Mojin Dong, Kwangjinker, Seoul, Korea

The presence of *Listeria* spp. in Korean market pork was evaluated by using the USDA, FDA, and Malthus methods. Pork samples were collected from local meat shops in Seoul, Korea. One hundred and thirty-three (55.4%) *Listeria* spp. were identified, including *L. monocytogenes*, with the highest detection rate of 31.6%, *L. innocua* 10.0%, *L. murrayi* 6.7%, *L. welshimeri* 4.6%, and *L. seeligeri* 6.7% respectively. But no *L. ivanovii* or *L. grayi* were detected in fresh pork samples. Detection rate of *Listeria* spp. by the USDA, FDA and Malthus methods were 70% (42/60), 68.3% (41/60), and 41.6% (50/150), respectively, and that of *L. monocytogenes* by USDA, FDA and Malthus methods were 46.7% (28/60), 38.3% (23/60) and 20.8% (25/1200), respectively.

(45) PREDICTIVE MODELING OF *LISTERIA* SPP. INACTIVATION IN WHOLE BOVINE MILK IN A HIGH-TEMPERATURE, SHORT-TIME PASTEURIZER

R. C. McKellar,* P. Punidadas and S. Liou, Centre for Food and Animal Research, Agriculture and Agri-Food Canada, Ottawa, Ontario K1A 0C6 Canada

Data was obtained on inactivation of *Listeria innocua* in whole milk in a pilot plant high-temperature short-time (HTST) pasteurizer. A computer program was designed to calculate the integrated lethal effect, or pasteurization effect (PE), using a modified Arrhenius equation. Times and temperatures in each section of the pasteurizer were integrated by an iterative search method for holding times and temperatures of 3-60 s and 60.5-69.5°C. A linear model was derived from 5 trials which related values of PE to log % residual counts (individual r^2 values of 0.728 to 0.995). Three validation trials performed with *L. monocytogenes* indicated that this pathogen was less heat resistant than *L. innocua*. Risk analysis simulations were performed using the Lotus 1,2,3 add-in @RISK to determine the probability of achieving a 4-log reduction of *L. monocytogenes*. These results suggest that predictive equations based on PE could be used to assess the effectiveness of commercial pasteurization processes in reducing the hazard posed by *L. monocytogenes*.

(46) SURVIVAL AND GROWTH OF *LISTERIA MONOCYTOGENES* SCOTT A IN BEEF AND PORK STORED AT DIFFERENT TEMPERATURES

A. Castillo,* N. E. Martinez-Gonzales and M. O. Rodriguez-Garcia, Texas A & M University, Department of Animal Science, 310 Kleberg Center, College Station, TX 77843-2471

Listeria monocytogenes is known to grow at low temperatures. In many places, meat is exposed at room temperature during sale, increasing the chance that this organism will grow. In this work, the behavior of *L. monocytogenes* Scott A in pork and beef stored at 4°C and 20°C was studied. The effect of changes in counts of lactic acid bacteria (LAB) and total psychrotrophic bacteria (TPB) on the survival of this pathogen was also studied. Portions of beef and pork were inoculated on the surface with 10^3 or 10^4 CFU/g *L. monocytogenes* Scott A and stored at 4°C or 20°C. Counts of *L. monocytogenes*, LAB and TPB, pH determination and sensory evaluation for off-odors were carried out at specific intervals. At 4°C, there was no increase in the numbers of *L. monocytogenes* on beef stored for up to 10 days, whereas in pork this pathogen showed a 1-log reduction in the same period. The growth of LAB and TPB were similar in beef and pork; therefore, the decrease in the *L. monocytogenes* counts in pork was not attributed to the growth of LBA or TPB. Sensory evaluation showed signs of spoilage from the 9th day of storage at 4°C for both types of tissue. At 20°C, a two-log increase was observed in the numbers of *L. monocytogenes* on both types of meat in 48 h of storage. The spoilage was detected by sensory evaluation during the first 24 hours of storage. No significant changes in pH were observed at 4°C or 20°C. The better survival of pathogens in pork than in beef has been observed in previous work.

(47) DISINFECTION EFFICACY AGAINST PURE-CULTURE AND MIXED-POPULATION BIOFILMS OF *LISTERIA INNOCUA* AND *PSEUDOMONAS AERUGINOSA* ON STAINLESS STEEL, TEFLON® AND RUBBER

F. Bourion* and O. Cerf, ASEPT, B. P. 49, 53020 Laval Cedex, France

The attachment of microorganisms is a common phenomenon throughout nature. Bacteria can stick to surfaces ranging from human teeth to submerged rocks. In food processing environments, spoilage or pathogenic microorganisms can attach to surfaces and proliferate into microcolonies; biofilms may form over time. Attached bacteria are a potential source of contamination and may cause food spoilage or transmission of diseases. As adherent microorganisms may adapt physiologically to this new environment, they have been shown to be more resistant to antibiotics and disinfectants than planktonic cells. Biofilm formation by *Listeria innocua* and *Pseudomonas aeruginosa* alone or in mixed culture was examined under dynamic conditions, on three materials commonly used in the food industry: stainless steel, Teflon® and rubber. The type of surface influenced biofilm development and production of extracellular materials. *L. innocua* was able to establish itself and grow among *P. aeruginosa* cells in mixed culture biofilms but couldn't maintain itself in single culture biofilms. The proportion of *L. innocua* in mixed materials reached about 32% on Teflon® against only 5 to 7% on steel or rubber. The effectiveness of two types of disinfectants was examined. Resistance to sodium hypochlorite and peracetic acid was strongly influenced by the type of surface and the structure of biofilms. *L. innocua* cells were much less sensitive to disinfectants in mixed biofilms where they benefited from the presence of *P. aeruginosa* cells.

(48) EFFECT OF TEMPERATURE AND PH ON THE GROWTH OF *LISTERIA MONOCYTOGENES* ON PORK PACKAGED IN CO_2

P. W. Bodnaruk* and B. J. Shay, Dept. of Food Science and Technology, University of Tennessee, Knoxville, TN 37901

Slices of pork striploin were inoculated with *Listeria monocytogenes*, vacuum skin packed in a highly permeable film, packaged in masterpacks filled with 100% CO_2 and stored at 0° and 5°C. *L. monocytogenes* cultures used for inoculation were grown at either 37°C/24 h or 5°C/10 days in TSB supplemented with yeast extract (0.2%) and glucose (0.2%). On high pH pork (pH>6) numbers of *L. monocytogenes* increased 3.0 $\log_{10}$ CFU/cm² when stored at 5°C for 48 days. At 0°C storage, *L. monocytogenes* numbers on high pH pork remained relatively constant over the 63 day storage period. *L. monocytogenes* numbers increased 2.9 $\log_{10}$ CFU/cm² on normal pH pork (pH<5.8) stored at 5°C for 48 days. Normal pH pork stored at 0°C appeared not to promote the growth of *L. monocytogenes*, numbers decreasing 0.8 $\log_{10}$ CFU/cm². The same trends were observed on sterile high pH pork inoculated with *L. monocytogenes*, indicating the normal meat microflora had a negligible effect on the growth of *L. monocytogenes* under the packaging and storage conditions described. The growth pattern of cold-adapted *L. monocytogenes* on pork indicated the organism did not possess any growth advantage when compared to *L. monocytogenes* cultures grown at 37°C. Cold-adapted *L. monocytogenes* demonstrated a 15 day longer lag period on normal pH pork stored at 5°C than cultures grown at 37°C.

(49) MICROBIAL COMPETITION: SUPPRESSION OF *LISTERIA MONOCYTOGENES* GROWTH BY *PSEUDOMONAS FLUORESCENS*

L. K. Bagi* and R. L. Buchanan, USDA-ARS-ERRC, 600 East Mermaid Lane, Wyndmoor, PA 19038

L. monocytogenes Scott A, alone and in co-culture with *Pseudomonas fluorescens* 33231, was studied to characterize the effects of microbial competition on the pathogen's growth. The bacteria were cultured in BHI broth, with a factorial design used to assess the impact of temperature (4°, 12°, 19°C) pH (5, 6, 7) and NaCl (5, 25, 45g/L). Samples were plated on Vogel Johnson and BHI agars to obtain *L. monocytogenes* and total counts, respectively. Growth curves were generated using the Gompertz equation. The primary effect of *P. fluorescens* was a suppression of the maximum population density (MPD) reached by *L. monocytogenes*. Under specific conditions, this was as great as a 10,000-fold MPD suppression, whereas under other conditions there was no suppression. Low incubation temperatures, low sodium chloride levels, and a neutral pH favored suppression. The effect appeared related to the impact of cultural conditions on the species' relative growth kinetics, an interaction noted in earlier studies with *L. monocytogenes* and *C. piscicola*.

(50) EVALUATION OF A NEW RAPID SCREENING TEST FOR *LISTERIA*

J. Gebler, Murray Goulburn Co-operative Co. Limited, 40 Commercial Road, Yarram, Victoria 3971, Australia

In routine monitoring programs in many food processing plants, it is necessary to quickly screen environmental samples of *Listeria*. Results from traditional cultural methods are too slow to enable rapid corrective action to be taken. A new screening meth-od developed by TECRA DIAGNOSTICS, the UNIQUE™ LISTERIA test, enables results to be obtained in only 32 hours using immunoenrichment technology. In this study, 199 routine environmental swabs from dairy factories were tested using the UNIQUE™ LISTERIA test and results were compared with the AOAC-approved TECRA® VIA method. Using UNIQUE™, 29 samples were found to be positive

and 25 were culturally confirmed. Four could not be confirmed directly from the UNIQUE™ module, but these were all shown to contain *Listeria*, by both the VIA and cultural methods. Otherwise, no unconfirmed positives or false negatives were recorded. The UNIQUE™ LISTERIA method was found to compare favorable with the AOAC-approved ELISA method. The ability to obtain a result in 32 hours, the convenient single-test and the ease of use make this test a useful tool for environmental monitoring.

(51) EVALUATION AND APPLICATION OF *LISTERIA MONOCYTOGENES* SPECIFIC ANTIBODIES

P. Schubert,* K. Kramer and A. Bubert, MERCK, KGaA, LPRO, UBA, Fo, 64271 Darmstadt, Germany

Recent outbreaks of listeriosis in humans have highlighted the importance of *Listeria monocytogenes* in contaminated food. Therefore, we developed different antibodies for the specific detection of *Listeria monocytogenes*. In this study we present a sandwich ELISA, including polyclonal or monoclonal antibodies, that were derived from the extracellular protein p60 of *Listeria monocytogenes*. Using our polyclonal antibodies (PepA, PepD), all of over 150 different wild-type strains of *Listeria* have been identified correctly, even ß-hemolysis-negative strains of *Listeria monocytogenes*. The method can be used with confidence to detect levels of about 10^6 CFU/ml. Similar results have been obtained with the monoclonal antibodies (mAb) regarding sensitivity and specifity, but mAb generated a five-fold reduction in background values with no cross-reaction to non-target organisms.

(52) PETRIFILM™ *LISTERIA* COUNT PLATE: A HIGHLY SELECTIVE METHOD FOR THE QUANTITATIVE RECOVERY OF *LISTERIA* FROM ENVIRONMENTAL SAMPLES

G. P. Sandberg,* M. Tochacek and R. F. Young, 3M, 3M Center, Building 270-3N-04, St. Paul MN 55144

A new dry rehydrated film method (PLP) has been developed which can quantify *Listeria* species in 24 hours, from environmental samples. The new method does not require a pre-enrichment step or special equipment. This easy, convenient, and highly selective method recovers both healthy and sublethally injured *Listeria* species. Recovery of *Listeria* with the new method equals and frequently exceeds recovery with traditional methods which utilize MOX, OXFORD and PALCAM agars, as well as rapid ELISA detection methods. We have run over 300 environmental swab and sponge samples. The samples have been run pre-enriched in the agar and ELISA methods (3 to 5 days) and then compared to our direct plating method (24 hours) which does not utilize an enrichment step. 36% of the samples have been negative by all methods, 48% have been positive by all methods, 3% of the samples have been negative by our test but positive by the other methods, and 13% of the samples have been positive by our rapid direct plating method, while the other methodologies were negative for *Listeria* growth. *Listeria* have been confirmed on the environmental samples which have tested positive by any of the detection methods used.

(53) TIME TO TOXIN PRODUCTION BY NONPROTEOLYTIC *CLOSTRIDIUM BOTULINUM* AS AFFECTED BY ENVIRONMENTAL FACTORS

P. H. Elliott* and D. W. Schaffner, Campbell Soup Co., Camden, NJ 08053

Time until neurotoxin production by nonproteolytic *Clostridium botulinum* was evaluated as a function of storage temperature (7-13°C), pH (5.75-6.5), % NaCl (0.25-1.75%) and spore inoculum level (10^0-10^5 spores/tube). Pooled spore inoculum (4 type E and 4 type B) were inoculated into prereduced TPGY broth adjusted to the appropriate pH and salt concentration. A full factorial design was used, and each combination was replicated nine times. Samples were tested for 60 days or until neurotoxin was evident using an amplified ELISA technique. A statistical (survival analysis) model was developed to predict time to toxin production as a function of the independent variables and their interactions. The most probable number (MPN) of spores required to produce toxin under the various combinations of environmental conditions on each day was used to calculate the probability of a single spore growing out and producing toxin. This information was used to create a generalized linear regression model to predict the probability of toxigenesis from a single spore.

(54) DEVELOPMENT OF A TWENTY-FOUR HOUR METHOD FOR THE DETECTION OF *BACILLUS CEREUS* SPORES IN RAW MILK

J. J. Quinlan* and P. M. Foegeding, North Carolina State University, Box 7624, Raleigh, NC 27695-7624

Bacillus cereus spores present in raw milk are able to survive pasteurization of the milk and may be able to germinate and grow, increasing the potential for spoilage of the pasteurized product and the occurrence of foodborne illness. A semi-quantitative method was developed to demonstrate the feasibility of detecting ≥10 *B. cereus* spores/ml of raw milk within 24 hrs. Immunomagnetic beads coated with a monoclonal anti-*Bacillus* spore antibody were used to isolate *Bacillus* spores from raw milk samples. Once isolated, the spores were heat shocked, plated on TSA and allowed to germinate and grow for 18-20 hrs. Resulting colonies were blotted with Immobilon-P membrane. The membrane was blocked and probed with a monoclonal antibody against a common H-antigen of *Bacillus cereus* cells that also cross-reacted with cells of some other *Bacillus* species. These experiments dem-

onstrated the feasibility of detecting low levels of *B. cereus* spores in raw milk and confirming the species based on serology of the vegetative cells within 24 hours.

(55) DEVELOPMENT OF A PCR ASSAY FOR THE DETECTION OF *BACILLUS CEREUS*

J. Czajka* and C. A. Batt, Department of Food Science, Cornell University, Ithaca, NY 14853

A PCR assay to detect *Bacillus cereus* was developed using base pair differences in the cereolysin AB gene that distinguish *B. cereus* from *B. thuringiensis*. A 700 bp PCR product generated from the cereolysin AB gene was sequenced from two strains of each species to reveal base differences between these species. The sequences differed at nucleotides 816, 825, and 1237. PCR primers were designed using the *B. cereus* sequence, positioning the sequence differences observed at nucleotides 825 and 1237 at the respective 3' base of two convergent PCR primers. These PCR primers, when used with the *Taq* DNA polymerase Stoffel fragment, amplified only the *B. cereus* and not the *B. thuringiensis* cereolysin. Each of the 22 *B. cereus* strains tested produced the 455 bp PCR fragment, whereas the four *B. thuringiensis* strains tested were negative. The minimum sensitivity of the PCR reaction for *B. cereus* was 25 colony forming units. These sequence differences are currently being used to develop a fluorogenic probe assay for the detection of *B. cereus* in nonfat dry milk.

(56) EFFECT OF MODIFIED ATMOSPHERE AND NACL TREATMENT ON THE GROWTH OF *YERSINIA ENTEROCOLITICA* ON MINIMALLY PROCESSED BROCCOLI STORED AT 4°C

P. W. Bodnaruk* and F. A. Draughon, Department of Food Science & Technology, University of Tennessee, Knoxville, TN 37901

Modified atmosphere (MA) packaging is widely used to extend the shelf life of fresh produce by decreasing the rate of respiration. Studies have indicated that alterations in package atmosphere can change the composition of the resident microflora and may enhance the growth of pathogens. This study was undertaken to investigate the growth of *Yersinia enterocolitica* in minimally processed fresh broccoli packaged in MA and stored at 4°C. Fresh broccoli heads were cut into florets, inoculated with 10^4 CFU/g *Y. enterocolitica*, dipped into a saturated salt solution and packaged in PD941 (Cryovac) film under air, vacuum, or MA. *Y. enterocolitica* growth was determined by direct plating on CIN agar. Growth of background microflora was determined using standard methods. *Y. enterocolitica* on NaCl-treated broccoli packaged in air, vacuum and MA grew to 10^7-10^8 CFU/g after 15 days storage, whereas *Y. enterocolitica* on untreated broccoli, packaged in the same manner, increased to 10^4-10^5 CFU/g. Growth of aerobic mesophiles, psychrotrophs, lactic acid bacteria, yeasts and molds were affected by NaCl treatment of the broccoli florets, resulting in more rapid spoilage of the salt-treated product. The type of atmosphere used for packaging broccoli did not have a significant ($P>0.05$) effect on the resident microflora. The shelf life of untreated minimally processed, packaged broccoli was approximately 9-12 days for each atmosphere treatment.

(57) USE OF A SINGLE PROCEDURE FOR SELECTIVE ENRICHMENT FOR PLASMID-BEARING VIRULENT SEROTYPES OF *Y. ENTEROCOLITICA* FROM GROUND PORK

S. Bhaduri* and A. Pickard, USDA-ARS-ERRC, 600 East Mermaid Lane, Wyndmoor, PA 19038

A single, selective enrichment, isolation and recovery procedure was developed for plasmid-bearing virulent serotypes of *Yersinia enterocolitica* (YEP^+) from ground pork. Appropriate dilutions of five different plasmid-bearing virulent strains, GER (O:3), EWMS (O:13), PT18-1 (O:5, O:27), O:TAC (O:TACOMA), and WA (O:8), representing five serotypes of *Yersinia enterocolitica* (YEP^+), were added to ground pork dispersed in modified trypticase soy broth containing yeast extract and bile salts and allowed to stand for 5 minutes. Volume was brought to 30 ml (10 × pork mass) and incubated at 12°C for 24 h. Irgasan (4µg/ml) was added and reincubated at 12°C for another 48 h. Selectively enriched cultures were diluted, plated on CIN and MacConkey's agar for presumptive isolation of YEP^+ and incubated at 28°C for 24 h. Presumptive colonies were streaked on Congo red (CR)-BHI-agarose (CR-BHO) and incubated at 37°C for 24 h. YEP^+ colonies were identified by CR binding technique. Concurrently, virulence of the CR^+ colonies were further confirmed by multiplex PCR using chromosomal *ail* gene (attachment-invasion locus) and *virF* gene (transcriptional activator for the expression of plasmid-encoded outer membrane protein *yop* 51) from virulence plasmid. The enrichment, isolation and identification for all five serotypes of YEP^+ was completed in 5 days using a single procedure. Published methods take at least 14 days and are not specific for the isolation of YEP^+, since not all YE are pathogenic. The identification of YEP^+ on CR-BHO also allows recovery of YEP^+ for further investigation, since, it does not facilitate the loss of virulence plasmid. The new method significantly speeds the identification of pathogenic YE.

(58) ENHANCEMENT OF RECOVERY BY REMOVAL OF BLOOD FROM STANDARD *CAMPYLOBACTER* CULTURE PROTOCOL

D. H. Bark, U.S. Food and Drug Administration, 22201 23 Drive SE, Bothell, WA 98041-3012

Campylobacter media were examined to determine if blood could be removed from standard protocol without compromising growth potential. Results with 2 clinical & 1 type culture demonstrated that broth supplemented with FBP (0.025% each of ferrous sul-

fate, sodium metabisulfite, and sodium pyruvate) significantly improved the recovery ($P<0.01$) of *Campylobacter jejuni*. Recoveries with FBP were, respectively, 4.4, 3.9 and 100,000 times greater than with blood, blood-FBP, or unsupplemented media. The average number of CFU recovered/ml were 2.1×10^7, 4.8×10^6, 5.4×10^6, and 2.1×10^2 for FBP, blood, blood/FBP and unsupplemented media, respectively. Data collected with mixed vibroid and coccal cultures produced similar results. These experiments were conducted in 50 ml erlenmeyer flasks containing 20 ml of nutrient broth #2-0.6% yeast extract and were initiated at approximately 100 CFU/ml. Cultures were incubated statically at 35°C in an atmosphere of the standard *Campylobacter* gas mixture and were enumerated by spread plating log dilutions of overnight growth to plate agar in duplicate. Data were recorded as CFU/ml and were analyzed relative to the value (number of bacteria) achieved for the FBP group. Experiments with 18 selected agars combinations (including agars with and without blood) failed to demonstrate a significant difference in the number of colonies recovered. Addition of either of the iron chelating agents, deferoxamine or EDDHA, inhibited growth on bloodless agars. Results from this study indicate that the absence of blood in the standard protocol did not compromise recovery. In fact, replacement of blood with FBP significantly improved enrichment success.

(59) MULTIPLEX POLYMERASE CHAIN REACTION FOR THE IDENTIFICATION AND DIFFERENTIATION OF *CAMPYLOBACTER COLI* AND *C. JEJUNI*

K. M. Harmon,* I. V. Wesley and G. M. Ransom, USDA-ARS-NADC, Iowa State University, 2300 Dayton Road, Ames, IA 50010

Enteropathogenic campylobacters are recognized as one of the most prevalent causes of human foodborne diarrheal illness, with *Campylobacter jejuni* and *C. coli* most often involved. Differentiation of these two species has traditionally relied on the hippurate hydrolysis test even though the test is only about 90% accurate. We have developed a multiplex polymerase chain reaction (mPCR) assay for the identification and differentiation of *C. coli* and *C. jejuni*. The assay uses two sets of primers and produces a characteristic amplification pattern for each of the two species. To date, 39 strains isolated from raw poultry have been examined in order to validate the efficacy of the method. In all but one case, the mPCR results agreed with those obtained by conventional biochemical methods. Ribotyping confirmed the PCR results. The mPCR assay may provide an alternative to traditional biochemical typing methods for the identification and differentiation of *C. coli* and *C. jejuni*. It is accurate and simple to perform, and it can be completed within 8 hours.

(60) COMPARISON OF SELECTIVE MEDIA FOR PRIMARY ISOLATION OF *CAMPYLOBACTER* USING NUMERICAL AND GRAPHICAL TOOLS TO INDICATE OPTIMAL MEDIA

B. J. Tenge, U.S. Food and Drug Administration, 22201 23 Drive SE, Bothell, WA 98041

The ability of 6 selective media to support growth of thermophilic *Campylobacter* in raw chicken, raw shellfish and raw milk was compared. Food matrices were artificially seeded with *Campylobacter jejuni* ATCC 29428 at three inoculation levels. The selective media included: Karmali agar, Cefex agar, Heart Infusion agar (HIA), Campylobacter Blood Free Selective agar (CBFCSA), Hunt-Abeyta agar (H-A) and Abeyta-Hunt agar (A-H). The media performance comparisons for *Campylobacter* recoveries were made graphically and numerically. Calculated test measures, called differential recovery (DR%) and ratiometric recovery, were used for the comparison. Side-by-side range boxplots visualized trends in media performance amongst media types as modified by inoculation levels. ANOVA from designed experiments further examined the trends in media performance of different media to selectively grow *Campylobacter* over background flora. Considering just low and medium inoculation levels, 12 experimental groups across 3 factors (food matrix, inoculation level, incubation period) were evaluated. HIA and A-H both showed highest performance exhibited by median ranks 1 (median DR% = 100) from the 12 experimental groups. Comparison of blood free media, CBFCSA and Karmali, indicates that CBFCSA had higher performance for supporting growth with a median rank (median DR%=100) from the 12 experimental groups. Comparison of blood free media, CBFCSA and Karmali, indicates that CBFCSA had higher performance for supporting growth with a median rank (median DR%=94) versus median rank 4.5 (DR% = 22.5) for Karmali media.

(61) RAPID DETECTION OF *CAMPYLOBACTER JEJUNI* IN CHICKEN PRODUCTS BY A NESTED PCR ASSAY

D. K. Winters,* A. E. O'Leary and M. F. Slavik, Dept. of Poultry Science and the Arkansas Biotechnology Center, University of Arkansas, Biomass Research Center, Fayetteville, AR 72701

C. jejuni is a major cause of human gastroenteritis, with many cases due to contaminated poultry. Rapid detection of *C. jejuni* by PCR directly from foods, without prior growth steps, would be beneficial for the poultry industry. We have reported a PCR assay that allows detection of this bacterium after plating on Campy cefex agar. We have now developed a more sensitive and rapid nested PCR assay using a second set of primers that specifically detects *C. jejuni* in chicken washes that have not undergone any lengthy growth steps prior to conducting PCR. This method takes only one day to identify *C. jejuni* in contaminated chickens. We detected *C. jejuni* in 80% of 4 sets of 15 chickens using this method. The cells

have been confirmed as such by standard microbiological techniques, by Southern blots with a probe specific for this bacterium and by our standard PCR assay. For the nested reaction, the external set of primers, C-1 and C-4, are used for 24 cycles. At this time, 0.1 µl of the PCR reaction is removed and added to a second reaction. The second PCR reaction is run with C-1 and an internal primer, C-2, for 24 cycles of 95°C for 1 min., followed by annealing at 53°C for 1 min. and elongation at 72°C for 1 min. A single band on a 4% NuSieve Blend agarose gel at 120 bp was apparent with *C. jejuni* cells at a sensitivity of 10^2 CFU.

(62) THE MEASURED HEAT-RESISTANCE OF NON-PROTEOLYTIC *CLOSTRIDIUM BOTULINUM* SPORES IS INCREASED BY ENDOGENOUS LYSOZYME ACTIVITY OF VEGETABLE EXTRACTS

S. C. Stringer* and M. W. Peck, Institute of Food Research, Norwich Research Park, Coloney, Norwich, Norfolk, United Kingdom NR4 7UA

Non-proteolytic *Clostridium botulinum* has the ability to form endospores that survive mild heat treatment and can grow and produce a powerful neurotoxin at a temperature as low as 3.3°C. It is thus a particular concern in minimally processed foods stored for prolonged periods under refrigeration. In order to ensure such products are safe, knowledge of the ability of spores of non-proteolytic *C. botulinum* to withstand heat treatments is required. Adding lysozymes of animal origin to recovery media improves recovery of heat-damaged spores of non-proteolytic *C. botulinum* thereby substantially increasing the measured heat-resistance. However, the importance of endogenous lytic activity in vegetable products had not been studied. We tested a range of vegetable extracts for their ability to promote growth from heat-damaged spores of non-proteolytic *C. botulinum* type B. Unheated spores or spores heated at 85°C for 0.5, 1, 2 or 10 minutes were enumerated on either a standard laboratory medium or the same medium containing one of eight vegetable extracts. Heating spores at 85°C for 2 minutes reduced the viable count by more than 10^4 when recovery was on a standard laboratory medium. However, heating at 85°C for 2 minutes, or even 10 minutes, reduced the viable count by a factor of only 10^2-10^4 when recovery was on media containing turnip, rutabaga, cabbage, potato or flat bean. Vegetable extracts with lytic activity therefore increase the measured heat resistance of spores of non-proteolytic *C. botulinum*.

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

R. N. Okagbue, Applied Biology and Biochemistry Department, NUST, P.O. Box 346, BYO, Zimbabwe

Thirty-eight cream samples from retail doughnuts from Bulawayo outlets were assessed microbiologically for compliance with the City Council's bacteriological standards and to identify probable contaminants. Analysis was by dilution-plating on nutrient, mannitol/milk salt and MacConkey agars. Aerobic plate counts (APC), staphylococcal and coliform counts per gram of 25 samples ranged from 1.6×10^2 to 9.2×10^3, from 1.1×10^2 to 5.6×10^4 and from 1.1×10^2 to 1.2×10^4, respectively. APC from 14 (56%) samples were acceptable but presence of coliforms made all the samples unsatisfatory bacteriologically. The remaining 13 samples were unsatisfatory as judged by their APCs, which ranged from 4.1×10^4 to 2.5×10^4. *Enterobacter aerogenes* (11), *E. cloacae* (3), *Citrobacter freundii* (3), and *Arizona* (1) were among 21(52.5%) coliforms that fermented lactose at 44.5°C. *Staphylococcus aureus* comprised over 80% of 48 staphylococci. *Micrococcus luteus* was found. Contamination of doughnut cream fillings by Micrococcaceae and coliforms suggest inadequate pasteurization or unhygienic handling of cream and necessitate regular microbiological monitoring.

(64) CROSS PROTECTION BY HEAT AND COLD SHOCK TO LETHAL TEMPERATURES IN *C. PERFRINGENS*

J. C. Limón,* N. Heredia and J. Santos Garcia-Alvarado, Universidad Autonoma de Nuevo León, F. C. Biológicas, Monterrey, Mexico 66451

Several bacteria subjected to a mild heat stress showed a protective response that enabled them to survive other stresses such as high salt concentration or lethal osmotic conditions. *Clostridium perfringens* is an important foodborne pathogen that is closely associated with food temperature abuse. In this report we demonstrate that in *C. perfringens*, a heat-shock treatment provides protection against subsequent cold-shock damage, and vice versa. *C. perfringens* FD-1041 was grown in fluid thioglycollate medium at 43°C until the cultures reached the mid log-phase. For cold tolerance analysis, cultures were heat-shocked at 50°C for 30 min and immediately submerged in a water bath at 10°C for selected times. For heat tolerance analysis, the cultures were cold shocked at 15°C for 30 min and immediately incubated at 55°C for selected times. In both cases, survivors were determined by plating anaerobically on a non-selective medium. Our results showed that heat-shocked cells were more cold tolerant (one log reduction in 29 min) than control cells (one log reduction in 18 min). On the other hand, cold-shocked cells were more thermotolerant (D_{55} = 17 min) than control cells (D_{55} = 7 min).

(65) EFFECT OF HEAT SHOCK ON SPORULATION, PROTEIN SYNTHESIS AND ENTEROTOXIN PRODUCTION OF *C. PERFRINGENS*

N. L. Heredia,* J. Santos Garcia-Alvarado and R. G. Labbé, Universidad A. de Nuevo, León, F. C. Biológicas, A. P. 124-F, Sn. Nicolás, Mexico

C. perfringens is responsible for human food poisoning because it produces an enterotoxin during sporulation. Spores can survive hostile environments and subsequently germinate. Several pro-

teins called heat shock proteins (HSP) have been shown to be related to acquisition of thermotolerance. In this study, *C. perfringens* FD-1041 was grown at 43°C in DS medium. After 1 and 3 h of incubation, the cultures were heat shocked at 50°C, and the cells were labeled with L-^{35}S methionine and cysteine. Cell proteins were analyzed by PAGE and autoradiography. The spore and enterotoxin levels were determined by plate count and counterimmunoelectrophoresis, respectively. Our results indicated that a heat shock at 1 h of incubation induced 4 HSP (100, 78, 71 and 36 kDa). Appearance of heat resistant spores and maximal enterotoxin production were delayed 1h compared to the control. When the heat shock was applied at 3 h of incubation, 3 different HSP were produced (100, 82 and 46 kDa). In this case, spore detection and maximal enterotoxin production were observed at the same time as in the control, but levels were slightly higher.

(66) USING A COMPUTER-BASED CD-ROM TUTORIAL TO STRENGTHEN UNDERSTANDING OF GOOD SANITARY PRACTICES IN RETAIL FOOD STORES

R. B. Gravani,* D. F. Berry, S. M. Kern, J. R. Tauer and K. Williams, Cornell University, Institute of Food Science, II Stocking Hall, Ithaca, NY 14853

Today the retail food industry employs about 3.2 million people in stores throughout the U.S. With this large number of people, food safety education and training has become an expensive and challenging situation. The logistics of training, high employee turnover, increasing numbers of part-time employees, and lack of uniformity of information presented all create unique training difficulties.

A computer-based CD-ROM education and training tutorial has been developed and used to strengthen food store employees' understanding of good sanitary practices, particularly good personal hygiene.

The CD-ROM utilizes current computer technology and motivational techniques to enhance learning. The use of sound, music, computer graphics, full-motion video, slides, and consumer testimonials are used to stimulate interest in the subject and to highlight the relevance of the information to the associates' daily activities.

The interactive CD-ROM tutorial is self-paced and enables associates to follow a sequence of activities, including why personal hygiene is important to customer health and safety, store image, and store profits. Good grooming habits, good work habits, and proper hand washing techniques are stressed in the training program. Each module is followed by a quiz to test retention of the information presented. The CD-ROM tutorial will be demonstrated during this presentation.

(67) DIFFERENT WAYS TO GET FOOD SAFETY INFORMATION TO CLIENTELE GROUPS

S. E. Barnard,* S. J. Knabel and T. S. Dimick, The Pennsylvania State University, University Park, PA 16802

Food safety activities have been conducted by many faculty and staff in a number of departments. This started with a one-day workshop for food service workers more than 20 years ago. Now much of the effort is centered in the Food Science Department under the extension plan of work, "Implementing the HACCP Concept Throughout the Food System to Prevent Foodborne Illness and Ensure Food Safety." Information on audiences and the variety of ways used to get food safety information to them will be shared. These include: an annual four-day Master Food Handler Workshop; electronic mail; a computer reference library of both short articles and scientific papers; fax and telephone with county extension staff. Other workshops, interactive television and regional meetings that have been conducted for food service managers, local health officers, and processors of meat, poultry, and dairy products. A newsletter for dairy processors has included information to improve safety. Eighteen lessons have been prepared on food safety for sixth to eighth graders. Both videotapes and slide/cassette tape sets have been produced for some commodities, including a videotape on Good Manufacturing Practices (GMP). Printed procedures have been developed for processors of some commodities and generic HACCP procedures are being developed for dairy farms and plants. A packet of some examples of program topics and specific information will be shared.

(68) CODEX ALIMENTARIUS: ITS EXPANDED IMPORTANCE IN FOOD SAFETY AND INTERNATIONAL TRADE

H. M. Wehr, TAS, Inc., 1000 Potomac Street, N.W., Washington, D.C. 20007

Codex Alimentarius (Codex) is an international governmental organization established in 1962 to develop standards to facilitate international trade in food and promote consumer health. Codex food standards and codes of practice have become important components in international food safety programs. The inclusion of Codex in international trade agreements, particularly GATT and NAFTA has substantially increased its importance. Codex is the reference food safety organization in both agreements and countries have a treaty obligation to use Codex standards unless they can scientifically justify their own standards. This presentation will discuss the history and structure of Codex, focus on its expanded role in international trade, and the increased attention of Codex in U. S. food safety programs, particularly those related to food hygiene and chemical contaminants in food.

(69) THE MANAGEMENT AND TECHNOLOGY OF RETAIL FOOD SYSTEM FOOD SAFETY

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

Government retail food operations HACCP regulations today have a confusing number of critical process time and temperature controls and rules for assuring the safety of food. What is needed is as few food safety processing rules as possible to make it as easy as possible for cooks to assure safe food for the consumer.

This paper will present a one-page universal HACCP plan for retail food processes. The critical process limits are derived from research data that has shown, in practice, to be effective, such as the *Salmonella* D- and z-values from the USDA cooked beef regulations and the cooling standard of 15 hours from 130° to 45°F with a 38°F driving force [Juneja, V. K., Snyder, O. P., and Cygnarowicz-Provost, M. 1994. Influence of cooling rate on outgrowth of *Clostridium perfringens* spores in cooked ground beef. *J. of Food Protect.* 57(12):1063–1067]. In addition, a revision to the 1992 NACMCF HACCP plan will be presented, which incorporates ISO 9000, EPA / OSHA Process Safety Management, Failure Mode Effect Analysis, Statistical Process Control and Continuous Quality Improvement, all of which are essential for a complete retail food safety program.

(70) ISO 9000/HACCP/FOOD HYGIENE PRACTICES: FOOD SAFETY AND QUALITY FOR THE FOOD AND BEVERAGE INDUSTRY

R. Dougherty, NSF International, 3475 Plymouth Road, P.O. Box 130140, Ann Arbor, MI 48113-0140

NSF had developed a specialized food safety and quality program for the food and beverage industry. The concept is based on EC Directive 93/43/EEC of 14 June 1993 on the Hygiene of Foodstuffs. The Directive suggests the integration of the hygiene practice of the Recommended International Code of Practice, General Principles of Food Hygiene of the Codex Alimentarius and the principles of HACCP (hazard assessment critical control points) with the quality management systems requirements of ISO 9000.

In developing this program, NSF has formed an advisory committee with high level US and international participation by regulators, users, and industry. Working with this committee, NSF has developed a guide document for industry to use in developing and implementing a food safety and quality management system using ISO 9000, HACCP and accepted food hygiene practices. NSF has also developed a third-party assessment and registration program. The goal is for a company to be able to have one management system that will assure food safety and quality. The requirement for "validation" of the HACCP plan, which is a separate assessment to determine that the plan assures food safety, is a unique aspect of NSF's program. This validation enhances the value of both ISO 9000 and HACCP.

(71) FOOD SAFETY EDUCATION FOR TEENS

M. B. Lee, School of Environmental Health, Ryerson Polytechnic University, 350 Victoria Street, Toronto, Ontario M5B 2K3 Canada

Although public health inspectors and sanitarians audit food processing in commercial establishments there are a few strategies to reduce foodborne illness which occurs in the home. Food safety education of teens is the focus of this multi-disciplinary project to reduce the risks of improper food handling in the home. The strategy centers on a food safety video, produced by the CBC TV show "Street Cents," which has an estimated viewing audience of over 600,000, primarily teens across Canada. Funding was obtained from: the Ontario Ministry of Agriculture, Food, and Rural Affairs; the Ontario Ministry of Health; Health Canada; and Agriculture and Agri-food Canada, to produce this 30 minute episode. Committee members organizing this project brought expertise from teaching (high school and university levels), nutrition, and public health (municipal, provincial, and federal) to deliver the food safety messages of prompt refrigeration, handwashing, preventing cross-contamination, and thorough cooking. The presentation includes a short clip from the video. Additionally, the video and teachers' guide, will be sold as a kit (at cost) for use by Family Studies and Home Economics teachers or community groups as secondary targets. An evaluation of the project is also planned.

(72) A NOVEL ENZYME-LINKED ANTIBIOTIC ASSAY FOR RAPID DETECTION OF GRAM-NEGATIVE BACTERIA

V. Lewandowski,* T. Bridgeman, E. Zottola and A. Olstein, Department of Science and Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108

The use of enzyme-linked immunosorbent assay (ELISA) techniques has led to the detection of numerous serotypes of specific bacteria, particularly human pathogens, from food. There still remains a need for a rapid test method that can provide sufficient signal-to-background detection of low bacterial numbers. A commercially available cationic lipopeptide antibiotic, Polymyxin B, was used to develop a surface probe capable of labeling cells at extremely high surface densities. Polymyxin B has been documented to bind to the lipopolysaccharide of gram-negative bacteria with both high frequency and high affinity. A probe was prepared by monobiotinylation of the lipopeptide of Polymyxin B. This probe was used to assay for cells directly in suspension or when deposited onto a microporous membrane. Cells concentrated on the membrane were counter labeled with avidin, exposed to a biotinylated enzyme, and then reacted with substrate. The color intensity of the substrate was related to the number of test organisms. This rapid and sensitive bioassay can be conducted as either a qualitative or a quantitative assay. This assay

can be executed within fifteen minutes and eliminates time-consuming pre-enrichment steps. Data demonstrating the sensitivity, biological range and selectivity will be presented.

(73) QUENCHING AND ENHANCEMENT EFFECT ON THE ATP BIOLUMINESCENCE SIGNAL USING DIFFERENT ATP EXTRACTANTS AND SANITIZERS

M. Velazquez,* H. Chan, A. Kirumira and J. Feirtag, University of Minnesota, 223 Food Science and Nutrition Department, St. Paul, MN 55108

ATP bioluminescence techniques are widely used as a rapid method for assessment of the cleanliness of food processing plants. Sanitizer residues could present a potential problem in the use of ATP bioluminescence techniques due to degradation of the luciferin-luciferase enzyme by these agents. The objective of this study was to evaluate the quenching and enhancement effects on the ATP bioluminescence signal using different ATP extractants and commercial sanitizers. Extractants evaluated were benzalkonium chloride (BKC), Triton X-100, benzethonium chloride (BZC), cetylpyridinium chloride (CPC) and trichloroacetic acid (TCA). Commercial sanitizers evaluated were DiverFoam Plus® (alkaline foam), Acid-O-Foam® (acid foam) and sodium hypochlorite (bleach). Of the extractants, CPC at 0.05% quenched the most, followed in descending order by X-100, BZC, TCA and BKC. Of the sanitizers, DiverFoam Plus® and Acid-O-Foam, which are recommended to be used at concentrations ranging from 2 to 5%, showed a 94 to 95% quenching effect when concentrations higher than 1.0% were used. Bleach showed a quenching effect greater than 80% when 0.1 to 5.0% concentrations were used. Results suggest that sanitizers may cause a significant reduction in the bioluminescence signal if present when swabbing surfaces, suggesting the surface is "clean and sanitized," but there may still be organic material or surviving microorganisms present that could lead to contamination.

(74) CHARACTERIZATION OF *ALICYCLOBACILLUS* SPECIES ISOLATED FROM FRUIT JUICES AND CANNED TOMATOES

I. Walls,* V. N. Scott and J. Webster, National Food Processors Association, 1401 New York Avenue N.W., Washington D.C. 20005

Alicyclobacillus spp. are sporeforming organisms that can survive a typical pasteurization process and then germinate, grow and cause spoilage in acid products. The three species in the genus are *A. acidocaldarius*, *A. acidoterrestris* and *A. cycloheptanicus*. Whereas *A. acidocaldarius* is associated with hot springs, *A. acidoterrestris* has been found in soil and water. Recently, acidophilic sporeformers were implicated in spoilage of fruit juices and canned tomatoes. Biochemical and physical characteristics, including ribotype patterns, were determined. These strains were clustered on the basis of pattern similarity in a database of 12,000 strains of about 200 species. Despite strain variation, the isolates were identified as *A. acidoterrestris* by the presence of embedded subsets of conserved fragments seen in their pattern types and the patterns of reference strains. In general, the isolates were Gram positive or variable, motile, catalase-positive facultative thermophiles. The temperature range for growth was 16°C - 55°C; growth was obtained over a pH range of 2.5 - 5.0. Most isolates required oxygen to grow, but some were able to grow anaerobically. Further studies are needed to determine the heat resistance of spores and to investigate potential inhibitors to germination.

(75) CHEMICAL, MICROBIOLOGICAL, AND PHYSICAL QUALITY OF PACKAGED ICE IN FLORIDA

R. Schmidt* and G. Rodrick, University of Florida, Food Science and Human Nutrition, Gainesville, FL 32611-0370

Packaged ice in the state of Florida was evaluated for chemical, physical and microbiological quality. The state-wide, random sample pool was determined based upon geographic, and population density parameters and represented 80% on-premise (bagged at retail outlet) and 20% off-premise (ice manufacturing plant) facilities. Only 11% of bags from on-premise, compared with 79% for off-premise, had sufficient label identification to allow tracing the ice back to the location of manufacture and bagging. Only one sample exceeded the regulatory limit for Heterotrophic Plate Count (<500 CFU/ml) while 13.5% of on-premise and 3.6% of off-premises samples exceeded the total coliform count limit (< 1/100 ml). Yeasts and molds were detected in 12% of the samples. *Listeria* were not detected in any of the samples. No significant differences in chemical composition, electrical conductivity and pH were detected between samples from on and off-premise facilities. All samples analyzed were in compliance with appropriate federal and state drinking water standards. Ice samples from on-premise facilities had higher levels of particulate material than did samples from off-premise facilities.

(76) ASSESSMENT OF THE MICROBIOLOGICAL QUALITY OF READY-TO-USE VEGETABLES FOR HEALTHCARE FOOD SERVICE IN ONTARIO, CANADA

J. Odumeru,* S. Mitchell, D. Alves, J. Lynch, A. Yee, S. Wang, S. Styliadis and J. Farber, Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario N1H 8J7 Canada

The microbiological quality of ready-to-use (RTU) vegetables (chopped lettuce, salad mix, cauliflower florets, carrot sticks, sliced celery, cole slaw mix, broccoli florets and green peppers) was determined before and after processing and during 11 days of storage at 4°C and 10°C. Total aerobic counts of RTU vegetables, with the exception of green peppers, decreased by as much as 1 log CFU/g immediately

after processing. Subsequently, bacterial counts increased to levels found in unprocessed vegetables by day 7 of storage at 4°C. Increased levels of *Listeria monocytogenes* were associated with temperature abuse (10°C) as demonstrated by counts > 100 CFU/g in 8 out of 120 samples stored at 10°C and 1 out of 176 samples stored at 4°C by day 11 of storage. *E. coli* (> 3.0 CFU/g) was detected in chopped lettuce stored at 10°C and in unprocessed green peppers stored at 4°C. Green peppers had higher bacterial counts and were more susceptible to spoilage than other RTU vegetables examined.

(77) THE EFFECT OF LACTIC ACID SANITIZER TREATMENT ON *LISTERIA MONOCYTOGENES* L-FORM BIOFILMS ON FOOD AND CLINICAL CONTACT SURFACES

S. Jassim,* A. Hibma and M. Griffiths, Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1 Canada

Listeria monocytogenes is a foodborne pathogen suspected of causing neurological disease when in a cell wall-deficient state (L-form). This, coupled with the finding that L-forms of other bacteria can attach to solid surfaces, prompted a study of the attachment to, and formation of *L. monocytogenes* L-forms on, relevant food and clinical contact surfaces. Biofilm formation for both parental (cell-walled) and L-forms on stainless steel and intravenous tubing was compared using viable counts and an *in vivo* bioluminescent phenotype of *L. monocytogenes*. Both the classic types adhered to and produced biofilms on food and clinical contact surfaces. Parental cell number attached to intravenous tubing was 1 log more than the L-form number, but on stainless steel, attached parental cell numbers were 1 log less than L-forms. When subjected to a lactic acid sanitizer treatment (130 ppm, pH 3.2) L-form biofilms on stainless steel were more susceptible than were parental cell biofilms. Bioluminescent provides a rapid monitor of biofilm formation for L-forms. These studies show for the first time adhesion and biofilm formation by a microorganism in a cell wall-deficient state.

(78) SURVIVAL OF *YERSINIA ENTEROCOLITICA* DURING FERMENTATION AND STORAGE OF YOGURT

R. C. Williams,* P. W. Bodnaruk and D. A. Golden, Department of Food Science and Technology, University of Tennessee, Knoxville, TN 37901

Yogurt fermentation is accomplished through acidification of milk to decrease the pH to around 4.5 using *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. *Yersinia enterocolitica*, a psychrotrophic foodborne pathogen, has a growth optimum around pH 7, although growth has been demonstrated at pH as low as 4.6. This study was undertaken to investigate the fate of virulent *Y. enterocolitica* during the manufacture and refrigerated storage of yogurt produced by three distinctly different starter cultures [commercial rapid acid (RA) and slow acid (SA) producers and a "home use" starter additionally containing *Lactobacillus acidophilus*]. *Y. enterocolitica* was added to a milk preparation (10^4 - 10^5 CFU/ml) immediately after starter culture addition. *Y. enterocolitica* populations were determined by direct plating on Yersinia Selective Agar throughout fermentation at 44°C and cold storage at 4°C. Titratable acidity and pH were also monitored throughout fermentation and storage. *Y. enterocolitica* levels increased slightly during the first 4-6 h of fermentation in all preparations, but decreased rapidly in the RA and SA preparations and reached undetectable levels under 4°C storage at 3 and 4 days, respectively. *Y. enterocolitica* populations decreased most slowly in the "home use" preparation, reaching undetectable levels at 6 days storage. Virulence in *Y. enterocolitica* was maintained throughout storage in a portion of the surviving population. These results indicate that *Y. enterocolitica* can survive for up to 6 days in refrigerated yogurt while maintaining virulence.

(79) EFFICACY OF CHLORINE AND HEAT TREATMENT IN KILLING *SALMONELLA STANLEY* ON ALFALFA SEEDS AND GROWTH OF THE PATHOGEN DURING SPROUTING AND STORAGE

C. B. Jaquette,* L. R. Beuchat and B. E. Mahon, Center for Food Safety and Quality Enhancement, University of Georgia, Griffin, GA 30223-1797

A study was undertaken to determine the efficacy of chlorine and hot water treatments in killing *Salmonella stanley* on alfalfa seeds. The behavior of *S. stanley* on alfalfa seeds subjected to conditions used commercially to grow and market sprouts was also investigated. Seeds were inoculated by dipping in a suspension of *S. stanley*, then dried at 21°C. Treatment of seeds containing 10^2 - 10^3 CFU/g in 100 µg/ml active chlorine solution for 5 or 10 min caused a significant ($P \leq 0.05$) reduction in population, and treatment in 290 µg/ml chlorine solution resulted in further significant reduction. However, dipping in solution containing active chlorine at 1020 µg/ml failed to result in further significant reductions. Treatment in a solution containing 2040 µg/ml chlorine reduced the *S. stanley* population to undetectable levels. Treatment of seeds in water for 5 or 10 min at 57°C reduced populations to ≤1 CFU/g; however, treatment for 10 min at ≥54°C caused a substantial reduction in viability of the seeds. An initial population of 3.29 $\log_{10}$ CFU/g of seed increased to 10^7 CFU/g of mature sprouts during the 102 h period of sprout production and remained constant throughout 10 days of subsequent refrigeration. These studies indicate that while populations of *S. stanley* can be greatly reduced, its elimination from alfalfa seeds may not be reliably achieved with traditional disinfection procedures.

(80) INHIBITION OF *LISTERIA MONOCYTOGENES*, *STAPHYLOCOCCUS AUREUS*, AND *BACILLUS CEREUS* BY THE HOP *B* ACID COLUPULONE AND ITS DERIVATIVE, HEXAHYDROCOLUPULONE

J. D. Meyer,* N. G. Faith, J. L. Schoeni, J. B. Luchansky, A. C. L. Wong, J. G. Cerveny and M. C. Barney, University of Wisconsin-Madison, 1925 Willow Drive, Madison, WI 53706

Brewers have recognized for many years that hops possess antimicrobial activity. However, the antimicrobial activity of individual hop constituents and their spectrums of activity are relatively unknown. The antimicrobial effect of the hop ß acid colupulone (0-50 µg/ml) and its derivative, hexahydrocolupulone (0-1.6 µg/ml), on the foodborne pathogens *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus* was determined in brain heart infusion broth at 25°C using an automated turbidimetric system. In brain heart infusion broth containing 3.2 µg/ml colupulone or 0.2 µg/ml hexahydrocolupulone, there was no detectable growth of *Staphylococcus aureus* after 96 hours of incubation at 25°C. The same inhibitory effect was achieved against *Listeria monocytogenes* and *Bacillus cereus* with 6.3 µg/ml colupulone or 0.4 µg/ml hexahydrocolupulone. Colupulone and hexahydrocolupulone effectively delay the growth of these foodborne pathogens even at very low levels. Unlike hop acids, ß acids are non-bitter, and incorporating them into a food system may provide an additional safety barrier.

(81) A RAPID DOT-BLOT IMMUNOASSAY FOR THE DETECTION OF *SALMONELLA ENTERITIDIS* IN EGGS, POULTRY AND OTHER FOODS

M. Yoshimasu* and J. Zawistowski, Economic Innovation and Technology Council, University of Manitoba, Food Science Department, Winnipeg, MB R3T 2N2 Canada

A simple, rapid and economical method for the detection of *Salmonella enteritidis* in eggs, poultry and other foods was developed. This method employs a two-step procedure: an enrichment step followed by a dot-blot immunoassay. Artificially inoculated eggs (1-500 cells/25 g of egg) were used to assess the effectiveness of the developed method. Eggs were homogenized, incubated for 20 h at 37°C and heated in the presence of sodium cholate to release the lipopolysaccharide antigen from the bacterial membrane. The antigen was uniformly dispersed within the gelled egg matrix that was formed upon heating. The antigen was transferred onto a solid support and detected by MAb 2F11 specific to the LPS O-9 antigen of *S. enteritidis*. To assess poultry and other foods, samples were inoculated with 1-50 cells/25 g, homogenized in lactose broth and incubated for 6 h at 37°C. One ml was transferred to homogenized eggs and incubated for an additional 18 h. The mixture was heated in the presence of sodium cholate and the antigen detected through the assay. *Salmonella*-contaminated egg and poultry samples at all concentration levels tested positive by the dot-blot assay. Positive results were confirmed by culture method, indicating a good correlation between the two methods.

(82) ANTIMICROBIAL PROPERTIES OF LINEAR FURANOCOUMARINS

J. Ulate-Rodriguez,* H. W. Schafer, E. A. Zottola and P. M. Davidson, Food Science and Nutrition Department, University of Minnesota, 1334 Eckles Avenue, St. Paul, MN 55108

The objective of this study was to evaluate the antimicrobial properties of the linear furanocoumarins (LFs) psoralen, 5-methoxypsoralen (5 MOP) and 8-methoxypsoralen (8-MOP) against *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Micrococcus luteus*, and their potential application as antimicrobials in a model food system. Minimum inhibitory concentrations (MIC) were determined in tryptic soy broth (TSB) and in a model food system consisting of a 25% dilution of baby food in peptone water. The effect of mixtures of the three LFs on the test microorganisms was evaluated by measuring the growth in TSB at room temperature for 48 hours. Growth rates were determined in a microplate reader by measuring changes in OD at 610 nm. Psoralen showed the lowest MICs for the three microorganisms. *L. monocytogenes* was the most sensitive of the three test microorganisms to LFs. Results were highly variable in the model system. Based on the MICs, the antimicrobial effect can be ranked as: psoralen > 5-MOP > 8-MOP.

(83) THE INFLUENCE OF DIVALENT CATIONS AND CHELATORS ON AFLATOXIN B_1 DEGRADATION BY *FLAVOBACTERIUM AURANTIACUM*

D. D'Souza* and R. E. Brackett, CFSQE, Department of Food Science & Technology, University of Georgia, Griffin, GA 30223-1797

Flavobacterium aurantiacum NRRL B-184 possesses the ability to degrade aflatoxin B_1, a potent carcinogen in liquid test media and in several foods. This study was undertaken to investigate the effects of added divalent cations (Mg^{2+} and Ca^{2+}) and chelators EDTA and 1, 10-phenanthroline on aflatoxin B_1 degradation by *F. aurantiacum*. Aflatoxin B_1 (10 µg/ml) was added to 72 h cultures of *F. aurantiacum* that had been washed and resuspended in phosphate buffer (pH 7.0). High performance liquid chromatography was used to determine aflatoxin concentration in these cultures ~10^{14} CFU/ml. Incubating cells with 0.1, 1, and 10 mM Ca^{2+} for 48 h significantly increased aflatoxin B_1 degradation by 11.78, 13.47, and 13.89%, respectively. Likewise, incubation with 0.1, 1, and 10 mM Mg^{2+} for 48 h significantly increased aflatoxin B_1 degradation, by 13.78, 13.28, and 13.12%, respectively. Incubating the bacterium with either divalent cation for 16 and 24 h did not significantly affect aflatoxin B_1 degradation after 24 h. Significantly less aflatoxin degradation was observed when 10 mM phenanthroline was added and incubated for 24 h. These re-

sults suggest the involvement of Mg^{2+} and Ca^{2+} cations in aflatoxin B_1 degradation by *F. aurantiacum.*

(84) DETERMINATION OF NISIN ACTIVITY USING AN HPLC METHOD

A. J. Larsson* and E. A. Zottola, University of Minnesota, Department of Food Science, 1334 Eckles Avenue, St. Paul, MN 55108

The method recommended for determining the nisin content of foods is a microbiological assay described by Tramer and Fowler (1964). This assay consists of putting prepared dilutions of a food sample in a well cut into an agar plate seeded with *Micrococcus luteus*, a culture that is sensitive to nisin. This agar plate is then incubated for 48 hours at 30°C to allow growth of the culture. The area of inhibition around the sample is determined and compared with a standard curve developed with the same assay. This procedure is not very reproducible and is frequently inaccurate. High Pressure Liquid Chromatography (HPLC) has been suggested as a method for determining the nisin content in foods. This study was carried out to determine if HPLC could be used to replace the microbiological assay. A pure sample of nisin, analyzed by HPLC to determine retention time, demonstrated one distinct nisin peak around 42 minutes. Cultures of several nisin-producing lactic acid bacteria were grown in milk, and the amount of nisin produced was determined using HPLC and the well assay method. Although the well assay did quantify nisin in the milk cultures, the HPLC method did not demonstrate a distinguishable nisin peak. The lower detection limit of nisin was determined to be approximately 1 µg/ml. The HPLC procedure may be effective for determining nisin concentration if the food samples being analyzed have concentrations above this detection limit.

(85) COMPARISON OF METHODS FOR COLIFORM AND *ENTEROBACTERIACEAE* COUNTS AMONG NATURALLY CONTAMINATED FOOD AND ENVIRONMENTAL SAMPLES

P. A. Mach* and K. O. Lindberg, 3M Company, St. Paul, MN, 55144-1000

Testing has been used for sanitation and hygiene monitoring in the dairy industry since the 1870's. However, the use of *Enterobacteriaceae* enumeration as an indication of sanitation levels has become prevalent in the food industry throughout most of Europe and the Pacific Rim countries. A recently commercialized 3M product, Petrifilm™ *Enterobacteriaceae* Count plate, was compared with violet red bile glucose agar for *Enterobacteriaceae* enumeration, using 14 naturally contaminated food groups and environmental swab samples from a turkey processing plant. Coliform counts were obtained from the same samples using Petrifilm™ Coliform Count plate and violet red bile lactose agar. ISO and APHA guidelines were followed for the comparisons. Statistical comparison of the coliform and *Enterobacteriaceae* enumeration methods showed that the Petrifilm™ and agar technologies were equivalent. However, *Enterobacteriaceae* counts were 0.65 log higher using the ISO methods and 0.7 log higher using the APHA methods than coliform counts for the samples tested. These differences may not be surprising, considering that coliforms are part of the *Enterobacteriaceae* taxon. However, these differences suggest that *Enterobacteriaceae* monitoring may be a more sensitive measure of the sanitation and hygiene levels for the food groups tested. This may be especially true in dry processes, where low-level contamination is usually anticipated.

(86) EVIDENCE FOR THE OCCURRENCE OF PLANT-SPECIFIC *BACILLUS CEREUS* IN THE DAIRY INDUSTRY

H. Schraft,* M. Steele, J. Odumeru, W. McNab and M. Griffiths, University of Guelph, Guelph, Ontario N1G 2W1 Canada

Biotype, fatty acid profile and restriction fragment length polymorphism of a PCR product (PCR-RFLP of the cereolysinABgene) were determined for 62 isolates of the *Bacillus cereus* group (*B. cereus*, *B. thuringiensis* and *B. mycoides*). Eleven isolates originated from various foods, and 51 isolates were obtained from pasteurized milk that had been processed by two different dairies. The isolates were clustered into 6 biotypes, 10 fatty acid groups or 7 PCR-RFLP clusters. Isolates with mesophilic or psychrotrophic characteristics were preferentially distributed into specific fatty acid or PCR-RFLP groups ($P < 0.003$). Unique fatty acid clusters were predominantly found in milk samples of each dairy ($P < 0.0001$), indicating that certain dairy plants harbour "plant specific" *Bacillus cereus* that constantly contribute to post-pasteurization contamination.

(87) APPLICATION OF A GENERALIZED EVALUATION PROCEDURE FOR COMPARING ISOLATION METHODOLOGIES FOR FOODBORNE *LISTERIA MONOCYTOGENES*

T. D. Hitchins, Food and Drug Administration, 200 C Street, SW, Washington, D.C. 20204

Evaluation of isolation methods for microbial pathogens in foods is complicated by the variability of the interference caused by competitive microflora in test samples. Evaluation can be simplified by using defined amounts of a standard competitor. For *Listeria monocytogenes* (Lm), triplicate test sample sets were prepared. Each set consisted of suspensions of variable levels of the standard competitor *Enterococcus faecium* (Ef) strain 111 (≈10 to 10^9 CFU/25 g) mixed with a low constant level (10 to 100 CFU/25 g) of Lm. These test samples were enriched at 30°C for 48 h in different media and streaked onto selective isolation agars. The input CFU ratio (Ef/Lm) permitting a 50% endpoint Lm recovery was 2.4×10^6 for the FDA 1-step enrichment and 0.8×10^6 for the ISO

2-step enrichment ($P>0.05$). These and other results, taken together, show that the evaluation method is feasible.

(88) THE ANTIBACTERIAL EFFECT OF TEA AND TEA CONCENTRATES ON *CLOSTRIDIUM BOTULINUM*

P. McClure,* and M.C. Cirigliano, Unilever Research, Sharnbrook, England

As hot-packed, unpreserved, Ready-to-Drink (RTD) teas have become more popular in the marketplace, manufacturers have relied on acidification to obviate any *C. botulinum* risk concerns associated with the native pH (i.e., 5.2-5.7) of these beverages. Recent reports documenting the *C. botulinum* inhibitory effects of certain tea constitutes, accompanied by a consumer demand for more "natural" products, have spawned an interest in unpreserved, non-acidified, ambient stable (*C. botulinum* sublethally processed) RTD tea beverages. Our objective was to determine if such a beverage line was safe and technically feasible. Three types of Ready-to-Drink (RTD) Tea (Brewed Black, Decaffeinated Brewed Black, and Black Instant), prepared in a range of concentrations from 0.12% to 5.6%, as well as 5 types of fresh-brewed and instant tea concentrates, prepared at a 40% concentration, were evaluated in the study. The low-concentrate teas also included variants supplemented with aspartame, HFCS, maltose, and milk to simulate retail beverage formats. The teas were UHT treated, inoculated with a cocktail of 15 strains of proteolytic and non-proteolytic *C. botulinum* spores, and stored at 30°C for 12 months. The samples were monitored for growth, toxin production and changes in appearance. Only the milk-supplemented teas supported growth and toxin production over the trial period. At the low tea concentration, the spores were observed to germinate but death followed shortly. At the high (40%) tea concentrate level, the spores failed to germinate during the course of the study. The results of our study indicate that plain (non-milk containing), non-acidified, hot packed, ambient stable, native pH, black tea beverage and beverage concentrates do not support *C. botulinum* growth and/or toxin production.

(89-95) SENSORY ATTRIBUTES OF DAIRY FOODS

J. C. Bruhn, S. Barnard, E. Spear, M. Smukowski, M. Bates, T. Dulmage, F. Bodyfelt, R. Marshall, T. Gottemoller, R. Bradley and T. Gruetzmacher

The objectives are to introduce participants to sensory properties of five dairy foods and to explain causes, prevention, and corrective measures for selected defects in the five products. This will be accomplished with presentations by dairy sensory experts and actual product tasting (evaluation) experience of six or seven samples of each product containing pronounced defects. The sensory attributes will include appearance, body/texture, and flavor. Printed material will be available that, along with the sensory experience, will provide the basis for a sensory based quality assurance program. Enrollment is limited to 100, making it necessary to preregister.

(96) THE ROLE OF PACKERS AND PROCESSORS IN ENHANCING CONTROL OF *E. COLI* O157:H7 IN MEAT PRODUCTS

J. Collins Williams, American Meat Institute, 1700 North Moore Street, Suite 1600, Arlington, VA 22209

The ability to control the microflora in meat and meat products is confounded by the complex and interactive systems used by the industry to produce an animal and then bring the meat to the retail market. Specifically, production and feeding techniques, transportation, and fabrication all impact the microbiological quality of animals, carcasses and meat. The packing and processing industry is in the middle of this chain and thus has the responsibility to apply sanitary practices to the slaughter and processing of animals coming from a variety of farms and other environments. In addition, packers and processors must assure that the products leaving their facilities are processed in such a manner as to reduce microbial levels while maintaining economic viability. Short of sterilization of the final products, it is not possible to produce meat which is free of microbes given current production and processing practices in the United States. Several intervention strategies used within packing and processing facilities have been demonstrated to enhance the microbial quality of meat that ends up in supermarkets across the country. Various antimicrobial rinses, washes, and specific treatments have been identified and several have been approved by the United States Department of Agriculture for use within the commercial facilities. The U.S. meat processing industry is committed to producing the best and safest products possible; however, it is important to recognize that pathogens and other microbes are an unavoidable part of the environment in which animals are raised and within their gastrointestinal tracts.

(97) FARM PREVALENCE OF VEROTOXIN-PRODUCING *ESCHERICHIA COLI* AND PRODUCTION INTERVENTION STRATEGIES

R. P. Johnson,* R. C. Clarke, J. B. Wilson, K. Rahn, S. A. Renwick, S. C. Read, M. A. Karmali and C. L. Gyles, Agriculture and Agri-Food Canada, Health of Animals Laboratory, 110 Stone Road West, Guelph, Ontario N1G 3W4 Canada

Escherichia coli O157:H7 and numerous other verotoxigenic *E. coli* (VTEC) associated with human illness are among the 200 or more VTEC serotypes isolated from cattle and other food animals. Over 90% of dairy farms have cattle shedding one or more VTEC in their feces, and within farms, a high proportion of cattle may carry VTEC of one or more serotypes.

E. coli O157:H7 has been found on approximately 10% of dairy farms and over 60% of beef cattle feedlots. Less than 2% of cattle are culture-positive for *E. coli* O157:H7, although higher infection rates have been reported. Controlling VTEC in the farm sector therefore may have substantial impact on the safety of meat products. Preliminary information suggests that vaccination is unlikely to prevent *E. coli* O157:H7 in cattle, but may reduce the level and duration of fecal shedding of this organism. As well, certain risk factors for VTEC infection on farms have been identified and suggest management practices may influence the introduction and maintenance of *E. coli* O157:H7 and other VTEC in herds. It has been noted, however, that farm studies may not reflect the VTEC status of animals at the time of slaughter. Dietary changes and other factors experienced by cattle prior to slaughter may dramatically affect the level of fecal excretion of VTEC and other enteric bacteria. Animals shedding high numbers of VTEC at slaughter constitute the major risk for product contamination. Consequently, detection and segregation of high-level shedders prior to slaughter and methods to reduce fecal contamination of carcasses at critical control points in processing may prove to be cost-effective control strategies.

(98) EFFECTS OF RED MEAT CARCASS DECONTAMINATION PROCEDURES ON MICROFLORA

W. J. Dorsa* and R. L. Hruska, U.S. Department of Agriculture, Agricultural Research Service, P.O. Box 166, Clay Center, NE 68933-0166

The fate of *E. coli* O157:H7 as well as other potentially pathogenic bacteria resulting from fecal and other sources of contamination on red meat carcasses is a major concern. The development and validation of various decontamination procedures for red meat carcasses is not a new research area. However, recent morbidity and mortality attributable to the presence of *E. coli* O157:H7 in ground beef has heightened the awareness of and rekindled a sense of urgency for this work. Over the last few years various intervention processes have been developed to decontaminate red meat carcasses. Some of these processes include carcass rinses with antimicrobial compounds, steam-vacuum, and hot water. An overview of the results of several investigations demonstrating the effects of these decontamination methods will be presented.

(99) ENTEROHEMORRHAGIC *E. COLI*: THE AUSTRALIAN PERSPECTIVE

P. M. Desmarchelier, Division of Food Science and Technology, Commonwealth Scientific and Industrial Research Organization (CSIRO), P.O. Box 3312, Tigalpa, D.C, Queensland 4173 Australia

Although sporadic infections of enterohemorrhagic *E. coli* (EHEC) have periodically occurred in Australia, it was 1995 when the first foodborne outbreak due to *E. coli* O111 occurred following the consumption of locally produced mettwurst. This resulted in a rapid response from both federal and state health and food authorities to bring the outbreak under control. Longer term responses include the introduction by regulatory authorities of a code of practice for uncooked fermented comminuted meat products for the small goods industry; the provision of government and industry funds to support the implementation of these codes and research into the ecology and epidemiology of EHECs and the safe production of meat and smallgoods; and a general awareness and stimulation of activities in the area of food safety control among all sectors concerned.

The pattern of EHEC serotypes in the Australian human and animal populations appears different to that in countries in the northern hemisphere. Serotype O157:H7 is not the predominant serotype isolated. A variety of serotypes including O111 are more common and possess a variety of virulence-associated determinants. Research into food safety and EHECs is therefore aimed at the development of detection methods for EHECs more appropriate for the Australian situation, also to determine the prevalence of EHECs in the meat and the meat animal population together with the farming and handling practices which influence EHEC carriage and transmission. Related studies are aimed at the identification of safe processing parameters for the production of fermented comminuted meat products. The results of these activities will contribute to an assessment of the hazards presented by EHECs in this country and recommendations for their control.

(100) BACTERIAL PHYSIOLOGY AND MEAT SAFETY

T. Ross, MRC Research Fellow, Department of Agricultural Science, University of Tasmania, GPO Box 252C, Hobart Tasmania, 7001 Australia

As a response to the increasing incidence and severity of meat-related infectious disease outbreaks, increasing attention is being focused on carcass decontamination strategies to improve the microbiological safety of meat. In the past, a variety of methods have been investigated such as hot or cold rinses, acid treatments, and chemical sanitizers, to reduce the microbial load on carcasses, but it appears that most decontamination procedures are empirically derived. As a result, the 'in-plant' effectiveness of many decontamination procedures is equivocal. Other important issues in the use of decontamination procedures relate to cost-effectiveness, comprehensiveness of the treatment, effect on carcass quality, and possible counter-productive effects on the microbiological quality and safety of the product.

Attention has also been focused on the physiology of *E. coli* O157:H7, which appears to be more resistant to a number of physiological stresses, in particular pH, than was previously thought for *E. coli*. A better knowledge of the physiology and physiological condition of bacteria which contaminate carcass surfaces would help to elucidate the effect and effec-

tiveness of carcass decontamination methods and the results reported to date. 'Habituation,' and the synthesis of stress proteins in bacteria which confer protection against a range of environmental insults, are examples of important physiological responses which may affect the efficacy of decontamination procedures 'in-plant'. In addition, an understanding of the physiology of bacteria on carcasses may allow the rational development of novel intervention strategies. For example, certain combinations of temperature shifts have been shown to cause unexpected effects on the growth responses of bacteria.

The literature regarding decontamination strategies will be critically reviewed and related to bacterial responses to water activity, temperature, and pH/organic acids, and the potential for better use of existing strategies, or for new strategies based on bacterial physiology, will be considered.

(101) VALIDATION OF PROCESSES FOR CONTROL OF *ESCHERICHIA COLI* O157:H7 IN FERMENTED SAUSAGE

C. W. Kaspar* and J. B. Luchansky, Food Research Institute, University of Wisconsin, Madison, WI 53706

A 1994 outbreak in California and Washington linked to consumption of pre-sliced, dry, fermented pork/beef salami contaminated with *Escherichia coli* O157:H7 prompted regulatory action that requires sausage manufacturers to ensure a 5-$\log_{10}$ reduction of this pathogen. This presentation will summarize research funded by the meat industry that addresses the fate of serotype O157:H7 strains in fermented sausage, with emphasis on salami. Briefly, the behavior of a five-strain mixture of *E. coli* O157:H7 was monitored in small (55 mm) or large (105 mm) diameter casings during fermentation at 70°, 90°, or 110°F to <pH 4.6 or >pH 5.0. After fermentation chubs were; i) dried directly [55°F, 65% relative humidity (RH)], or ii) held for an additional 7 days at the fermentation temperature and targeted pH (i.e., "cured") and then dried, or iii) heated using the method 7 process and then dried. Regardless of the temperature or pH, fermentation alone was not sufficient to eliminate high levels of the pathogen. For the 70°F fermentation, method 7 heating followed by drying delivered a 5-D kill, whereas curing followed by drying did not. For the 90°F fermentation to <pH 4.6, curing for seven days or method 7 heating delivered a 5-$\log_{10}$ reduction for small-casing chubs. Fermentation at 90°F to >pH 5.0 and method 7 heating followed by drying resulted in a 5-D kill for the large casing product, but curing followed by drying did not. For the 110°F fermentation to <pH 4.6, curing for ca. 4 days reduced counts of the pathogen by 5 $\log_{10}$ units for both large and small casing chubs. To effect a 5-D kill following fermentation at 110°F to >pH 5.0 it was necessary to cure the product for 7 days or use themethod 7 process. In general, fermentation at higher temperatures (90° or 110°) to a lower pH (<pH 4.6) followed by curing or method 7 heating and then drying was required to deliver a 5 $\log_{10}$ reduction of *E. coli* O157:H7 in salami. These data will assist manufacturers in setting priorities for validating processes to control this pathogen in fermented sausages.

(102) ASSESSING MICROBIAL HAZARDS FROM CHILLED/FROZEN FOODS EXPOSED TO REFRIGERATION FAILURE

R. V. Lachica* and R. Worfel, U.S. Army, Natick Labs, Natick, MA 01760-5056

A guide was developed in 1987 and subsequently adopted in military installations to facilitate deciding which foods exposed to refrigeration failure (RF) are still salvageable. The present study is a reassessment of the scientific basis of the guide to address more adequately the hazards from cold tolerant pathogens and other so-called emerging pathogens in foods exposed to RF (FERFs). On the basis of six criteria, the following pathogens were considered relevant to FERFs: *Bacillus cereus*, *Clostridium botulinum*, salmonellae, *Staphylococcus aureus*, and *Listeria monocytogenes*. FERFs were categorized into three groups: (i) frozen foods that are damaged by the process of defrost, (ii) those that are safe but may suffer in quality or reduced shelf life as a result of RF and (iii) potentially hazardous FERFs, which are further subdivided into five groups based on the types of potential microbial hazards that they present to the consumer. The classification scheme is based on whether or not they are ready-to-eat, frozen, hermetically sealed, or cured/salted. These factors impact on the time and temperature of exposure to RF that a product can tolerate before it becomes potentially hazardous as a result of pathogen proliferation.

(103) MICROBIAL QUALITY OF VACUUM PACKAGED COOK/CHILL FOODS PREPARED IN A HOSPITAL

B. E. Langlois,* K. Akers, S. Bastin and J. O'Leary, University of Kentucky, Lexington, KY 40546-0215

Microbial quality of 1,137 vacuum packaged cook/chill food samples representing 77 food products prepared for regular diets or modified for low sodium, low fat, diabetic or strained diets were analyzed during a 27-month period. Products from the same production batch were analyzed after manufacture (AM; 581 samples) and again after storage (AS; 556 samples) at 1±1°C for up to 21 days in the hospital's food bank. Products were analyzed from one to seven times during the study for aerobic counts, at 26°C (AP26) and 35°C (AP35), counts of coliforms, *Escherichia coli*, *Clostridium perfringens*, *Bacillus cereus*, staphylococci, and *Staphylococcus aureus*, and for the presence of *E. coli* O157:H7, *Listeria* and *Salmonella*. Pathogens were not detected in any of the samples.

Mean $\log_{10}$ aerobic counts were 1.6 (AP26) and 1.5 (AP35) AM and 1.5 (AP26) and 1.6 (AP35) AS. None of these differences were significant. Within some food products, significant differences were obtained between AM and AS aerobic counts as well as between counts of regular and modified products. Mean $\log_{10}$ coliform and staphylococci counts were 0.1 or less. Results indicate that cook/chilled food products produced using GMP can be stored up to 21 days without significant increases in aerobic counts.

(104) AUTOMATED RIBOTYPING-BASED ASSESSMENT OF DIVERSITY IN BOVINE MASTITIS–CAUSING MICROORGANISMS

J. L. Bruce,* A. L. Rivas, C. A. Batt, M. Wiedmann, C. McDowell and R. Gonzalez, DuPont Experimental Station, Building 357, Room 1011, Wilmington, DE 19880-0357

A total of 48 *Staphylococcus aureus* strains isolated from individual milk samples from 12 dairy farms and 15 *Streptococcus agalactiae* strains from 6 dairy farms were characterized by automated ribotyping (RiboPrinter™ Microbial Characterization System) and antibiotic resistance profiles (Kirby-Bauer method). *S. aureus* strains were characterized into 18 RiboGroups; in nine of the farms, more than one RiboGroup was isolated, suggesting a diverse population and an endemic rather than an epidemic distribution of this species. Six RiboGroups represented the *S. agalactiae* strains; two distinctive RiboGroups were isolated from each of three farms; and the remaining three farms, with multiple isolates sampled, had only one RiboGroup. Except for two isolates, strains within a *S. aureus* RiboGroup showed the same antibiotic resistance profile for all isolates from a given farm, indicating horizontal transmission of antibiotic resistance genes. This study demonstrates that automated ribotyping allows discriminative typing of *S. aureus* and *S. agalactiae* isolated from milk samples. In conjunction with antibiograms, this method of characterization offers a promising tool for the analysis of strain diversity and the study of the transmission of antibiotic resistance in mastitis-causing microorganisms.

(105) A COMPARISON OF VARIOUS PHENOTYPIC AND GENOTYPIC METHODS FOR TYPING *ENTEROBACTER SAKAZAKII*

M. Nazarowec-White* and J. M. Farber, Microbiology Research Division, Health Canada, Banting Bldg., Ottawa, Ontario K1A 0L2 Canada

Enterobacter sakazakii, designated a unique species in 1980, has been implicated as the causal organism in a rare but severe form of neonatal meningitis. Dried infant formula has been implicated as a potential source of the organism. Strains of *E. sakazakii* were isolated from dried infant formula available on the Canadian market, whereas clinical strains were obtained from Canadian hospital culture collections. Identification of strains was confirmed using the API 20E identification system. The relatedness of the bacterial isolates was evaluated using both phenotypic and genotypic methods. From the 16 strains examined, 3 biotypes and 4 antibiogram patterns were observed. Ribotyping using the Dupont Riboprinter™ microbial identification system categorized the test strains into 10 clusters. After initially screening 100 decamer primers, two were selected for random amplification of polymorphic DNA (RAPD) typing. Banding patterns were similar for 3 strains isolated at the same hospital, although each strain was isolated in a different year. All three of the food isolates from Company A showed similar profiles, while two food strains isolated from dried infant formula produced by Company B revealed different profiles. Pulsed-field gel-electrophoresis (PFGE) using the restriction enzymes *Xba I* and *Spe I* revealed a similar number of bands to that of RAPD (a total of 12 different banding patterns), giving distinguishable patterns that allowed the strains of *E. sakazakii* to be compared. It was found that RAPD and PFGE were the most discriminatory methods for distinguishing clinical and food isolates of *E. sakazakii*.

(106) COMPARATIVE RECOVERY OF COLIFORMS FROM MEAT AND MILK USING M-COLIBLUE24 AND DIRECT PLATING

J. S. Dickson,* J. Erdman and M. A. Grant, Iowa State University, Department of MIPM, Ames, IA 50011

Membrane filtration (MF) methods are available for monitoring Total Coliforms (TC), Fecal Coliforms (FC) and *E. coli* (EC) in water, beverages and foods. A new medium for membrane filtration, m-ColiBlue 24 (mCB), which allows simultaneous detection of TC and EC, was compared to direct plating methods. Twenty-eight samples of ground pork and ground beef were analyzed by membrane filtration with mCB and Violet Red Bile agar (VRBA). The recovery of TC was not significantly different between the two media. Presumptive EC were recovered in 18 of these samples. Representative isolates were identified by the BBL Crystal system. Of the mCB isolates, 64.6% were classical Total Coliforms, 89.6% were *Enterobacteriaceae* and 8.3% *Vibrionaceae*. Of the VRBA isolates, 63.0% were classical Total Coliforms, 86.9% were *Enterobacteriaceae* and 13.0% *Vibrionaceae*. In a second set of meat samples, the mean TC population capable of growth in Brilliant Green was 3.07×10^2 CFU/gm with mCB and 2.88×10^2 with VRBA. In raw milk samples, the mean TC populations verified by Brilliant Green were 3.05×10^2 CFU/ml for mCB and 6.35×10^2 CFU/ml for VRBA. In the milk samples, the mean value for EC populations simultaneously detected on mCB was 7.33×10^1 CFU/ml.

(107) RAPID COLIFORM COUNTS OF RAW MILK

P. Tuitemwong,* and K. Tuitemwong, Department of Microbiology, Kasetsart University, Thailand, Food Science and Technology, KMIT Thonburi, Thailand

Poor sanitation standards of some farms and milk collection centers in rural areas of Thailand result in high counts of total bacteria and coliforms (10^4-10^5 CFU coliforms/ml). In order to monitor sanitation standards, an attempt was made to reduce the detection time of coliforms in raw milk to within working hours (8 h) using rehydrated dry film (Petrifilm Series 2000, P2000). One hundred and two raw milk samples from 25 cooperatives and 77 individual farms in Thailand were tested for coliform counts as a sanitary index of each farm and collection center by using 2 methods, the conventional and P2000. In the case of P2000, readings were taken every hour. Pure cultures of *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp. and non-coliforms, (*Proteus* spp., *Salmonella* spp., and *Shigella* spp.) were used to monitor sensitivity of P2000. Results from both methods were not significantly different ($P<0.05$), with r^2 of 0.94. High counts (10^5-10^7 CFU/ml) of coliforms/non-coliforms were detected in 4 h. Longer incubation times could detect fewer counts (10-10^3 CFU/ml), and 8 h and 14 h were required for formation of colonies and gas bubbles, respectively. Non-coliform bacteria failed to show typical colonies after 14 h. In conclusion, P2000 gave faster results than the conventional method and required much less time to detect the customary contamination level of coliforms in raw milk.

(108) MICROBIOLOGICAL AND SENSORY QUALITY OF MILK

C. R. Hackney,* S. E. Duncan, H. Williams and W. Hartman, Virginia Polytechnic Institute and State University, Department of Food Science and Technology, Blacksburg, VA 24061-0418

Raw milk, in-line processing samples and product samples were taken at ten dairy plants over a two year period. All plants were visited at least twice. Samples were subjected to microbial, chemical and sensory analysis upon arrival at our laboratory and after storage at 3° and 7°C until code and code plus four days. Raw milk quality continues to be a problem. Pasteurization was adequate for all plants tested; however, post-process contamination continues to limit shelf life. The temperatures in cold storage rooms were often greater than 3°C and were usually uneven. The air quality in the areas around the fillers was often very high. Seals of cardboard cartons were found to be defective in some plants. Sensory analysis detected various off flavors. For the most part, samples made code and code plus 4 days at 3°C with only a "moderate lacks freshness" character, but many failed to make code at 7°C. Sensory description for those samples included mostly "fermented/fruity" flavor or "pronounced lacks freshness." The Virginia Tech shelf life test worked best in plants where Gram-negative bacteria caused the main spoilage problem. The Virginia Tech shelf life test was not a good predictor of the shelf life of milk from plants that had very low Gram-negative populations, but that had problems with Gram-positive bacteria spoiling milk at temperatures above 5°C.

(109) FERMENTED MILK CONTAINING *BIFIDOBACTERIUM LONGUM* POTENTIATES IMMUNE RESPONSE OF THE HOST

C. Ferreira,* M. R. Moulin and J. M. Mezencio, Departamento de Tecnologia de Alimentos/ Universidade Federal de Vicosa, 36570-0000 Vicosa-MG-Brazil

For many decades, the literature has indicated beneficial effects of consuming lactic acid/bifidobacteria. However, it is not clear whether the viable cells have the same effect on the host as fermented or nonfermented milk containing such cells. Mice (BALB/c 25-30g) were randomly chosen and fed sterilized nonfat dry milk (NFDM) reconstituted to 10% containing viable or nonviable cells and fermented milk containing *Bifidobacterium longum* (*B. longum*). The culture was grown for 24 h/37°C in modified MRS broth. After centrifugation, it was concentrated and resuspended in NFDM. The animals were immunized with sheep red blood cells at 6, 7 and 8 days after initial feeding. Blood samples were collected on days 5, 7, 10, 13 and 15 after immunization. The sera were diluted and tested for circulating antibodies using the agglutination technique. The entire experiment was repeated (10 animals/group). It was observed that the titres from mice fed nonviable and unfermented milk containing viable cells were similar. However, the titre from that of mice fed fermented milk was 2.5 times higher. It is suggested that *B. longum* carried in a fermented milk can potentiate the immune response of the host.

(110) SURVIVAL AND GROWTH OF *AEROMONAS HYDROPHILA* AND *LISTERIA MONOCYTOGENES* ON RAW CABBAGE AND CELERY

R. V. Diaz,* R. M. Raybaudi and A. J. Martínez, Instituto de Ciencia y Tecnología de Alimentos, Universidad Central de Venezuela, P.O. Box 47097, Caracas, 1041A Venezuela

The survival and growth of *A. hydrophila* and *L. monocytogenes* on raw cabbage and celery stored at 5°C were evaluated. Vegetables were shredded, placed in plastic bags, inoculated (10^4-10^5 CFU/g) separately with each organism and stored at 5°C. Uninoculated samples served as controls. Populations of *A. hydrophila* and *L. monocytogenes* and lactic acid bacteria were evaluated using selective recovery media and direct

plating technique on the initial day of inoculation and at various intervals for 28 days. In cabbage samples, significant ($P<0.05$) decreases were detected in populations of *A. hydrophila* and *L. monocytogenes* during storage. In celery samples, significant ($P<0.05$ decreases of populations of *A. hydrophila* occurred during storage. No significant changes in populations of *L. monocytogenes* were detected. Behavior of lactic acid bacteria were similar in both vegetables, with a significant ($P<0.05$) decrease of populations through storage. Results indicate that *A. hydrophila* and *L. monocytogenes* do not proliferate well in raw cabbage and celery. Associated microflora other than lactic acid bacteria, pH, antimicrobial activity of substrates, etc, may account for this effect. Even if no growth was observed, *A. hydrophila* and *L. monocytogenes* can survive on raw cabbage and celery, and this in turn may represent a health hazard to the consumer.

(111) ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM BEAN SPROUTS WHICH INHIBIT *LISTERIA MONOCYTOGENES*

J. M. Farber,* Y. Cai and L-K. Ng, Health Canada, Bureau of Microbial Hazard, P. L. 2204A2, Ottawa, Ontario K1A 0L2 Canada

Bacterial isolates from mung bean and soy bean sprouts were screened for bacteriocin production using a well diffusion method, and 34 of them were found to inhibit *Listeria monocytogenes*. Ten isolates producing the biggest inhibition zones were further characterized and presumptively identified as Enterococci by the VITEK test. However, testing with an *Enterococcus*-specific gene probe showed that these strains were not enterococci. 16s ribosomal DNA sequencing analysis demonstrated that the isolates were *Lactococcus lactis* subsp. *lactis*. One strain, #80, exhibited the strongest inhibition against *L. monocytogenes* and was selected for further testing. In MRS broth, the isolate survived at 3 to 4.5°C for at least 20 days, grew at 4°C and produced anti-Listeria material at 5°C. When co-cultured with *L. monocytogenes*, the isolate inhibited *L. monocytogenes* at 4°C after 14 days and at 10°C after 2 days. Primary characterization showed that the anti-*L. monocytogenes* material was proteinaceous (inactivated by proteinase K, proteinase, typsin, α-chymotrypsin, pepsin and papain), and the antimicrobial effects were not caused by pH, phage or H_2O_2, indicating that it was a bacteriocin. Plasmid profiling of the ten strains placed them into three different groups. A plasmid cured-strain was obtained by increasing the incubation temperature of MRS broth containing acridine orange. This strain still produced bacteriocin and had the same antibiotic resistance pattern as the wild type strain, indicating the bacteriocin gene was most likely on the chromosome. Bacteriocin-producing isolates had immunity to nisin, suggesting that they produced nisin, a finding later confirmed by PCR. Nucleotide sequencing revealed that the form of nisin produced was nisin Z.

(112) OCCURRENCE OF *LISTERIA MONOCYTOGENES*, *SALMONELLA* SPP., *E. COLI*, AND *E. COLI* O157:H7 IN VEGETABLE SALADS

C. Lin,* S. Fernando, T. Huang and C. Wei, University of Florida, Gainesville, FL 32611-0370

Fresh produce sometimes contains pathogenic bacteria, including *L. monocytogenes*, *Salmonella* spp., *Shigella sonnei* and *E. coli*. Therefore, freshly prepared vegetable salads can serve as a vehicle for foodborne illnesses. In this study to examine the occurrence of *L. monocytogenes*, *Salmonella* spp., *E. coli*, and *E. coli* O157:H7 in vegetable salads, 20 samples collected from restaurants and supermarkets in the Gainesville area were homogenized and incubated in enrichment and then in selective enrichment media, and the cultures streaked onto sets of selective agar plates specific for each bacterial species. Colonies with characteristic features were checked biochemically and immunologically for confirmation. One of the four supermarket salads and two of the 16 restaurant samples contained *E. coli*. This bacterial contamination could be of human origin.

(113) GROWTH OF *LISTERIA MONOCYTOGENES* ON MINIMALLY PROCESSED BROCCOLI WITH ANTIMICROBIAL TREATMENT

R. D. Smiley,* D. A. Grindstead, J. R. Mount and F. A. Draughon, Department of Food Science and Technology, University of Tennessee, Knoxville, TN 37901

This study was undertaken to determine the growth and survival of *Listeria monocytogenes (LM)* on minimally processed osmotically dehydrated broccoli treated with chlorine or metabisulfites under modified atmospheres. Fresh broccoli was cut into florets, inoculated to achieve a target level of log 3.0 CFU/g *LM*, osmotically dehydrated to approximately 2% NaCl (w/w) and/or treated with 200 ppm hypochlorite or 100 ppm metabisulfite. Control samples were prepared that were not osmotically dehydrated for all treatments. Samples were packaged in a gas permeable film using vacuum or air and stored at 4° or 10°C for 15 d. Analyses for *LM* were performed at 3-day intervals using direct plating on PALCAM agar and enrichment, differential plating and confirmation by the FDA BAM procedure. On osmotically dehydrated broccoli not treated with antimicrobials, *LM* increased approximately log 5.0 CFU/g at 10°C and approximately log 2.0 CFU/g on broccoli stored at 4°C for 15 days. Osmotic dehydration did not significantly reduce counts of *LM* at either 4° or 10°C. Metabisulfite treatment significantly ($P<0.05$) reduced recovery of *LM*, and *LM* declined steadily on broccoli after 3 days of storage at 4° or 10°C. Hypochlorite treatment did not significantly ($P>0.05$) reduce *LM* on broccoli.

(114) APPLICATION OF ATP-BIOLUMINESCENCE FOR CLEANING VALIDATION OF FOOD PROCESSING EQUIPMENT

E. E. Ehrenfeld,* J. Scheld, S. A. Miller and C. R. Carpenter, IDEXX Laboratories, One IDEXX Dr., Westbrook, ME 04092

LIGHTNING™ is an ATP-bioluminescence system consisting of a hand-held luminometer and a disposable device containing the swab and the pre-measured firefly reagents (luciferin and luciferase). In this system the amount of biological residue (food material as well as microbial contamination) present on a given surface is detected by measuring the amount of ATP contained in the sample. The ATP therefore acts as an indicator of cleaning effectiveness.

The system is able to detect very small quantities of food residues: 1 microliter of raw milk, 1 milligram of yogurt, 0.1 microliter of orange juice, 3 milligrams of tofu, and 2 milligrams of raw chicken. This level of sensitivity allows differentiation between dirty and clean surfaces in food processing environments. Typical data collected in food processing plants from both dirty (prior to cleaning) and clean sites will be presented.

(115) APPLICATION OF A RAPID ATP-BIOLUMINESCENCE METHOD FOR ASSESSING CLEANLINESS OF MILKING EQUIPMENT

K. Strutz,* C. Fong and P. Vasavada, University of Wisconsin-River Falls, River Falls, WI 54022

A commercial ATP-bioluminescence system was used to assess cleanliness of milking machines on a dairy farm. The ATP levels of the milking machine parts, viz. claws, inflations and milk jars, were determined after routine cleaning and sanitization following a protocol recommended by the instrument manufacturer. The microbial levels of these parts were assessed by the standard swabbing technique and plating procedure. The experiment was repeated 5 times. The cleanliness of the milker parts was indicated in terms of "zones of cleanliness", where a zone reading of < 2.5 and > 3.0 represented "clean" and "dirty" surfaces, respectively. The average ATP values of milking machine claws, inflations, and milker jars were 2.7, 2.4 and 1.8, respectively, with instrument background values varying from 0.6-1.2. The average ATP values for pipelines and bulk tank outlet were 1.5 and 3.0, respectively. Although direct comparison of the ATP levels with the bacterial counts was not possible with the protocol used, surfaces with ATP readings of > 2.5 generally showed APC > 100 CFU/in^2 and some surfaces showing ATP readings of < 2.5 also showed APC >100 CFU/in^2. The results showed that the ATP-bioluminescence method may have application as a rapid method for assessing and monitoring cleanliness of milking equipment on a dairy farm.

(116) MONITORING CLEANLINESS OF FOOD CONTACT SURFACES USING A RAPID ATP-BIOLUMINESCENCE METHOD

C. Foong* and P. Vasavada, University of Wisconsin-River Falls, River Falls, WI 54022

Cleanliness of three food contact surfaces was evaluated using a commercial ATP-bioluminescence system. The ATP levels of stainless steel, plastic and wood surfaces soiled with orange juice or raw milk were determined initially (unclean), after cleaning with warm water, after recleaning and sanitizing, and after autoclaving following a protocol recommended by the manufacturer. Duplicate samples were taken using standard swabbing technique at each step of the cleaning process. The residual ATP levels were indicated in terms of "zones of cleanliness," where a zone reading of > 3.0 and < 2.5 represent "dirty" and "clean" surfaces, respectively. The average zone values for the wooden, steel and plastic surfaces were 2.2, 1.7 and 1.4, respectively. Washing of the surfaces resulted in a decreased reading of up to two zones, and re-washing (which included scrubbing) and sanitation decreased the reading an additional zone. The swabs of autoclaved surfaces gave much lower zone readings (< 2.0), which were similar to those for the unsoiled surfaces (i.e., control background). The results showed that reduction in the background ATP levels indicative of cleaning efficiency of the food contact surface can be readily monitored by using a bioluminescence technique.

(117) A NEW MEDIUM FOR THE QUANTIFICATION OF BACTERIA IN FOOD AFTER 24 HOURS

D. Townsend,* A. Croteau and A. Naqui, IDEXX Laboratories, Westbrook, ME 04092

During the past several years great efforts have been made to develop better pathogen tests for the food industry which utilize state of the art technology. In contrast, not much effort has been expended in developing new nonpathogenic microbiological tests. In this presentation we report on the results of performance studies with a new microbiological medium called Total Plate Count (TPC). TPC allows quantification of the total bacterial concentration of food in 24 hours.

Sixty-five different food samples representing a variety of different food matrixes were tested. Microbiological counts on TPC were determined at 24 hours and plotted against values obtained from Petrifilm total aerobic (PF), standard plate count (SPC) and Redigel total count (RG) after 48 hours. Correlation coefficients between the tests were 0.98, 0.92 and 0.95 for PF, SPC and RG, respectively. Certain foods such as liver, nuts, and flour yielded false positive results due to endogenous enzyme activity of the food itself; however, these foods are not routinely tested for total viable bacterial counts. It is concluded that TPC is a suitable alternative for microbiological analysis of foods.

(118) REAL TIME MONITORING OF LACTIC FERMENTATIONS USING IMPEDANCE MICROBIOLOGY

J. P. Coombs,* A. M. Marshall, A. P. Pridmore and P. Silley, Bioscience International Inc., 11607 Magruder Lane, Rockville, MD 20832

Impedance microbiology was first introduced and developed as a rapid means of determining microbial loading in raw materials and final product where the time to detection (TTD) is inversely proportional to the number of organisms in the sample. Although it is suited to such applications, the detection system primarily measures the metabolic activity of the target population, and as such, is highly appropriate to monitoring the activity of starter cultures used within the food industry, where metabolic activity might be considered more important than absolute numbers. The presented data show typical impedance curves for a range of starter cultures, including *Lactobacillus* spp., *Streptococcus* spp., *Bifidobacterium* spp., and *Lactococcus lactis*, inoculated into milk and monitored over incubation periods ranging from 6 to 12 hours using the RABIT system. The study measured respective impedance parameters and pH at the end of the lactic fermentation. In the presence of ampicillin at residue levels (0 - 0.2 μg/ml) there was an increase in TTD (0.8 to >4.6 h) and a decrease in total metabolic activity, measured as the total conductance change, as antibiotic concentration increased for *Lactobacillus plantarum*, *Streptococcus* spp. and *Lactococcus lactis*. *Bifidobacterium* sp. showed no change for TTD, but there was a reduction in metabolic activity. In all cases the changes in impedance parameters were related to inhibition of acid production (0 - 55%) by the test organisms as ampicillin concentrations increased from 0 to 0.2 μg/ml. Inhibition of acid production is a standard parameter for assessing performance of lactic starter cultures and is expressed as a function of that acid normally produced by the culture in the absence of antibiotic.

(119) THE EFFICACY OF WASHING AND SANITIZING ANIMAL HAULING TRUCKS

K. Rajkowski, USDA-ARS-ERRC, 600 East Mermaid Lane, Wyndmoor, PA 19038

Swine can shed *Salmonella* into the truck environment under the stress of being transported to market. The *Salmonella*-contaminated trucks, if not cleaned between trips, can infect other farms, abattoir environments, and other animals. The floors of single haul trucks (long haul [>500 miles] *vs.* short [<500 miles]) were sampled for *Salmonella* and coliforms before and after washing and sanitizing. To determine the effect of season of the year, the sampling was done over a one-year period. Samples were initially screened for *Salmonella* by the Tecra immunoassay and then, if positive, subjected to the standard cultural assay (MPN). Coliforms were determined by the Petri film method. Before washing and sanitizing, the haulers were positive for both groups of bacteria regardless of the season of the year. There was no significant difference ($P>0.05$) between the number of positive haulers and the distance travelled. Washing and sanitizing the haulers reduced the level of *Salmonella* from >1100 to <2 MPN/cm^2 and the coliform level from 10^5 to <1 CFU/cm^2, thereby minimizing the possibility of contaminating other farms, the abattoir, and other animals.

(120) ENHANCED DETECTION OF PATHOGENS IN MEAT PRODUCTS USING AUTOMATED MALTHUS CONDUCTANCE ASSAYS

D. M. Gibson, BIODON, 43 Brighton Place, AB10 6RT Aberdeen, United Kingdom

Automated conductance assays using the Malthus System V instrument have been developed for many of the routine microbiological tests for both the detection and enumeration of potentially pathogenic and spoilage microbes on foods. The protocols for detection of *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., *E. coli* and coliforms are described and the results obtained with naturally contaminated meat products (raw and cooked) using Malthus and reference methods are presented. The Malthus methods yielded more positive results, confirmed by recognized procedures, than the reference methods, there being 32 out of 338 samples positive for *Salmonella* compared with 30 by ISO and 32 by USDA methods, 63/152 samples for *Listeria* compared with 60 by the USDA method, 76/330 samples for *Campylobacter* compared with 64 by the FDA method, 41/156 samples for coliforms compared with 30 by ISO, and 2/156 samples for *E. coli* by both Malthus and ISO methods. Malthus assays had improved performance criteria, took less time and needed much less sample handling.

(121) GENETIC CHARACTERIZATION AND IDENTIFICATION OF LACTIC ACID BACTERIA IMPORTANT TO THE FOOD INDUSTRY USING AUTOMATED RIBOTYPING

A. McCardell,* J. Bruce, E. Cole and M. Corby, DuPont Experimental Station, Wilmington, DE 19880-0357

Lactic acid bacteria are of economic significance to the food processing industry. Lactics are used in food fermentations and have been implicated in food spoilage. The difficulties associated with characterizing and identifying these organisms classically have been an impediment for improvement in the food processing industry. The Riboprinter™ Microbial Characterization System is an automated ribotyping system that genetically characterizes bacteria. The Riboprinter™ System has the capability of creating custom characterization and identification databases. Lactic acid bacteria from a variety of food and food-related sources, identified as *Lactococcus*, *Lactobacillus*, *Leu-*

conostoc, and *Pediococcus* species, were used to create a custom identification database. This database was then challenged with suspect lactic acid bacteria. The Riboprinter™ system identified each isolate to the species level. The systems ability to generate a ribosomal DNA fingerprint from these organisms allowed them to be genetically characterized. Isolates could be grouped at the genus, species, and subspecies levels based on their DNA fingerprints. The ability to accurately and reliably genetically describe and discriminate among these organisms should be of significant value to the food industry.

(122) BIOPRESERVATION OF VACUUM-PACKAGED COARSE GROUND BEEF BY *LEUCONOSTOC GELIDUM* UAL187

R. J. Worobo,* G. G. Greer, M. E. Stiles and L. M. McMullen, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton AB T6G 2P5 Canada

Biopreservation by *Leuconostoc gelidum* UAL187 was studied in commercially produced vacuum-packaged coarse ground beef. Commercially produced trim was immersed in a bacterial suspension of *L. gelidum* UAL187 (bac^+), *L. gelidum* UAL187-13 (bac^-) or a sterile control suspension. Samples were inoculated at a concentration of 10^5 CFU *L. gelidum*/g of meat. After dipping, all three batches were coarse ground, vacuum packaged and stored at 4°C for 35 days. After 0, 10, 21 and 35 days of storage, vacuum-packaged samples were removed for microbial analysis, and the coarse ground beef was fine ground and tray-wrapped for retail display. Microbial analysis was done at 0, 1, 3, 5, 7 and 9 days of aerobic storage, at 2°C. For vacuum and aerobic storage the inoculated and control samples were enumerated for *L. gelidum, Enterobacteriaceae*, lactic acid bacteria, pseudomonads and total psychrotrophs, and they were qualitatively analyzed for odor and appearance using an experienced five-member panel. At each aerobic storage interval, color reflectance coordinates (L*, a*, b*) were measured and pH was recorded. Vacuum-packaged samples after extended storage showed levels of enterics, pseudomonads and lactic acid bacteria that were 1 to 2 logs lower in inoculated samples than in the control. This reduction in bacterial load under vacuum was also evident after aerobic storage. Odor shelf-life was also extended by addition of *L. gelidum*. Color stability of ground beef prepared for retail display was increased in inoculated samples compared with the control.

(123) OREGON CONSUMERS' USE OF USDA SAFE HANDLING INSTRUCTIONS LABEL ON MEATS AND POULTRY AND THEIR KNOWLEDGE OF FOODBORNE ILLNESS RISKS

M. Woodburn* and C. Raab, Oregon State University, Corvallis, OR 97331

USDA has required that a safe handling label be on each retail package of raw or partially cooked meats and poultry since July 6, 1994. This study was designed to evaluate its use more than one year later. Data on consumer knowledge and practices related to foodborne illness and use of the label were collected from 100 Oregonians by telephone interviews. The random digit dialing household sample was drawn proportionate to county populations by Survey Sampling, Inc. (Fairfield, CT). Meats and poultry were the foods most frequently considered to present a high risk of food poisoning. Nearly all respondents had heard of *Salmonella* and *E. coli* as problems in food. They most frequently identified meats, poultry and eggs as related to salmonellae and ground beef as related to *E. coli*, although a few said "all foods." About 75% of the respondents recalled having seen a safe handling instructions label on meat or poultry packages. If they had, most read it or glanced at it only the first few times. Few were able to recall the information. Educational efforts should capitalize on published outbreaks and problems, include specific information on food handling practices, and use varied visual/word formats. (Partially funded by the Oregon Beef Council.)

(124) AN EVALUATION OF THE EFFICACY OF TWO BEEF CARCASS DECONTAMINATION METHODS

S. Kochevar,* J. Sofos and G. Smith, Colorado State University, Fort Collins, CO 80523-1171

The objective of the present study was to determine the efficacy of a steam-vacuuming system and of a high temperature (74°C)/high pressure (20.68 bar) spray-washing process as compared to knife-trimming for reducing microbiological and physical contamination on beef carcass surfaces. The steam-vacuuming system effectively reduced ($P<0.05$) aerobic plate counts and total coliform counts on carcass surfaces with or without visible fecal contamination. When feces were present, the steam-vacuuming system and knife-trimming were both effective in removing visible contaminants, but steam-vacuuming was more effective in reducing microbial contamination. Spray-washing of brisket samples contaminated with a bovine fecal paste inoculated with *Enterobacter aerogenes* effectively improved the visual appearance and decreased the microbiological populations of the samples. The use of higher pressure (20.68 bar) water for spray-washing did not spread contamination of any form to other areas on the sample surface.

(125) ISOLATION OF *HAFNIA ALVEI* FROM COMMERCIALLY PREPARED CHUB-PACKED GROUND BEEF, AND ITS IMPORTANCE IN MEAT SPOILAGE

S. Gamage,* S. Ingham and J. Luchansky, University of Wisconsin-Madison, Madison, WI 53706

Ten pound chubs of coarsely ground beef were monitored for microbial sources of gaseous spoilage. Total aerobic and anaerobic counts after 18 days at

2°C and 7°C were about 3×10^7 CFU/g. Homofermentative *Lactococcus* spp. were the dominant microorganisms recovered at both atmospheres and temperatures. In one of three trials, Gram negative coliform counts after 18 days were 3.1×10^5 CFU/g, with *Hafnia alvei*, a known gas producer, predominating. Sixteen representative *H. alvei* isolates produced gas in Cooked Meat medium, but no evidence of lipolysis, proteolysis or H_2S production was noted. Pulsed-field gel electrophoresis, fatty acid analyses and biochemical profiles of the 16 *H. alvei* isolates indicated that they were the same clone. However, these meat isolates differed from a *H. alvei* isolate subsequently recovered at the meat-packing plant. The predominance of *H. alvei* in one trial, its ability to grow at refrigeration temperatures, and the production of gas in a synthetic medium suggest that this microorganism may contribute to gaseous spoilage of chub-packed ground beef.

(126) MICROBIOLOGY OF AQUACULTURED STRIPED BASS GROWN IN EARTHEN PONDS, FLOW-THROUGH TANKS, AND RECIRCULATING TANKS

P. C. Nedoluha* and D. Westhoff, University of Maryland Department of Animal Sciences, College Park, MD 20742

The microbiology of the skin (S), gills (G), intestines (I) and culture water (W) of striped bass (*Morone saxatilis*) and hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) grown in earthen ponds, flow-through tanks and recirculating tanks was examined. Randomly selected isolates (n=1742) from APCs were identified using MIDI and BIOLOG systems. Enrichments were used to detect foodborne pathogens. A mixed model ANOVA was used to determine significant differences ($P<0.05$) among plate counts. Aquaculture system had no significant effect on the plate counts. Aerobic plate counts were significantly higher than anaerobic counts for the S, G and W but not for the I. Counts at 35°C and 22°C were not significantly different but were significantly higher than counts at 7°C. The types of bacteria randomly isolated were similar for all systems, but their prevalence varied; incubation temperature affected the genera isolated. The predominant groups were *Aeromonas* (19%), *Flavobacteria/Cytophaga* (16%), *Bacillus* (7%), *Moraxellaceae* (6%), *Pseudomonas* (6%), coryneforms (5%), *Plesiomonas* (5%) and *Micrococcaceae* (4%). The incidence of bacteria that can cause serious foodborne illness was very low; no *Salmonella* or *Yersinia enterocolitica* were isolated, and *Listeria monocytogenes*, *Shigella dysenteriae* and *Y. pseudotuberculosis* were isolated from ponds on only one sampling day. *Staphylococcus aureus* and *Vibrio* spp. occurred in all systems in low numbers. There was no indication that healthy, aquacultured striped bass present a higher risk of foodborne illness than wild fish.

(127) GROWTH OF PSYCHROTROPHIC PATHOGENS ON REFRIGERATED AQUACULTURED RAINBOW TROUT AND CHANNEL CATFISH FILETS

C. F. Fernandes,* T. B. Thomas and G. J. Flick, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Aquacultured rainbow trout *(Oncorhynchus mykiss)* and channel catfish *(Ictalurus punctatus)* filets were inoculated with the psychrotrophic pathogens *Listeria monocytogenes* and *Aeromonas hydrophila,* and growth of these pathogens was monitored during refrigerated (2-4°C) storage. Each pathogen was grown in nutrient broth at 35°C for 18 h and used as a standard inoculum. To individual filets of both catfish and trout contained in sterile bags, cell suspensions (@ $10^{4.7}$ CFU/g) of each individual and a combination of psychrotrophic pathogens were added and mixed to ensure uniform distribution. The filets were stored at 2-4°C and *A. hydrophila, L. monocytogenes* and aerobic counts were determined on filets on days 1, 3, 6, 8, 10, 13 and 15. The *A. hydrophila, L. monocytogenes* and aerobic plate counts were determined using AOAC procedures. Individually inoculated *A. hydrophila* and *L. monocytogenes* grew on catfish and trout filets during the 15 days of the study. There was no inhibition of either pathogen by the natural flora on the catfish or trout filets. Both psychrotrophic pathogens grew equally well in catfish and trout filets inoculated with a combination of *A. hydrophila* and *L. monocytogenes*. In all three treatments, the counts of the psychrotrophic pathogens were lower than the aerobic plate counts. Both the individual and combination of inoculated psychrotrophic pathogens grew on refrigerated filets. Hence, the psychrotrophic pathogens, if present in raw products, could cross-contaminate ready-to-eat products and result in a health risk to normal and particularly to immunocompromised individuals.

(128) EFFECT OF ORGANIC ACIDS ON THE MICROFLORA OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

C. F. Fernandes,* J. Cohen, T. B. Thomas and G. J. Flick, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

A microfloral suspension obtained from aquacultural catfish *(Ictalurus punctatus)* filets was suspended in 0, 1, 2 and 4% organic (*viz.* acetic, propionic and lactic) acids at 24°C and their survival was studied during a 20 min holding period. Catfish filets were also sprayed with organic acids to reduce their microbiological populations. Aerobic counts were enumerated on Standard Methods Agar following neutralization of the sample to pH 7.2 with dilute NaOH. The plates were incubated at 35°C for 48 h. All organic acids examined were detrimental to the survival of suspension microflora obtained from the catfish filets. The number of surviving bacteria decreased with increasing concentration and time of exposure to the organic acids. A decreasing order of

detrimental effect was observed, with propionic acid followed by acetic and lactic acids. Spray washing of catfish filets with water did not significantly ($P < 0.05$) alter the microbial populations of the filets. However, catfish filets sprayed with organic (e.g., lactic and propionic) acids significantly ($P < 0.05$) reduced the microbial counts by one log cycle. Lactic and propionic acids were not significantly ($P > 0.05$) different from each other in influencing the aerobic counts of the aquacultured channel catfish filets.

(129) COMPARISON OF QUALITY IN AQUACULTURED FRESH CATFISH FILETS II—PATHOGENS *E. COLI* O157:H7, *CAMPYLOBACTER*, *VIBRIO*, *PLESIOMONAS*, AND *KLEBSIELLA*

C. F. Fernandes,* G. J. Flick, J. L. Silva and T. A. McCaskey, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Fresh aquacultured channel catfish (*Ictalurus punctatus*) filets were procured from three catfish processors in southeastern United States during the four annual seasons (e.g., summer, fall, winter and spring) and screened for selected human pathogens. Five catfish filets were randomly selected for aerobic plate counts and twenty filets were screened for five pathogenic bacteria *viz. Campylobacter jejuni/coli*, *Escherichia coli* O157:H7, *Klebsiella pneumoniae* subsp. *pneumoniae*, *Plesiomonas shigelloides* and *Vibrio cholerae*. The aerobic plate counts were enumerated using 3M™ Petrifilm™ aerobic count plates. After incubation at 35°C for 48±2 h, the *E. coli* O157:H7 counts were determined using the 3M™ Petrifilm™ test kit HEC. *C. jejuni/coli* and *V. cholerae* were isolated according to the methods described in the Food and Drug Administration Bacteriological Analytical Manual. *P. shigelloides* and *K. pneumoniae* subsp. *pneumoniae* were screened following procedures described in the Compendium of Methods for Microbiological Examination of Foods. There was a significant difference ($P < 0.05$) in the aerobic plate counts (3.00 to 6.03 log CFU/g) due to differences in unit processing operations and processing season. *C. jejuni/coli*, *E. coli* O157:H7 and *K. pneumoniae* subsp. *pneumoniae* were not isolated. Only *P. shigelloides* and *V. cholerae* were isolated during the warm weather. Occurrence of pathogens in raw products could cross-contaminate ready-to-eat products, consequently impairing the health of normal, and specifically that of immunocompromised individuals.

(130) MICROBIAL EVALUATION OF SALMON ROE PROCESSED IN ALASKA

B. H. Himelbloom* and C. A. Crapo, University of Alaska-FITC, 900 Trident Way, Kodiak, AL 99615-7401

Salmon roe is a raw, salt-cured delicacy primarily exported to Japan as egg skeins or single eggs (ikura). The project objective was to determine the microbial content of salmon roe processed at one plant in Alaska. Ikura prepared from fresh pink or chum salmon was collected aseptically over thirty days of processing. Ikura was composed of 31.3-39.9% protein, 9.8-13.6% fat, 2.3-3.1% salt and 48-52% water; water activity was 0.98-0.99 and pH was 6.0-6.2. Aerobic plate counts varied with a trend of increasing counts towards the season's end. Aerobic bacterial counts ranged from less than 10^2/g to 2.6×10^6/g (median = 2.3×10^3/g). Coliform counts were variable and ranged from less than 3/g to 2.4×10^3/g (median = 9/g). *Escherichia coli*, *Staphylococcus aureus*, yeasts or molds were not detected. Improved roe handling procedures especially during peak salmon processing should reduce the incidence of high bacterial counts.

(131) BIOGENIC AMINES IN FISH SAUCES

Y. W. Huang,* M. Zheng, H. E. Amos, K. W. Gates and M. A. Froetschel, University of Georgia, Athens, GA 30602-7610

Fish sauce is a fermented product made from many varieties of small fish and invertebrates, including anchovy, herring, mackerel, shrimp and squid. All the commercial fish sauces in U.S. markets are imported from Asia, where the simple traditional fermentation techniques in small scale have been used. The quality of products is not uniform. Biogenic amines may be produced by decarboxylation of certain amino acids during fermentation. However, little is known about the biogenic amines in fish sauces. The objective of this research was to determine levels of biogenic amines in imported products. Ten brands of fish sauces and pastes (three of each) were purchased from Oriental markets in Atlanta, Georgia. Amino acid profiles and biogenic amines (including putrescine, cadaverine, tryptamine, 2-phenylethylamine, spermidine, spermine, histamine, and tyramine) were determined, using an amino acid analyzer and HPLC, respectively. The test samples contained putrescine (at levels up to 160 µg/ml), cadaverine (up to 206 µg/ml), histamine (up to 147 µg/ml), tryptamine (up to 165 µg/ml) and tyramine (from 23 to 134 µg/ml). Results indicated that variation in level of amines among imported samples might be due to the raw materials, processing conditions and degree of microbiological contamination.

(132) QUALITY OF SURIMI MADE FROM TILAPIA AND CARP

Y. W. Huang,* H. Abdel-Aal and A. H. Awad, Center for Food Safety & Quality Enhancement, University of Georgia, Athens, GA 30602

Freshwater species tilapia (*Orechromis* spp.) and grass carp (*Ctenopharyngodon idella*) were used to produce surimi. Proximate composition, amino acids, minerals, Hunter color L, a, b values, protein solubility, and expressible water of surimi were determined using standard methods. Gel forming ability of cooked surimi was measured by using the penetration test, torsion test and folding test. Yields of surimi were 20.54% and 19.77% from whole tilapia and carp, respectively. Proximate compositions of

surimi made from tilapia were 14.81% for protein, 2.31% for fat, 73.91% for moisture, and 0.55% for ash, while that of surimi made from carp were 13.45% for protein, 0.78% for fat, 76.63% for moisture, and 0.56% for ash. Amino acid profiles of tilapia and carp surimi were comparable to that of Alaska pollock surimi. Hunter color value of raw surimi from tilapia was L=71.41, a=-0.83, and b=+2.68, while that of the raw surimi from carp was L=71.40, a=-1.24, and b=+2.41. Compared to surimi made from the fillet, surimi made from tilapia frames had much less Ca, Mg, K, and P and higher Cu, Fe, Ni, and Cr. The cooked tilapia surimi exhibited higher ($P<0.05$) gel strength (1061.5 g × cm) than carp surimi (560.3 g × cm). Results indicated that tilapia is an alternate source for production of high quality surimi in addition to Alaska pollock.

(133) FECAL COLIFORMS IN TEA: WHAT'S THE PROBLEM?

M. P. Doyle, University of Georgia, Griffin, GA 30223

Recent news media reports of fecal coliforms in iced tea and leaf tea have inaccurately and sensationally blamed the industry for feces in tea. The reports further indicated that a health hazard existed. Fecal coliform populations of 10^3 to $>10^6$/ml were present in many of the samples assayed; however, further analysis by food microbiologists revealed that the dominant bacterium present in tea was *Klebsiella pneumoniae*. *Enterobacter* sp. and other *Klebsiella* sp. were also detected but *Escherichia coli*, the indicator of fecal contamination, was not isolated from any samples of iced tea or leaf tea we assayed. Furthermore, to date there is no definitive evidence that iced tea is a vehicle of foodborne illness. Considering the large populations of *K. pneumoniae* frequently consumed by iced tea drinkers and the lack of reported illnesses associated with iced tea consumption, there is no evidence to implicate iced tea as a health hazard. The frequent occurrence of false-positive fecal coliform results from tea and vegetables puts in question the value of the fecal coliform test for foods. Perhaps the fecal coliform test should be abandoned for food testing.

(134) THE MICROBIOLOGY OF TEA AND TEA BREWING

M. C. Cirigliano, T. J. Lipton Company, 800 Sylvan Avenue, Englewood Cliffs, NJ 07632

The recent concern over the safety of fresh brewed iced tea has posed a number of questions about the appropriateness of using the coliform, and specifically the fecal coliform, test in assessing public health risk potential where tea and vegetables are concerned. It also casts doubt on the efficacy of traditional tea brewing, storage and handling practices, in both the restaurant and home venues, in obviating any risk concern. To assess the reliability of the fecal coliform test in predicting what the test is used to imply, where shellfish growing waters are concerned, this study undertook to correlate the presence of fecal coliform in more than 3,000 ingredient and retail leaf tea blends, representing the 1995 crop year, with the presence of *E. coli*. Restaurant and "in-home" tea brewing modeling studies were also conducted to assess the risk potential associated with traditional, and abuse, brewing, handling, and storage conditions. *E. coli* was not detected in any of the leaf tea samples tested. Where "fecal" coliforms were found, *K. pneumoniae* accounted for 80% of the "false positives", followed by *E. cloacae* (10%) and *E. agglomerans* (5%), all three common plant epiphytes. Both traditional home steeping and restaurant "autobrew" methods were used in the brew modling studies. "Hot" brew water trial temperatures ranged from 145°F-195°F. Abuse brews were also done at RT (@25°C) and 35°C. No native coliform bacteria survived brew water temperatures at or greater than 150°F for 3 minutes. At abuse temperatures, or when fresh tea was seeded by contaminated equipment, a lag phase of from 12-16 hrs. occurred. Uncleaned tea urn spigots were found to be the principal nidi for iced tea contamination.

(135) HOW DID ICED TEA BECOME SUCH A HOT TOPIC?

T. L. Schwarz, Division of Cooperative Programs, CFSAN, U.S. Food and Drug Administration, 200 "C" Street, S.W., Washington, DC 20204

The issue of bacteria in brewed iced tea surfaced briefly in 1986 and the Agency addressed the issue with some guidance concerning cleaning and sanitizing of the dispensers. Beginning in September, 1995, FDA was deluged with questions on this topic from health agencies, trade association and the media. The story spread widely that iced tea was somehow contaminated and dangerous. Is it safe? Is it sanitary? What did FDA do to respond?

(136) ICED TEA — IS IT SAFE? GETTING IT RIGHT IN THE MEDIA

B. Howard, CDC, 1600 Clifton Road, Mailstop D-25, Atlanta, GA 30333

The issue of fecal coliform presence and the possible bacterial contamination of iced tea is an issue that captured the attention of both local and national print and broadcast media over the past nine months. TV stations and newspapers around the country have taken to acquiring samples from restaurants, cafeterias and fast food outlets and having these samples tested at private labs and comparing the findings in a sort of "Top Ten" showing the highest test levels of fecal coliforms. In response to a number of these incorrect and misleading reports, the CDC issued an advisory to state public health departments in January 1996 reafirmings that in nearly 10,000 foodborne outbreaks, iced tea was never identified as the source of foodborne illness. This document went on to provide

steps that could be taken in homes and eateries that would help in making tea a safe product for people to enjoy. This advisory was identified by a national wire service as an "alert" or "notice" to states that there may be a problem with iced tea nationally. This discussion will explore the manner in which the CDC responded to these reports, and lessons learned from this "media encounter."

(137) ICED TEA: HOW DID IT GET A BAD RAP?

J. Aronow, Aronow and Pollock Communications, Inc., 524 Broadway, New York, NY 10013

Food-related crises occur-and when they do, the public is entitled to learn the facts. Unfortunately, the media too often do not understand the facts or, leaning toward sensationalism; veer from the truth. The result is the same: distorted reporting, leading to unnecessary concern by the public.

One case in point: the "discovery" of bacteria, labeled pathogenic through improper laboratory testing, in black tea and then reported as an "epidemic." Even a subsequent statement by the CDC that the bacteria were benign was phrased in such scientific jargon that the truth was obscured.

It is essential that organizations involved with risk-related issues develop professional criteria for communications, to cover spoken communications both with the media and within the organization itself; the proper handling of data; spokesperson training, and other elements.

Only with proper communication guidelines can food and health-related organizations properly fulfill their responsibility to provide accurate, complete information to the public.

(138) THE ICED TEA SCARE: HOW THE MEDIA FAILED AND WHY

H. Kome, Health Reporter, Spartanburg (SC) Herald-Journal, P.O. Box 1657, Spartanburg, SC 29304

1) The failure of the media to report the iced tea story is symptomatic of larger inability to deal with science stories.

2) News reporting regularly involves "dumbing down" information for mass consumption. Part of this process involves pulling down the most compelling information out of reports and discarding information that is less clear, less dramatic or contradictory.

3) An examination of the chronology of the iced tea scare reveals that numerous reporters and editors had a chance to question the validity of tests supposedly showing fecal bacteria in iced tea.

4) By examining other science-related stories that have reached a mass audience, it is possible to see the symptoms of the problem that led to the iced tea scare. Stories on the recent "mad cow" problem in Great Britain, for example, reveal a lack of scientific information and an unwillingness to explain the complexities of the problem to a general audience.

5) All is not lost. By understanding the approach of the media, health professionals can help prevent inaccurate stories.

(139) MICROBIAL INDICATORS-PURPOSES AND USES

L. A. Jaykus, Food Science Department, Box 7624, North Carolina State University, Raleigh, NC 27695-7624

A microbial indicator is defined as an organism or group of organisms whose presence is taken as a surrogate for the presence of a particular group of microorganisms of interest, generally indicative of a potential product quality or safety problem. The purpose of this presentation is to introduce the concept of microbiological indicators, describe the characteristcs of the ideal indicator microorganism, and discuss the general value of microbial indicators for application to food safety issues. The presentation will conclude with an introduction to the specific microbial indicators to be covered in detail by subsequent symposium speakers.

(140) THE USE OF INDICATOR ORGANISMS IN HACCP PLANS

A. M. McNamara, USDA-FSIS, 300 12th Street, Washington, D.C. 20204

In February 1995, USDA's Food Safety and Inspection Service (FSIS) initiated rulemaking to reduce pathogenic bacteria and to implement HACCP (Hazard Analysis and Critical Control Points) in all U.S. meat and poultry plants. HACCP is a preventative approach to food safety which consists of identifying food safety hazards, controlling these hazards at critical control points (CCP's), determining critical limits, monitoring CCP's, and implementing corrective action, record-keeping, and verification procedures. The use of indicator organisms plays a key role in monitoring CCP's and in HACCP verification procedures. The role of indicator organisms in HACCP, the generic HACCP plans developed by the National Advisory Committee on Microbiological Criteria for Foods, and the microbiological sampling and testing results of FSIS pilot testing of HACCP systems will be presented in order to define the use of indicator organisms in HACCP plans.

(141) THE USE OF MICROBIAL INDICATORS: INDUSTRY PERSPECTIVE

D. L. Zink, Nestlé, USA, Inc., 800 North Brand Blvd., Glendale, CA 91023

Today, the goal of food manufacturers is to be able to ship their products to customers within only a few hours of the final manufacturing and packaging steps. It is often too costly to store large quanitites of product for days or weeks while lengthy microbio-

logical tests are performed. Therefore, the food industry is reexamining the value of testing for indicator organisms. The industry is particularly interested in indicator organisms that can be detected or enumerated by rapid methods. Tests for indicator organisms can be performed on samples from the plant environment, food contact surfaces and in-process products. *Enterobacteriaceae* counts can be used as an indicator of proper food contact equipment sanitation and post process contamination. The selection of an indicator organism and the determination of microbiological criteria for its use must be done with care. The improper use of indicator organisms can lead to disaster. When properly used, indicator organisms can reduce the cost of microbiological testing yet verify proper process control and equipment sanitation.

(142) VALUE OF MICROBIAL INDICATORS IN ENVIRONMENTAL MONITORING

J. J. Frank, Food Science Building, University of Georgia, Athens, GA 30602

Some pathogenic microorganisms, such as *Listeria monocytogenes* and *Salmonella* spp., can survive or grow in the food processing plant environment. Food products that are exposed to the environment after heat processing are at risk of being contaminated by these pathogens. Controlling this risk involves reducing the opportunity for growth and survival of pathogenic microorganisms in the food processing environment. This is accomplished by various practices including cleaning, application of chemical sanitizers, water control, and reducing product spillage. Environmental sanitation efforts can be monitored by testing surfaces for the presence of pathogens, or by using microbial or biochemical indicators tests. The value of a microbial indicator is that it provides a warning that conditions are suitable for pathogen growth or survival. Even if a pathogen is not present, such conditions should be corrected. The indicator microorganisms should have better survival and growth characteristics than the pathogens of concern. For example, a test for Gram positive microorganisms provides a better indicator of conditions that support growth and survival of *L. monocytogenes* than does a coliform test.

(143) MICROBIAL INDICATORS AND FOODBORNE PATHOGENS-*SALMONELLA*

J. S. Bailey, USDA-ARS-RRC, Russell Research Center, P. O. Box 5677, Athens, GA 30613

The use of 'indicator' or 'index' organisms in ready-to-eat foods, water and environmental monitoring is widespread. In each of these sample types, the likelihood of a specific pathogen being present is low and 'indicator' bacteria are often used to test whether proper processing or sanitation procedures are being followed. Implementation of HACCP plans for raw meat and poultry processors will require some form of microbiological testing to monitor and verify the efficacy of the HACCP system. Ideally, one would test the food for the pathogen of concern. However, salmonellae are infrequently present and are usually found in very low numbers with heterogenous distribution compared to other bacteria. Therefore, other bacteria have been considered as 'indices' of pathogens. Published literature and research data concerning the criteria needed for an effective 'index organisms' the problems associated with different 'index organisms,' and the use of total aerobic bacteria, coliforms and *Enterobacteriaceae* as possible indicators of salmonellae contamination will be discussed.

(144) *E. COLI* O157:H7 IN BEEF — ARE INDICATOR ORGANISMS RELEVANT?

R. Nickelson II, National Cattleman's Beef Association, Chicago, IL 60611

Indicator organisms are used for two purposes, determination of the probable presence of a pathogen that is more difficult to isolate and the indication of improper process control. In the first case, it is unlikely that any of the traditional indicators (aerobic plate counts, coliforms, *E. coli* or *Enterobacteriaceae*) would be correlated to the presence of *E. coli* O157:H7. In a nationwide survey of raw ground beef, only 3 of 6000 samples were positive for *E. coli* O157:H7. It is improbable that data on any of the indicator organisms would have been useful in predicting the presence of *E. coli* O157:H7. Second, the presence of *E. coli* O157:H7 on beef carcasses or beef products has not been shown to be related to improper sanitation or cooling. Proposed microbiological performance standards for raw beef will offer little information about pathogens that occur in small numbers and at low incidence levels. *E. coli* O157:H7 is the best indicator of *E. coli* O157:H7.

(145) BACTERIOPHAGE INDICATORS OF ENTERIC PATHOGENS IN FOODS

M. D. Sobsey, University of North Carolina, CB# 7400, Rosenau Hall, Chapel Hill, NC 27599

Fecal contamination of foods continues to be an important source of consumer exposure to, and illness from, enteric pathogens. Because of the difficulties of detecting enteric pathogens in foods, there is a need for improved indicators of fecal contamination and enteric pathogens in foods. Enteric bacteriophages of *E. coli* (coliphages) and *Salmonella* are promising candidate indicators of at least some of the important enteric pathogens in foods, especially the human enteric viruses and *Salmonella* bacteria. Using simplified new mehtods, these bacteriophages can be detected quickly (6-8 hours) in foods. Typing male-specific RNA coliphages using oligonucleotide ("gene") probes may provide a rapid and simple system to distinguish human from animal fecal contamination in a variety of foods, including bivalve molluscan shellfish. This may aid in determining the risk of food as a source of human pathogens.

(146) ACID AND HEAT TOLERANCE OF ACID HABITUATED *ESCHERICHIA COLI* O157:H7

H. Thippareddi,* D. Retzlaff, R. K. Phebus and D. Y. C. Fung, Dept. of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506

Habituation of microorganisms to acidic conditions generally increases the acid tolerance on subsequent exposure to more severe acid conditions. *Escherichia coli* O157:H7 strains were grown in tryptic soy broth (TSB) and TSB supplemented with dextrose (TSBG). Cultures were used directly or washed in peptone water (PW). Heat resistances were evaluated at 55°C in PW, and $D_{55°C}$ values (min) were calculated. Acid resistances were evaluated in lactate buffer (pH 4.0) and decimal reduction times (min; D_A) were calculated. Medium pH after growth at 35°C/ for 18 h was measured. Fatty acid profiles were evaluated using gas chromatography.

Dextrose supplementation resulted in lower medium pH (4.95 and 5.03; and 5.96 and 6.00 for cells grown in TSBG and TSB for FSIS 45753 and salami outbreak strains, respectively). Growing cells in TSBG vs. TSB resulted in lower heat resistance ($D_{55°C}$ values of 11.6 min and 27.3 min for FSIS 45753 and 5.9 min and 16.3 min for outbreak strain, respectively). Washing of cells resulted in lower heat resistance. Acid habituation resulted in higher acid tolerance (D_A of 66.7 min vs. 49.9 min for FSIS 45753 and 31.2 min vs. 22.3 min for the outbreak strain, respectively). Washing resulted in lowering of acid resistance of the cells. Growing of cells in TSBG resulted in lower levels of 16:1 and 18:1 fatty acids in the outbreak strain. Acid habituation of cells resulted in higher acid tolerance and lower heat tolerance of both strains of *E. coli* O157:H7.

(147) CHANGES IN HEAT-RESISTANCE OF *E. COLI* O157:H7 FOLLOWING HEAT SHOCK AND ACID SHOCK

N. C. Williams,* and S. C. Ingham, University of Wisconsin-Madison, Department of Food Sciences, Madison, WI 53706-1565

Heat is the primary process for destroying *Escherichia coli* O157:H7 in meats. This study investigated whether short-term exposure of *E. coli* O157:H7 to a supraoptimal but nonlethal temperature (heat shock) or a suboptimal pH (acid shock) would enhance the subsequent heat resistance (expressed as D-value) of *E. coli* O157:H7. Heat shock and acid shock treatments, although done in a laboratory medium, were designed to be similar to short-term temperature abuse and marinating of meat, respectively. *E. coli* O157:H7 ATCC 43894 was grown to stationary phase and then either directly acid shocked (pH 4.0 acetic acid for 15 min) or stored at 4°C for 24 h and then heat shocked at 45°C for 30 min. Control cells were used directly. Duplicate experiments were done to determine D-values of control, acid-shocked and heat-shocked cells in Trypticase Soy Broth at 54 and 58°C. Survivors were enumerated by spread plating on Trypticase Soy Agar. Acid shock led to D-value decreases of 43% at 54°C and 21% at 58°C. In contrast, heat shock led to D-value increases of 24% at 54°C and 36% at 58°C. Although acid washing of beef carcasses appears not to eliminate *E. coli* O157:H7, marinating meats may increase the lethality of subsequent cooking against this pathogen. Temperature abuse immediately prior to cooking, however, may decrease lethality.

(148) INFLUENCE OF A_W AND TEMPERATURE ON VIABILITY OF UNHEATED AND HEAT-STRESSED *ESCHERICHIA COLI* O157:H7 IN SALAMI

R. S. Clavero* and L. R. Beuchat, Center for Food Safety and Quality Enhancement, University of Georgia, Griffin, GA 30223-1797

The influence of a_w (0.95 or 0.90) and temperature (5 or 20°C) on the viability of unheated and heat-stressed *E. coli* O157:H7 cells, surface inoculated into salami at populations of 10^5 (high) and 10^2 (low) CFU g^{-1} and stored for 32 days, was investigated. The performance of tryptic soy agar (TSA), modified sorbitol MacConkey agar (MSMA), and modified eosin methylene blue agar (MEMB) for supporting recovery of inoculated cells was determined. Regardless of the physiological condition of the cells, decreases of 1 to 2 $\log_{10}$ CFU g^{-1} occurred within 2 hours after inoculation. Non-selective TSA did not support recovery of viable cells from salami containing a low inoculum. However, enrichment of low-inoculum salami in modified tryptic soy broth (mTSB) resuscitated unheated cells from salami (a_w 0.95) stored at 5°C for 32 days and at 20°C for 16 days; no viable cells were detected for heat-stressed cells at either 5 or 20°C within 1 day. Decreases in populations of *E. coli* O157:H7 in salami receiving high inocula occurred but test cells did survive for 32 days at 5°C. Viability was observed higher for unheated cells compared to heat-stressed cells and when salami was stored at 5°C compared to 20°C. Results showed that surface contamination of salami with *E. coli* O157:H7 at populations of 10^4 - 10^5 CFU g^{-1} after processing may pose a health risk for at least 32 days if storage is at 5°C. Regardless of treatment conditions, performance of media in recovering *E. coli* O157:H7 cells followed the order of TSA > MSMA > MEMB agar.

(149) ISOLATION AND CHARACTERIZATION OF SUBSTANCES INHIBITORY TO *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES*

T. A. Bridgeman* and E. A. Zottola, Department of Food Science and Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108

Pseudomonas aeruginosa and *Serratia liquefaciens*, isolated from ground beef, were found to be inhibitory to *Escherichia coli* O157:H7. The agar flip and streak method was used initially to screen for inhibition. It was hypothesized that these Gram negative

bacteria may prevent the growth of *E. coli* O157:H7 if present in ground beef. The nature of the inhibitor was found to be a protein, which suggests a bacteriocin. These organisms did not produce the inhibitory substance when grown in a liquid broth system. Therefore, an agar extraction and concentration method was developed to isolate and identify the inhibitory substance. A 24 h broth culture of each isolate was swabbed over the surface of 40 brain heart infusion agar plates and were incubated for 48 h at the optimum growth temperature of the isolates, 30°C to 37°C. The agar was then removed from the petri dishes and placed in a sterile blender with 100 ml of a 10% ethanol solution and 100 ml of a 0.1 M citric acid solution. The crude extract obtained in this manner was shown to be inhibitory to *E. coli* O157:H7 and *Listeria monocytogenes* using an agar well diffusion assay procedure. The inhibitory substances were heat stable and inactivated with trypsin and pepsin.

(150) OUTER MEMBRANE PROTEINS AND ADHERENCE OF TEMPERATURE AND IRON-STRESSED ENTEROHEMORRHAGIC *ESCHERICHIA COLI* GROWING AT 9.5°C TO HEP-2 CELLS

T. S. Schwach* and E. A. Zottola, Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenue, St. Paul, MN 55108

Changes in outer membrane proteins (OMPs) by SDS-PAGE and adherence to HEp-2 cells of 3 strains of enterohemorrhagic *Escherichia coli* (EHEC) were monitored. Strains were grown at 9.5°C for 12 days with and without iron-restriction in 0.3% Trypticase Soy Broth plus 1% Nobel Agar. Ethylenediaminedihydroxyacetic acid was added (0.75 mM and 2.0 mM) to induce low and high levels of iron-restriction and 100 mM $FeCl_3$ was added to induce iron-replete conditions. Microbial populations, adherence at 37°C after 6 h (standard method) or 24 h to HEp-2 cells, and OMP profiles were analyzed every 3 days for each treatment. Microbial populations slowly increased from 10^2 to 10^7 CFU/ml over the experimental period for both control and iron-replete treatments while both iron restricted treatments showed less than a 0.5 log increase in microbial numbers during the first 3 days followed by no further changes in population levels. A 6 h adherence assay was used to monitor levels of adherent EHEC in the control and iron-replete samples, which showed a gradual increase from 1 to 15% over the 12 days. A 24 h adherence assay was necessary for the iron-stressed samples as their growth, even at 37°C, was adversely affected by iron-restriction. Although adherence of iron-restricted EHEC showed a slight increase during the first 3 days (from 0.1 to 2%), a steady decline in adherence levels was seen during the remainder of the study with the more iron-stressed sample showing no apparent adherence after 12 days. Changes in OMPs, particularly those involved in iron regulation (70-85 kDa), were noted over the 12 days for each treatment as well as between the 4 treatments. Results suggest that EHEC growing under conditions of both temperature and iron stress exhibit metabolic changes which affect their ability to grow at 9.5°C, their ability to recover and grow at 37°C, and their ability to adhere to HEp-2 cells at 37°C.

(151) SURVIVAL OF *ESCHERICHIA COLI* O157:H7 DURING FERMENTATION OF APPLE CIDER

J. J. Semanchek* and D. A. Golden, Department of Food Science and Technology, University of Tennessee, Knoxville, TN 37901

The ability of *Escherichia coli* O157:H7 to survive for extended periods in acid foods such as apple cider and mayonnaise suggests that this pathogen has unusual tolerance to low pH. This investigation was undertaken to determine the survival characteristics of *E. coli* O157:H7 in fermenting and non-fermenting apple cider. Fresh apple cider was inoculated with *Saccharomyces cerevisiae* and *E. coli* O157:H7, for fermenting cider, or with *E. coli* O157:H7 alone, and incubated at 20°C for 10 d. Populations of *E. coli* O157:H7 were determined daily using Sorbitol MacConkey Agar (SMAC) and Trypticase Soy Agar supplemented with cycloheximide (TSAC). Ethanol was measured using gas chromatography. Populations of *E. coli* O157:H7 were reduced to non-detectable levels in fermenting cider within 3 d at 20°C. In non-fermenting cider, *E. coli* O157:H7 populations were reduced from about 6.5 log CFU/ml to 3 log CFU/ml after 10 d. Recovery was poorer on SMAC than on TSAC, particularly from fermenting cider, suggesting that substantial portions of *E. coli* O157:H7 populations were sublethally injured. Inactivation of *E. coli* O157:H7 in fermenting cider is attributed to a synergistic effect between pH and ethanol (up to 6.01% in fermenting cider). These results indicate that *E. coli* O157:H7 is capable of survival in fresh apple cider at 20°C and that fermentation of cider is an effective method of destroying this pathogen. Alternative means of controlling *E. coli* O157:H7 in fresh apple cider are needed to improve the safety of this product.

(152) APPLICATION OF A FLUOROGENIC 5' NUCLEASE PCR ASSAY FOR DETECTION OF *LISTERIA MONOCYTOGENES* IN RAW MILK

C. A. Batt,* D. M. Norton, B. Miller, R. Behari, S. J. A. Flood, V. Lunge and P. Witham, Cornell University, Department of Food Science, Ithaca, NY 14853

A 5'nuclease, fluorogenic probe-based PCR assay to detect *Listeria monocytogenes* in milk has been refined and tested. The probe is labeled with two fluorophores, a reporter and quencher, and hybridizes to an amplicon from the *L. monocytogenes hly* A gene. It is hydrolyzed during amplification by the 5'nuclease activity of Taq DNA polymerase, releasing the reporter from the dampening effect of the quencher, causing the reporter's emission to increase. The PCR product is then detected directly by measur-

ing the fluorescence of the sample assay after PCR. This assay is quantitative and can detect from 1 to 5 CFU of *L. monocytogenes* in pure culture. Due to the occasional presence of compounds in raw milk that inhibit Taq DNA polymerase activity, a template purification protocol employing a guanidinium hydrochloride extraction procedure and absorption of the DNA onto silica particles was used. The sensitivity of direct detection of *L. monocytogenes* was approximately 100 CFU/ml raw milk. To monitor PCR inhibition, an internal positive control was incorporated into the reaction mix. The control is amplified by the same two PCR primers as the *L. monocytogenes* target but contains a different internal sequence and is detected by a probe labeled with a second reporter dye. By multiplexing the *L. monocytogenes* detection system with the internal positive control, qualitative or quantitative results can be obtained with threshold cutoffs set to signal PCR inhibition and minimum *L. monocytogenes* detection limits.

(153) DEVELOPMENT OF A BACTERIOPHAGE-MEDIATED ATP-BIOLUMINESCENT DETECTION SYSTEM FOR *LISTERIA MONOCYTOGENES*

L. McIntyre,* S. A. A. Jassim and M. W. Griffiths, Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1 Canada

The success of rapid detection methods for foodborne pathogens such as *Listeria monocytogenes* is reliant on both the specificity and sensitivity of the technique. One approach is to exploit the innate host specificity of bacteriophages to selectively infect and lyse only the target pathogen. Subsequent release of bacterial ATP can then be detected by its presence above background levels using a bioluminescence assay. For this study, *Listeria monocytogenes* ATCC 23074 and its specific bacteriophage (ATCC 23074-B1) were chosen to establish an effective detection system. ATP bioluminescence, impedance microbiology, and microscopy were employed to: (i) determine optimal conditions of temperature, time, and phage/bacteria ratios required for cell lysis; and (ii) adapt this protocol for detection of *Listeria monocytogenes* in a milk system. All three techniques confirmed that this combination of bacteria and phage produced successful cell lysis of *Listeria monocytogenes*. Bioluminescent detection in a broth system was achieved within 60 minutes of exposure to the phage at 30°C, while impedance microbiology demonstrated a two-to three-fold increase in time required to detect cells in the presence of phage in both broth and milk systems. This detection protocol is specific for *Listeria monocytogenes*, but requires further development to increase assay sensitivity.

(154) USE OF NISIN TO CONTROL *LISTERIA MONOCYTOGENES* IN QUESO FRESCO CHEESE

A. J. Degnan,* N. Y. Farkye, M. E. Johnson and J. B. Luchansky, Food Research Institute, 1925 Willow Drive, Madison, WI 53706

Queso Fresco (QF) cheese prepared from pasteurized whole milk was challenged with a 3-strain mixture of *Listeria monocytogenes* (LM; 10^4 CFU/ml) and stored at 4 or 12°C for 21 days. Treatments included whole milk prefermented with nisin-producing *Lactococcus lactis* subsp. *lactis* (PFM+N; 5% v/v, 10^8 CFU/ml; 25,000 activity units [AU]/ml; pH 5.5), or Nisaplin™ added to cheese milk (up to 1g/liter or 1000 AU/ml), or Nisaplin™ "salted" onto cheese curd (up to 10g/kg curd or 10,000 AU/hg). In nisin-free control batches (pH 6.3), LM counts increased 1.3 (4°C) or 3.4 (12°C) $\log_{10}$ CFU/g within 21 days. Counts of LM in PFM+N samples declined 1.4 $\log_{10}$ CFU/ml (day 1) and remained about 3 $\log_{10}$ units lower than control population at either storage temperature. The combination of nisin (up to 10,000 AU activity/g cheese) and more acidity (pH 5.3) in PFM+N samples contributed to the decreased counts. Counts of LM in batches to which Nisaplin (1000 AU/ml) was added directly to inoculated milk declined about 2 $\log_{10}$ below inocula levels by day 1 at 4 and 12°C but recovered to control levels within 21 days at 12°C only. Counts of LM in batches to which Nisaplin (10,000 AU/g) was "salted" onto cheese curd declined below detection (10 CFU/g; >3 $\log_{10}$ drop) for up to 14 days at 4°C, while at 12°C, counts lagged 2-3 $\log_{10}$ CFU/g below control populations for 21 days. These data establish the potential for nisin in controlling *Listeria monocytogenes* in fresh Hispanic-style cheese.

(155) RESPONSE OF *ESCHERICHIA COLI* O157:H7 IN THE PRESENCE OF SODIUM LACTATE DURING REFRIGERATED STORAGE WITH AND WITHOUT TEMPERATURE ABUSE

D. E. Conner* and O. Oyarzabal, Auburn University, Auburn, AL 36849

Addition of lactates to processed meats can inhibit bacterial growth, thereby increasing quality and safety. In contrast, sodium lactate (SL) can stimulate growth and survival of *Escherichia coli* O157:H7 under some conditions. Therefore, the effects of SL on the response of this pathogen during refrigeration and when temperature abused were determined. Brain heart infusion containing 0 (control), 1, 2, 3, or 4% (V:V) SL (60%) was inoculated (10^4 CFU/ml), and held statically at constant temperatures of 4°C for 42 d or at 4°C plus temperature abuse at 21 d, in which media were held at 25°C for 24 h and then returned to 4°C. *E. coli* O157:H7 were enumerated weekly. In all SL treatments, populations decreased by 2.6-3.0 $\log_{10}$ CFU/ml during storage, and populations were lowest

($P<0.05$) in the control. When samples were temperature abused, populations increased by 1.3-3.2 $\log_{10}$ CFU/ml. The greatest increase occurred in the control and the least in the presence of 4% SL. However, at 28-42 d, populations were equal ($P>0.05$) in the control and 4% SL treatment and both were higher ($P<0.05$) than in the other treatments. Although SL can limit outgrowth of *E. coli* O157:H7 during temperature abuse, it can enhance survival during refrigeration following temperature abuse.

(156) COMPUTERIZATION IN PASTEURIZATION CONTROLS

R. Coffman, Masterleo and Associates, 771-H Dearborn Park Lane, Worthington, OH 43085

For many years, the food and dairy industries have utilized mechanical instrumentation to monitor processes, electromechanical devices for control and manpower for product routing. While proven safe and effective over the years, each has limitations. Mechanical instrumentation is very application specific, increasing the need for a variety of shelf spares. Electromechanical devices can and do fail, and are often difficult to adjust or trouble shoot. Manpower is subject to turnover and "personalization" of a task, resulting in quality assurance concerns.

Technology is available today to minimize these effects. Micro-processor based recording and control devices are reliable and adaptable to different processes and inputs. Programmable Logic Controllers support growth or modifications in the process, and can be relied upon to provide consistent process performance. Position feedback from field devices can ensure proper valve position before moving product. Because these devices are not foolproof, the FDA developed criteria regarding the use of micro-processor based equipment in public health controls. Adherence to these rules helps ensure a safe, consistent product.

Processors must continue the move away from mechanical devices to micro-processor based instrumentation and control. This will reduce process downtime and improve the quality, consistency and shelf life of products.

(157) ROUND TABLE DISCUSSION: INCREASING DAIRY PRODUCT SHELF-LIFE FROM 16 TO 21 DAYS

J. Delaney,* R. Fuqua, T. Boufford and V. Mills, Prairie Farms Dairy, Inc., Evergreen Packaging, Carlinville, IL 62626

In today's market, most HTST processed fluid milk will consistently hold up to a 14 day shelf-life. There is, however, a pressing demand for longer shelf-life products which is driven by such things as rising distribution costs, a diminishing number of dairy processors and market competition. Going from 14 to 21 day shelf-life requires much more investment by the dairy processor than just purchasing an extended shelf-life filler.

Various dairy industry specialists will participate in a round table discussion where they will share some of the practical aspects of achieving an extended shelf-life on fluid milk products on a consistent basis. Topics to be discussed range from raw product quality, equipment concerns and environmental aspects. Time will be provided for the audience to participate in a question-and-answer session.

(158) PLANT DESIGN AND EQUIPMENT FOR ASEPTIC AND NEAR-ASEPTIC PROCESSING OF MILK

D. Henyon,* C. Reinhart and R. Simpson, Elopak, Inc., 30000 South Hill Road, New Hudson, MI 48165

As the demand to increase product shelf-life continues to grow, many dairy processors are converting part or all of their processing area to the production of UHT or ultra-pasteurized products. The panel will discuss not only the equipment that is needed to meet this challenge, but also the changes in plant design and employee philosophy that play a vital role in the transition from HTST to UHT processing.

The panel will address the package and the packaging aspects, consider differences between direct and indirect processing and discuss some of the various specialized equipment that is needed such as aseptic valves, pressurized storage tanks and steam-block flow diversion systems that meet both the PMO and CFR Section 113. A question and answer period will be provided for audience participation.

(159) GRADE A REGULATION FOR ASEPTIC AND NEAR ASEPTIC PROCESSING EQUIPMENT: FOR MILK AND MILK PRODUCTS

S. Sims, Food and Drug Administration, 200 C Street, Washington, D.C. 20204

This presentation is a practical examination of the principles of operation and Grade A requirements for current and future aseptic and near aseptic milk processing equipment.

(160) PREVALENCE OF *LISTERIA MONOCYTOGENES* IN DAIRY PRODUCTS

R. Byrne,* J. Kozak, T. Balmer and K. Fisher International Dairy Foods Association, 1250 H Street, NW, Suite 900, Washington, D.C. 20005

Over the past ten years, *Listeria monocytogenes* has become increasingly recognized as an important foodborne pathogen. Incidents involving dairy products caused the United States Food and Drug Administration to implement a series of *Listeria* surveillance programs, most notably the FDA Dairy Safety Initiatives. These surveillance programs resulted in the discovery of this pathogen in many varieties of domestic and imported cheese, ice cream, and other dairy products and prompted numerous product recalls. These recalls caused lawsuits, as well as significant financial losses for the dairy industry. Since 1985, an extraordinary amount of research and information regarding

foodborne *Listeria* has been generated resulting in a greater understanding of its impact on the food industry, particularly the dairy foods industry. This paper will discuss the prevalence of *Listeria* in the context of the dairy environment, from farm to processing plant to finished product; examine the favorable conditions in dairy products which make them susceptible to contamination; discuss the results of the FDA Dairy Safety Initiatives regarding the prevalence of *L. monocytogenes* in dairy products; discuss mitigating factors which may provide protection from growth in certain products; delineate the actions and preventive measures which can be taken by the dairy industry and regulatory agencies to minimize public concern; and offer recommendations in addressing the social, political, and scientific issues surrounding *L. monocytogenes*.

(161) CONSUMER PERCEPTIONS OF FOOD SAFETY ISSUES

R. B. Gravani, Cornell University, Department of Food Science, II Stocking Hall, Ithaca, NY 14853

Although the U. S. food supply is among the most varied, affordable and safe, as well as one of the most sanitary and highly regulated in the world, there are still many concerns about the safety of foods.

Food safety is especially important to women, households with children, the high-school educated, and consumers who buy food for a household member on a medically restricted diet. One in five U.S. consumers has doubts about the safety of foods.

Consumers and the scientific community have distinctly different perceptions of food safety risks. Underlying reasons for these differences will be discussed. In addition, data from several national surveys on consumer perceptions of issues including microbiological hazards, pesticide residues in foods, products of biotechnology, and food irradiation will be presented.

(162) CHANGING NEWSPAPER COVERAGE OF MICROBIAL FOOD SAFETY RISKS IN NORTH AMERICA AND IMPLICATIONS FOR RISK COMMUNICATION

D. Powell, Department Food Science, University of Guelph Guelph, Ontario N1G 2W1 Canada

For North American consumers, most knowledge of food safety issues is transmitted and translated through modern media outlets. Media coverage of food and health related issues is increasing dramatically, especially in specialty magazines and cable television shows (or even channels, such as The Food Television Network—because everybody loves to eat). And while the effect of press messages on consumer behavior remains uncertain, recent research suggests that media content typically leads public opinion, at least on a societal level. A comprehensive print media analysis was undertaken to better understand the public discussion of microbial food safety risks in the context of other health-related coverage. Print and electronic indexes for the N.Y. Times and the Toronto Globe and Mail were used to identify all microbial food safety news stories appearing from 1985 to 1993. These stories were analyzed quantitatively and qualitatively to provide a historical perspective on food safety news. From Oct. 1, 1993, to Dec. 31, 1994, all print stories relating to food, health and medicine were collected from the Associated Press, the N.Y. Times, the Toronto Globe and Mail, and the Kitchener-Waterloo Record, a local paper based in Kitchener, Ont., 100 km west of Toronto. A total of 4,029 stories were collected. These four media outlets can be deemed representative of North American newspaper coverage of medical, food and health issues because of the agenda-setting effect of the outlets chosen, and because of the tremendous homogeneity amongst news outlets. Medical stories dominated the coverage, accounting for 41% of the story database. The number of stories dealing with food and health, and with food (and water) safety, were almost identical, at 20% each. Biotechnology stories accounted for 12% of the database, while food biotechnology and pesticides were relatively minor, accounting for 5 and 2%, respectively. The number of food safety stories increased proportionally in the later half of 1994. Qualitatively, news reports of foodborne illness outbreaks in the U.S. typically lead to questions about regulation, science and control of the food production system, much more so than in Canada. Incorporating media analysis research into a comprehensive risk communication plan will be discussed.

(163) EFFECTS OF PROFESSIONAL AND MEDIA WARNINGS ABOUT THE HAZARDS OF *ESCHERICHIA COLI* O157:H7 PRIOR TO AND AFTER THE 1993 JACK-IN-THE-BOX OUTBREAK

L. J. Harris, University of California, Davis, CA, 95616

Governments, scientists, and professional health associations are faced with a dilemma-at what point does sufficient evidence exist to justify changes in policy or public warnings about the potential dangers of a specific hazard? In 1982, *E. coli* O157:H7 was found to be responsible for outbreaks of human illness in Oregon and Michigan after patrons of a fast-food restaurant ate contaminated, undercooked hamburgers. Soon after, reports on the epidemiology, physiology, pathogenicity, and control of this relatively unusual "emerging pathogen" began to appear in the scientific literature. However, this information was not widely diseminated beyond scientific circles. The key event that promoted policy makers, industry, and public awareness of this organism was the 1993 "Jack-in-the-Box" outbreak, due largely to intense media coverage of the outbreak and of the organism since that time. The role of scientific

and public perceptions of health risks from *E. coli* O157:H7 and how these evolving perceptions influenced decision making and public policy initiatives will be discussed.

(164) COMMUNICATING TO THE PUBLIC ABOUT NEW TECHNOLOGIES

C. M. Bruhn, Center for Consumer Research, University of California, Davis, CA 95616

Many consumers recognize personal responsibility in selecting and maintaining safe food. Microbiological safety has been consumers' most frequently volunteered food safety concern for several years, and in the last three years the percentage of people expressing concern has increased from 36% in 1992 to over 50% in 1995. This consciousness and concern could enhance consumer receptivity to newer technologies which enhance microbiological safety, such as food irradiation, laser or high pressure treatment.

Educational programs on new technologies must begin with consumer benefits. Enhanced microbiological safety, even though it is an invisible benefit, can be potent when the benefit can safeguard people you care about.

While some consumers will maintain a cautious stance toward an unfamiliar technology, many will try a new product if the benefits seem real and other safety and quality concerns are addressed. When considering food irradiation, consumers have expressed concern about flavor, nutritional quality, and worker and environmental issues. Even though the latter is not within the usual scope of educational programs, if not addressed consumers may reject the process.

Since consumers get science and food safety information from television, newspapers, and magazines, this medium should be used to convey information about food irradiation or other new technologies to safeguard their food. The value of "irradiation pasteurized" food for children and older adults could be emphasized through the media used by the individual responsible for their food purchase and preparation, such as "Modern Maturity" or "Parenting."

Consumers evaluate the believability of a message by the credibility of the person conveying the message, their personal judgment if the message makes sense, and the frequency of the message. The most highly respected health and safety authorities is the American Medical Association, followed by the American Dietetic Association and the FDA/USDA. These groups have endorsed the safety of irradiated food. Activist groups express concern about irradiation, however their credibility appears to have decreased.

Experience with food irradiation indicates products processed by newer and less familiar technologies which offer consumers quality or safety benefits will be accepted.

(165) AVAILABILITY OF FOOD SAFETY AND HACCP TRAINING MATERIALS

C. Roberts, USDA-FDA Foodborne Illness Education Information Center, National Agricultural Library, Room 304, 10301 Baltimore Blvd., Beltsville, MD 20705-2351

Shrinking budgets for education and training don't have to lead to a decrease in quality of training. Rather, business owners and others responsible for training food workers need to broaden their horizons by looking outward to see what materials others have developed. This presentation will focus on available training resources and how to access those materials both conventionally and electronically. Gaps in available resources will be discussed for those thinking of developing new training materials.

(166) DESIGNING FOOD SAFETY AND NUTRITION INFORMATION, EDUCATIONAL MATERIALS AND CAMPAIGNS: A COMPARATIVE REVIEW OF FOUR CAMPAIGNS

C. Schiffman, FDA, Center for Food Safety and Applied Nutrition, Washington, D.C. 20204

Health education campaigns require careful planning based on the application of research and communication theory. A comparative study of four health education campaigns (two food safety; two nutrition) details the thinking behind the message design decisions, and materials and channels chosen. Significant issues which influence visual and verbal message design choices, and material and channel format arise when developing information and campaigns for ethnic and hard-to-reach at-risk audiences.

(167) A DEFINITIVE AND RAPID METHOD FOR IDENTIFYING ATYPICAL *SALMONELLA* FROM SELECTIVE AGAR PLATE

E. M. Cole,* S. Tseng, M. Barbour, D. Macool, L. Ecret, C. I. McDowell, H. K. White and B. Krieger, FQMS, L.L.C., an affiliate of E. I. Du Pont de Nemours & Company, Experimental Station, Wilmington, DE 19880-0357

Atypical *Salmonella* isolates from food and environmental samples are often overlooked on selective agar plates because they resemble many other coliform bacteria; therefore, testing of these isolates is often delayed because microbiological work ups are being performed on the more typical looking suspect colonies. In some cases, identification of suspect colonies by classical conventions can be cumbersome and difficult to interpret. The amount of time, labor, expense, and experience needed for accurate identifications can be prohibitive to many food plants. We have developed a rapid, accurate, sensitive, and easy-to-use assay to identify *Salmonella*, including atypical colonies, directly from selective agar plates.

The method uses suspect colonies from selective agar plates and combines PCR amplification with homogeneous fluorescence for detection. A panel consisting of 250 known *Salmonella* serovars from our collection of over 1800 strains in addition to 150 non-*Salmonella* organisms (*Citrobacter, Hafnia, Proteus,* and others) were tested on BS, HE, and XLD agar. The assay gave positive results for the *Salmonella* strains and negative results for the non-*Salmonella* organisms. Results of the assay allow definitive identification of suspect colonies as *Salmonella* in only 2.5 hours.

(168) CONTROL OF ENTERIC PATHOGENIC BACTERIA ON FRESH PRODUCE

D. L. Peters,* S. S. Sumner, J. A. Albrecht and L. B. Bullerman, University of Nebraska, Lincoln, NE 68583-0919

Enteric pathogens on fresh produce have been implicated in numerous outbreaks of foodborne illness. The effectiveness of different wash treatments as controls of *Shigella* spp., *Escherichia coli* O157:H7 and *Salmonella* spp. was tested. Lettuce and green pepper inoculated with *Shigella* and broccoli florets and cherry tomatoes inoculated with *E. coli* O157:H7 were washed by dipping or spraying. Wash treatments included water, 2% or 5% acetic acid (AA), 20 μg/L monolaurin, or 3% hydrogen peroxide (H_2O_2) alone or in combination with 2% or 5% AA. Nisin with ethylenediaminetetraacetic acid was also used as a wash treatment. Produce samples were analyzed immediately and after 3 days storage at 12°C. Two wash treatments (2% AA + 3% H_2O_2 and 5% AA + 3% H_2O_2) were significantly ($P < 0.05$) different from the other wash treatments, with an average log reduction in bacterial numbers of 4.5, which eliminated the inoculum. Cantaloupe and honeydew melon inoculated with *Salmonella* were spray washed with 5% AA + 3% H_2O_2, or 2% AA + 3% H_2O_2, with water as a control. The average log reductions in bacterial numbers were 4 and 3.5 on smooth rinds, and 3 and 2 on rough rinds, respectively. Neither treatment eliminated the *Salmonella* from the rinds. The two wash treatments were significantly ($P<0.05$) more effective on the honeydew melon rinds.

(169) EVALUATION OF THE *SALMONELLA* BAX™ SYSTEM. A RAPID PCR BASED METHOD FOR THE ANALYSIS OF FOODS FOR FOODBORNE *SALMONELLA*

C. L. Sobities,* A. R. Bennett, D. J. Greenwood, R. P. Betts and J. G. Banks, DuPont Experimental Station, Wilmington, DE 19880-0357

BAX is a commercially available one-day *Salmonella* detection method based on a PCR amplification of *Salmonella*-specific nucleic acid. Food samples are non-specifically preenriched for 20h. After pre-enrichment, samples undergo a secondary enrichment in Brain Heart Infusion Broth for 3h. Cells are lysed and added to the PCR tubes, which contain all reagents required to perform amplification. *Salmonella*-specific PCR product is detected using a simple gel electrophoresis procedure. The evaluation reported here tested the BAX *Salmonella* procedure against the International Standards Organization (ISO) *Salmonella* methodology. The evaluation considered sensitivity, specificity, and comparison of method performance between the BAX and ISO method. The method sensitivity was tested using 20 serotypes, which form the top ten food poisoning *Salmonella* in the USA and UK. Specificity was evaluated by testing 100 *Salmonella* serotypes, together with thirty-five potentially cross reacting organisms (i.e., organisms that can cause false positive reactions in conventional or other rapid tests). Comparative results were obtained through tests on 50 different foods inoculated with low levels of *Salmonella* and 20 additional naturally contaminated samples. The results show the *Salmonella* BAX test to be a sensitive (10^4 - 10^5 CFU/ml), rapid (28h) test with excellent specificity and giving results that are comparable to those of the ISO method.

(170) ESTABLISHING BASELINE RISK FOR *SALMONELLA ENTERITIDIS* IN SHELL EGGS

R. A. Morales,* L. A. Jaykus and P. Cowen, North Carolina State University, Raleigh, NC 27695-7620

Foodborne *Salmonella enteriditis* (S.e.) infection is a significant public health concern, frequently epidemiologically linked to the consumption of grade A shell eggs. Recently promulgated food pathogen control programs and strategies seek to reduce the risk of foodborne disease yet involve definitive costs for a finite level of risk reduction. This paper presents the first step in a comprehensive, integrated risk and economic assessment approach to the evaluation of HACCP-based interventions for the control of S.e. contamination in shell eggs. Within the framework of probabilistic scenario analysis, first-iteration estimates are presented for the traditional exposure assessment (probability of exposure 1.2×10^{-3} - 1.2×10^{-6}, dose-response assessment (probability of disease given exposure 1.3×10^{-1} - 1.6×10^{-2}), and risk characterization (individual risk estimated at 1.6×10^{-4} - 1.9×10^{-8}) components of a quantitative risk assessment to evaluate potential human health risk due to the consumption of S.e.-contaminated shell eggs. The second-iteration method for characterizing risk using a Monte Carlo approach and the method for evaluation of HACCP-based interventions for risk mitigation will be briefly discussed.

(171) ELIMINATION OF *SALMONELLA* AND *STAPHYLOCOCCUS AUREUS* FROM BISON, OSTRICH, ALLIGATOR AND CAIMAN MEAT BY GAMMA IRRADIATION

D. W. Thayer* and G. Boyd, USDA-ARS-ERRC, 600 East Mermaid, Wyndmoor, PA 19038

There is an expanding industry for the marketing of high-value meats from animals other than the typical domesticated species, including, but not limited to, bison, ostrich, alligator, and caiman. In

this study we compared the gamma radiation resistance of a mixture of *Salmonella* (*S. dublin, S. enteritidis, S. newport, S. senftenberg* and *S. typhimurium*) and one of *Staphylococcus aureus* (ATCC 13565, ATCC 25923, and B124) when present on ground bison, ostrich, alligator, and caiman meats at 5°C. A minimum of five doses were used to establish the D-values, and the studies were replicated three times. The radiation D-values for *Salmonella* and *S. aureus* were 0.53 ± 0.02 and 0.37 ± 0.01 kGy, respectively. These are composite values, as the D-values obtained with the various meats did not differ significantly. The authors conclude that both of these foodborne pathogens can be eliminated from these meats by gamma radiation doses between 1.5 and 3.0 kGy at 5°C.

(172) DETECTION OF *SALMONELLA* IN FOOD USING A NOVEL PCR-BASED FLUOROGENIC 5' NUCLEASE ASSAY

C. T. Yamashiro,* R. Behari, S. J. A. Flood, C. Thio, P. Witham, D. M. Norton, S. Kawasaki, B. Kimura, T. Fujii and C. Paszko-Kolva, Perkin Elmer, 850 Lincoln Center Drive, Foster City, CA 94404

Specific and rapid detection of *Salmonella* in food, including meat and dairy products, is an international concern for both food processors and consumers alike. We have developed a total system for food sample preparation and subsequent detection of *Salmonella*. Preenrichment media, including tryptic soy broth and Universal Preenrichment Broth, were evaluated for growth rates of *Salmonella* in a variety of food products and amenability towards DNA extraction methods for polymerase chain reaction (PCR)-based analysis. Several DNA extraction methods were evaluated for ease of use, efficiency of lysis, and their ability to eliminate PCR inhibitors. Detection of PCR exploits the endogenous 5' nuclease activity of AmpliTaq® DNA Polymerase to digest an internal probe which is labeled with both a fluorescent reporter dye (i.e., FAM) and a quencher dye (TAMRA). FAM fluorescence is quenched by TAMRA on the intact probe. During PCR amplification, the probe is hydrolyzed and the FAM fluorescence intensity increases. Immediately after the PCR the samples are analyzed on a 96 microwell fluorescence plate reader, which eliminates the need for gel electrophoresis. We also implemented an internal control for detecting inhibition of PCR in a multiplex format. This control uses a specific probe containing a different reporter dye (i.e., TET) unique to the control amplicon. Detection of *Salmonella* by this system has excellent correlation to standard culture detection methods. This total system approach facilitates rapid and specific screening of foods for *Salmonella*.

(173) RAPID MOLECULAR METHOD FOR THE DETECTION OF HUMAN ENTERIC VIRUSES IN CLAMS

A. B. Dix, Department of Food Science, Box 7624, North Carolina State University, Raleigh, NC 27695-7624

Application of methods to extract and concentrate human enteric viruses from hard-shelled clams (*Mercenaria mercenaria*) for detection using reverse transcriptase-polymerase chain reaction (RT-PCR) and confirmation by oligoprobe hybridization (OP) were evaluated. Fifty-gram clam samples were processed by an absorption-elution-precipitation method and seeded with 10^3-10^5 plaque forming units (PFU) of poliovirus (PV1) and hepatitis A virus (HAV). Seeded clam concentrates were further purified by fluorocarbon (Freon) extraction and concentrated by polyethylene glycol (PEG) precipitation and elution. Virus recoveries after Freon extraction were 51% for PV1 and 70% for HAV. Both viruses were effectively precipitated at PEG concentrations greater than 6%. Susbequent elution of PEG pellets was less efficient. To further concentrate viruses and reduce sample volume, the protein precipitating agent Pro-Cipitate® was employed. PEG eluants processed with Pro-Cipitate® resulted in virus recoveries from 20-50% and direct compatability with RT-PCR amplification after a 0.5-log dilution. Investigation of various RT-PCR enhancements agents revealed that supplementation of reactions with 2.5-5.0% PEG 4000 resulted in a one-log increase in virus detection at low levels of target viruses.

(174) THE EFFECTS OF SOME EXTRUSION AND CANNING PROCESSES ON DEOXYNIVALENOL

C. E. Wolf-Hall,* M. A. Hanna and L. B. Bullerman, University of Nebraska-Lincoln, Lincoln, NE 68583-0919

The effects of extrusion processing of corn grits spiked with deoxynivalenol (DON) under different conditions, including moisture content variations (18, 22 and 26%), temperature variations (120, 140 and 160°C) and mixing versus nonmixing, on DON concentrations were examined. Moisture content and temperature effects were not apparent, but more DON was lost in the nonmixed extrusion (0-40%) than in the mixed (0-27%). When the effect of extruding spiked dry dog food on DON under simulated industry conditions was examined, no significant ($P < 0.05$) loss in the amount of DON was detected. Also tested were the effects of canning processes on DON in spiked cream style corn, a mixed cereal product baby food, and a dog food. Only the cream style corn process showed a significant decrease in DON levels (12%). The use of an α-amylase in the extraction method for analysis by an enzyme-linked immunosorbant assay greatly improved the recovery of DON from the extruded and canned products. Overall, DON showed heat stability with the thermal processes tested.

(175) ELECTRON MICROSCOPY OF FUNGAL SPORES PRODUCED UNDER REDUCED WATER ACTIVITY

M. Blaszyk* and G. Blank, University of Manitoba, Winnipeg, MB R3T 2N2 Canada

Spores of *Penicillium roqueforti*, *Penicillium cyclopium* and *Penicillium viridicatum* were produced on potato dextrose agar having water activity (A_w) levels of 0.99 and 0.88. Either glycerol or NaCl was used as a controlling solute. Scanning electron microscopy indicated size and surface appearance differences among spore crops. In general, *Penicillium* spores produced on media with 0.99 A_w were uniform in size (e.g., av. diam. of *P. roqueforti* spores was 1.71 ± 0.08 μm) and were covered with a smooth, dense outer sheath. In contrast, spores produced on 0.88 A_w were more variable in size (e.g., av. diam. of *P. roqueforti* spores produced on glycerol and NaCl were 1.83 ± 0.16 and 1.76 ± 0.11 μm, respectively). The outer layer appeared either thin and translucent (*P. roqueforti* and *P. viridicatum*, PDA-glycerol 0.88 A_w) or dense and fractured (*P. roqueforti* and *P. viridicatum*, PDA-NaCl 0.88 A_w; *P. cyclopium* either PDA-glycerol or PDA-NaCl, 0.88 A_w). Examination of transmission electron sections revealed that the outer layer of *Penicillium* spores produced on media 0.88 A_w was loosely attached to the spore surface and often fractured (cabbage like). Although spore cytoplasm appeared dense and amorphous, making internal differentiation difficult, spores produced on PDA 0.88 A_w containing glycerol did appear to contain large structures resembling lipid bodies. However, spore treatment with a chloroform and methanol mixture during fixation did not eliminate these structures.

(176) STABILITY OF FUMONISIN B_1 (FB_1) DURING EXTRUSION COOKING

S. Katta,* M. Castelo, S. Sumner, M. Hanna, and L. Bullerman, University of Nebraska, Lincoln, NE 68583-0919

Stability of fumonisin B_1 (FB_1) during extrusion cooking of spiked corn grits (5 μg/g) in a twin screw extruder was investigated. A 3 × 3 × 2 factorial design was used to determine the effects of temperature (120, 140, and 160°C), moisture content of the corn grits (18, 22, and 26%), and screw type (mixing and nonmixing) on FB_1. Extruded samples were analyzed using a commercial enzyme-linked immunosorbent assay (ELISA). Fumonisin recovery from unextruded corn grits was found to be about 96%. Extrusion cooking resulted in the partial reduction of FB_1, which ranged from 12.3-56.6% depending on extrusion conditions. Extrusion cooking with a nonmixing screw resulted in an increased reduction of FB_1 with increasing moisture contents. At a moisture content of 26%, more reduction of FB_1 was observed at 120°C compared to at 140°C or 160°C. On the contrary, in case of extrusion cooking with the mixing screw, more reduction was observed at 160°C compared to at 120°C or 140°C. Of all the parameters studied in this experiment, extrusion cooking with a mixing screw at 160°C and 22% moisture content (db) resulted in the maximum reduction of FB_1 (56.6%).

(177) INHIBITION OF GROWTH AND MYCOTOXIN PRODUCTION OF PENICILLIUM BY LACTOBACILLUS SPECIES

H. Gourama, Penn State University, Berks Campus, P.O. Box 7009, Reading, PA 19610-6009

Lactic acid bacteria have been reported to affect mold growth and mycotoxin production. In this study, two lactobacilli isolates that were isolated from food products were found to inhibit or reduce mold growth and mycotoxin production by *Penicillium* species. The molds investigated were *Penicillium citrinum* and *Penicillium expansum*. Two types of mycotoxins were tested, patulin and citrinin. The inhibitory activity in the two lactobacilli cell free supernatants was shown to be unrelated to the production of lactic acid or hydrogen peroxide and was found to be sensitive to proteolytic enzymes such as trypsin. The inhibition was eliminated by heating the supernatant at 100°C for 10 minutes. The addition of *Penicillium* spores into the cultures of lactobacilli caused a decrease in mycotoxin production. The antimycotoxigenic activity was not affected by the addition of glucose used by lactobacilli species during incubation.

(178) AN EASY SCREENING TEST FOR DETECTING YEAST CONTAMINATION IN RINSE WATER SAMPLES

C. M. Chen,* K. Doherty and A. Naqui, IDEXX Laboratories, One IDEXX Drive, Westbrook, ME 04092

An enzyme-based yeast test for the rapid screening of yeast contamination in rinse water samples from soft drink bottling plants is described. This method uses a quality control (QC) measure for yeasts in rinse water of 15 CFU/100 ml as the pass/fail standard. The pre-packaged powder reagent is used with 100 ml water collected in a sterile vessel. After adding a liquid reagent, the sample vessel is incubated at 30°C for 72 hours. Positive samples, which indicate the presence of >15 CFU of yeasts in the 100 ml water, exhibit green fluorescence when viewed under a UV_{365nm} lamp. The results obtained with pure cultures showed that 92% of the 140 yeast isolates were positive in 3 days with an inoculum of 16-50 CFU/100 ml and 95% were positive in 4 days with the inoculum of <15 CFU/100 ml. No cross reactivity with bacteria (~10^5 CFU/100 ml) was observed within 5 days of incubation at 30°C. This screening test was evaluated in parallel with the 5-day "Monitor" membrane filtration method using 364 rinse water samples from 4 soft drink bottling plants. The index of agreement between these two methods was 84%. This 3-day yeast screening test is easy to use, does not require trained microbiologists to perform the test, provides consistent result interpretation, and is a useful tool for the routine QC for detecting yeast contamination in rinse water from soft drink bottling plants.

(179) FUMONISIN CONCENTRATIONS IN COMMERCIAL CORN-BASED FOOD PRODUCTS

M. Castelo,* S. Sumner and L. Bullerman, University of Nebraska-Lincoln, Lincoln, NE 68583-0919

Corn-based food products purchased in three different parts of the country, Maryland, Arizona and Nebraska were analyzed for total fumonisin using a commercial enzyme linked immunosorbant assay (ELISA) and for fumonisin B_1 (FB_1) using high-performance liquid chromatography (HPLC). All 18 samples collected from Maryland were found positive for fumonisins (200-7450 ng/g) by ELISA and 15 of the 18 samples were found positive for FB_1 (27-5916 ng/g) by HPLC. All 15 samples collected from Arizona were also found positive for fumonisins (50-1450 ng/g) by ELISA and either of the 15 samples were found positive for FB_1 (33-1565 ng/g) by HPLC. Twenty-one of 23 samples collected from Nebraska were found positive for fumonisins (100-2500 ng/g) by ELISA, with only ten samples containing detectable levels of FB_1 (33-927 ng/g) by HPLC. Overall, the highest fumonisin concentrations were found in corn meals and corn muffin mixes, and ranged from 400 ng/g to 7450 ng/g for fumonisins by ELISA and from 36 ng/g to 5916 ng/g for FB_1 by HPLC. These findings indicate that there may be a high risk of human exposure to fumonisins through the consumption of some corn-based foods.

(180) RETENTION OF ACID TOLERANCE AND ACID SHOCK RESPONSES IN *ESCHERICHIA COLI* O157:H7

D. Garren,* M. Harrison and S. Russell, University of Georgia, Department of Food Science and Technology, Athens, GA 30602-7610

Escherichia coli O157:H7 and non-O157:H7 survival due to the retention of an induced acid tolerance or shock responses when exposed to lactic acid was studied over time. Induced acid tolerance or shock responses could allow pathogens, like *E. coli* O157:H7, to survive in acidic foods during storage. *E. coli* O157:H7 strains (932 and E009) and a non-O157:H7 strain (23716) were used to determine if cells could adapt to a nonlethal acidic pH and then survive extreme acidic conditions over time. *E coli* cells were grown to stationary phase at 32°C and were exposed to one of two treatments: acid shock or acid adaption. Acid shocked cells were directly challenged with lactic acid at pH 3.5 or 4.0. Acid adapted cells were initially exposed to a pH of 5.5, and then challenged at pH 3.5 or 4.0. *E. coli* samples were incubated at either 25°C or 32°C and sampled to monitor cell survival using a Vitek bactometer at time intervals over 21 d. All strains appeared to have significantly higher populations and survived longer at pH 4.0 and 25°C. A significant difference was observed in the two responses with acid shocked cells having a slightly higher survival rate than acid adapted cells after extended storage. Survival rates differed for the strains regardless of pH and temperature storage conditions, with strain 932 the most acid resistant and strain 23716 the least.

(181) A MODEL FOR THE INTERACTIONS OF TEMPERATURE, PH, LACTATE, SALT AND NITRITE ON THE SURVIVAL OF *E. COLI* O157:H7

R. Whiting* and M. Golden, USDA-ARS-ERRC, 600 East Mermaid Lane, Wyndmoor, PA 19038

To develop a tool for estimating the survival of *E. coli* O157:H7 during food storage, a four-strain mixture was inoculated into brain heart infusion broths having adjusted pH (3.5 - 7), added salt (0.5 - 15%), lactate (0 - 2%) and nitrite (0 - 75 ppm) and stored at 4 to 37°C. Their survival was followed in 81 combinations of the five environmental factors by enumerating for up to 4 months on tryptic soy agar plates. The patterns of declining numbers of viable cells were fitted to a logistic model and the times for 3 to 6 logs of decline were then described by a polynomial regression equation with confidence intervals. Survival times were shortened when temperatures exceeded 11°C and pH values were below 5.0. Increasing the concentration of lactate or nitrite reduced the survival times at low pH's. Salt had a protective effect. Survival times ranged from less than a day to exceeding the 4 month experimental period. By providing a prediction of the pathogen's ability to survive, this model can assist in estimating the fate of *E. coli* O157:H7 that may contaminate uncooked meat products or other foods.

(182) EFFECTIVENESS OF SANITIZERS VS. *E. COLI* O157:H7

Z. L. Wang* and M. J. Banner, Diversey Corporation, 46701 Commerce Center Drive, Plymouth, MI 48170

This study raised the question, would certain sanitizers commonly used by the food industry and known to be effective vs. common *E. coli* be as effective against *E. coli* O157:H7? Sanitizers based on peracetic acid, quaternary ammonium compounds (QAC) and phosphoric acid were evaluated via a suspension test method. A common *E. coli* was tested against 2 strains of *E. coli* O157:H7. The suspensions were exposed to the sanitizers for 0.5, 1.0, 2.0 and 5.0 min. (and 10 min. for 1 of the QACs) under clean conditions or under dirty conditions. Peracetic acid induced a 5-log kill vs. all strains in as little as 0.5 minutes under both clean and dirty conditions. QACs induced a 5-log kill in 0.5 min. under clean conditions, but not under dirty conditions even after 5 and 10 min. of exposure vs. the 3 strains of *E. coli*. The effectiveness of phosphoric acid sanitizer varied with the soil conditions, contact times and the strains of *E. coli*. Some differences in the resistance of the *E. coli* O157:H7 vs. the common strain of *E. coli* were observed. When the sanitizers were used at the concentrations and contact times normally used, they were effective vs. the

E. coli O157:H7 as well as the common *E. coli* strain. In general, all the sanitizers were effective vs. the *E. coli* O157:H7 under clean conditions. The presence of soil reduced the preformance of the QAX and phosphoric acid sanitizers, which shows the importance of cleaning prior to sanitizing.

(183) HEAT SHOCK RESPONSE PROTECTS *ESCHERICHIA COLI* O157:H7 AGAINST LETHAL ACIDITY

G. Wang* and M. Doyle, University of Georgia, Department of Food Science, Griffin, GA 30223-1797

Escherichia coli O157:H7 has unusual acid tolerance which has been associated with acid-induced proteins. The influence of heat shock on acid tolerance of *E. coli* O157:H7 was studied. Seven strains of *E. coli* O157:H7and *E. coli* K-12 were tested for their ability to survive in minimum glucose medium (pH 2.5) at 37°C. Survival of heat-shocked (10 min at 48°C) cells was about 10 to 100 times greater compared with nontreated cells depending on the strain. No significant difference ($P > 0.05$) for *E. coli* O157:H7 strain 932 was observed between heat shock-induced and acid adaptation-induced (pH 5.0) acid tolerance. Chloramphenicol (100 µg/ml) pre-vented heat shock-induced acid tolerance, indicating the requirement of newly synthesized protein(s). Two outer membrane proteins (22 and 14 kD) were synthesized within 10 min of heat shock and were expressed for at least 6 hours by cells growing at 37°C. N-terminal amino acid sequence analysis revealed that the 22 kD protein contains a redox active disulfide, which is likely involved in H^+ transport. Results indicate that sublethal heat treatment of *E. coli* O157:H7 cells substantially increases their tolerance to acidic conditions. This could have practical implications for foods that received a mild heat treatment and rely on acid as a preservative.

(184) SURVIVAL OF *ESCHERICHIA COLI* O157:H7 IN DRINKING AND RECREATIONAL WATER

G. Wang* and M. Doyle, Univeristy of Georgia, Department of Food Science, Griffin, GA 30223-1797

Several recent *Escherichia coli* O157:H7 (O157) outbreaks associated with both drinking and recreational water raise concerns about waterborne infection caused by this pathogen. The survival characteristics of a mixture of five nalidixic acid-resistant O157 strains (10^3 CFU/ml) in samples of autoclaved, filtered municipal water, reservoir water, and two recreational lake water were studied for a period of 91 days at 8, 15, or 25°C. Greatest survival was in autoclaved, filtered municipal water and least in lake water, suggesting that O157 needs minimum nutrients to survive. At 15°C, O157 populations rapidly decreased within 21 days, whereas total aerobic microorganisms only decreased < 1.0 $\log_{10}$ CFU/ml within 28 days. Regardless of the type of water sample, survival of O157 was greatest at 8°C and least at 25°C. SDS-PAGE of surface antigens of surviving cells revealed that there was no alteration in lipopolysaccharide pattern but outer membrane protein composition did change. These studies indicate that O157 is a hardy pathogen that can survive for a long period of time in water, especially at cold temperatures. The competition or predation for nutrients and antagonistic activity from other microorganisms likely affect the survival of O157 in an aquatic environment.

(185) HEAT INACTIVATION AND INJURY OF *E. COLI* O157:H7 CULTURED AT 10 AND 37°C

J. Semanchek* and D. Golden, University of Tennessee, Department of Food Science, Knoxville, TN 37901

When subjected to harsh environments, bacteria may become injured and escape detection by routine enumeration procedures, resulting in an inaccurate assessment of the viable population in foods. Outbreaks of *Escherichia coli* O157:H7 foodborne illness associated with consumption of cooked foods has raised questions concerning the ability of this pathogen to survive during exposure to heat. This study was undertaken to evaluate inactivation and injury of three strains of *E. coli* O157:H7 (ground beef, cider, and salami isolates). Test strains were grown in Trypticase Soya Broth at 10 and 37°C, heated in peptone water at 52, 54, and 56°C for up to 90 min, and surface plated on Trypticase Soya Agar (TSA) and TSA containing 2% NaCl. The ground beef isolate exhibited the greatest amount of cell death and injury during heat treatment, whereas, the salami isolate was the most heat resistant strain tested. All strains grown at 10°C were more sensitive to heat treatments than strains grown at 37°C, indicating that sub-optimum temperature impedes the ability of *E. coli* O157:H7 to withstand subsequent thermal stress. Results obtained from this study reveal substantial variability in the heat resistance among the three test strains of *E. coli* O157:H7. Minimal heat processing may be inadequate for rendering foods free of *E. coli* O157:H7 but may result in the development of sublethal injury. Sublethally injured *E. coli* O157:H7 may not be detected using selective isolation procedures, as evidenced by the inability of heat-injured cells to grow on TSA containing 2% NaCl.

(186) STUDIES ON THE SURVIVAL OF *ESCHERICHIA COLI* O157:H7 IN FERMENTED MEAT PRODUCTS

D. Riordan,* G. Duffy, J. Sheridan, R. Whiting, B. Eblen, D. McDowell and I. Blair, The National Food Centre, Dunsinea, Castleknock, Dublin 15 Ireland

Recent food poisoning outbreaks have linked *E. coli* O157:H7 with fermented ready-to-eat meat products. This study investigated its survival during pepperoni manufacturing and validated the models previously developed in broth systems. The salami out-

break strain (30-2C4) was inoculated into a standard pepperoni batter and its survival monitored throughout the 37°C fermentation and drying (15°C for 14 d) processes using TSA plates overlaid after 2 h with sorbitol-MacConkey agar. The results showed a reduction in numbers during fermentation of approximately $\log_{10}$ 0.5 CFU/g and during drying of an additional 1.0 log. The survival was affected by the interaction of different acidities (dextrose levels), and salt and nitrite addition. The survival of three *E. coli* O157:H7 strains (salami; Ent C9490 hamburger; and a clinical food isolate) in a fermented meat matrix at 50, 55 and 60°C showed mean D-values of 92.67, 18.92 and 1.212 min, respectively. No significant differences in thermal resistance between the three strains were observed. These data suggest that this pathogen is relatively heat resistant, and this has implications for introducing a heating step into the manufacturing process.

(187) EVALUATION OF AN ELISA SYSTEM FOR DETECTING VEROTOXIN PRODUCED BY ENTEROHEMORRHAGIC *E. COLI* (EHEC)

W. Tsai,* C. Miller and E. Richter, Silliker Laboratories Group of Ohio, Inc., Columbus, OH 43212

Verotoxins (*Shiga*-like toxins) produced by EHEC are one of the major virulence factors contributing to this type of infection. In this study, a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) system for detecting verotoxin was evaluated. Four EHEC (*E. coli* O26:Hll, *E. coli* O46:H38, and two *E. coli* O157:H7) were inoculated into two batches of 375 g raw ground beef (10^4 APC/g, and 10^6 APC/g) with 10^{-1}, 10^0, and 10^1 EHEC cells per g of sample. All samples were diluted 1:10 in TSB + mitomycin C (25 ng / ml) and incubated for 22 ± 2 h. at 37 °C. The results demonstrated that the assay could detect verotoxin when 10^1 cells/g were present in the ground beef samples with low (< 10^4 APC/g) background flora. The target/competitor ratio plays a major role affecting the assay sensitivity. In addition, 500 raw ground beef samples obtained from local retailers were tested with the assay. Nine samples were found to be verotoxin positive. With further confirmation, including immunoblot techniques, three samples were confirmed to have been contaminated with non-O157:H7 EHEC.

(188) A MULTIPLEX PCR ASSAY FOR DETECTING VEROTOXIN-PRODUCING *ESCHERICHIA COLI* O157:H7

J. Meng,* S. Zhao and M. Doyle, University of Georgia, Georgia Experimental Station, Griffin, GA 30223

A PCR assay (SZ-PCR) for detecting *E. coli* O157:H7 was described in our previous study that detected a 633-bp DNA sequence upstream of the *eae* gene. But it also detected some strains of enteropathogenic *E. coli* O55:H7 and O55:H-. To overcome the limitation of this PCR assay that failed to differentiate some *E. coli* O55:H7 and O55:H- strains from O157:H7, we developed a multiplex PCR assay that simultaneously identified *E. coli* O157:H7 and verotoxins (VT) it encodes. Two pairs of oligonucleotide primers were designed to amplify a 214-bp and a 430-bp fragments for VT 1 and 2 genes, respectively, based on the gene sequence data obtained from the GenBank database. We evaluated the multiplex PCR using 122 isolates of *E. coli* O157:H7, VT-producing *E. coli* (VTEC), non-VTEC, and other bacterial genera. All 40 isolates of *E. coli* O157:H7 were correctly identified by SZ primers along with the VT types produced by each isolate. Eleven *E. coli* O55:H7 and O55:H- isolates were negative for the VT1 and 2 genes. No VTEC strains other than O157:H7 were amplified by SZ primers. Therefore, this multiplex PCR is able to differentiate *E. coli* O55:H7 and O55:H- from O157:H7 and is specific for VT-producing *E. coli* O157:H7.

(189) THE BEHAVIOR OF *ESCHERICHIA COLI* O157:H7 IN FERMENTATION SYSTEMS WITH THERMOPHILIC AND MESOPHILIC DAIRY STARTER CULTURES

J. Soudah* and K. Boor, Cornell University, 415 Stocking Hall, Ithaca, NY 14853

The recent surprising isolation of *E. coli* O157:H7 from patients who became ill following consumption of fermented foods, including salami and yogurt, suggests a critical need to understand the interactions between this pathogen and commercial starter cultures commonly used in the food industry. To that end, we have examined the survival of *E. coli* O157:H7 in UHT milk in the presence of various starter cultures. 100 ml of milk were inoculated with low (10 cells/ml), medium (10^3 cells/ml) or high (10^5 cells/ml) levels of *E. coli* O157:H7 (SEA 6396) alone (control) or with the various levels of *E. coli* and 1% (v/v) of a single starter culture or an equal blend of two thermophilic or two mesophilic cultures. Thermophilic starter cultures included *Streptococcus thermophilus* and *Lactobacillus delbruekeii* ssp. *bulgaricus*; mesophilic starter cultures used were *Lactococcus lactis* ssp. lactis and *Lactococcus lactis* ssp. cremoris. Thermophilic fermentations were held at 37° or 42°C for 24 hours and mesophilic fermentations were held at 21° or 30°C for 24 hours. In all cases, *E. coli* O157:H7 survived the 24 hour incubation periods. Pathogen numbers were reduced in the presence of the lactic starter cultures. Mixtures of *Streptococcus thermophilus* and *Lactobacillus delbruekeii* ssp. *bulgaricus* or of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* appeared to be more effective at reducing *E. coli* numbers than when these cultures were used alone. This may be a consequence of the ability of the combined starter cultures to synergistically produce acid. These results suggest that a combination of starter cultures chosen for rapid acid production will provide an increased margin of safety in the production of fermented dairy products.

(190) THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN MEAT

O. P. Snyder, Jr.,* K. Juneja and B. S. Marmer, USDA-ERRC, 600 East Mermaid Lane, Wyndmoor, PA 19038

Thermal inactivation of a four-strain mixture of *E. coli* O157:H7 was determined in 90% lean (10% fat) ground beef, lean chicken, turkey, pork and lamb. Inoculated meat was packaged in bags which were completely immersed in a circulating water bath and held at 55°, 57.5°, 60°, 62.5° and 65°C for predetermined lengths of time. D-values, determined by linear regression, in 90% lean ground beef were 21.12, 4.85, 3.18, 0.93 and 0.39 min, respectively (z = 6.0°C.). D-values at 60°C in lean chicken, turkey, pork and lamb were significantly less and ranged from 1.77 to 1.89 min. This shows that if 90% lean ground beef is used to validate the safety of a process for *E. coli* O157:H7, that process will also be safe for lean chicken, turkey, pork and lamb at 60°C. The heat resistance of *E. coli* was not changed after refrigerated or frozen storage of inoculated 90% lean ground beef for 48h. The results of this study will be beneficial to the food industry in designing HACCP plans to effectively eliminate *E. coli* O157:H7 in the meat products used in this study.

(191) VALIDATION OF PEPPERONI PROCESSES FOR CONTROL OF *ESCHERICHIA COLI* O157:H7

N. Faith,* J. Hinkens, T. Lorang, P. Bailey, D. Buege, J. Luchansky and C. Kaspar, University of Wisconsin Food Research Institute, 1925 Willow Drive, Madison, WI 53706

The outbreak of *Escherichia coli* O157:H7 linked with dry-cured salami in late 1994 prompted regulatory action that required manufacturers to ensure a 5 $\log_{10}$ reduction in counts of the pathogen in fermented meats. Thus, pepperoni batter (75% pork: 25% beef with a fat content of ca. 32%) was inoculated with a pediococcal starter culture and a 5-strain mixture of *E. coli* O157:H7 ($\geq 2 \times 10^7$ CFU/g) and stuffed into 55-mm fibrous casings 47 cm in length. The viability of the pathogen was monitored before stuffing, after fermentation, after thermal processing, and/or after drying. Fermentation at 96°F and 85% relative humidity (RH) to pH 5.1 and then drying at 55%F and 65% RH to a moisture/protein ration of ≤1.6 only reduced counts of the pathogen about 0.74 $\log_{10}$ units. Heating chubs after fermentation to internal temperatures of 145°F instantaneous or 128°F for 60 minutes resulted in a ≥6-$\log_{10}$ unit decrease in numbers of *E. coli* O157:H7. By visual inspection,heating did not adversely effect the texture or composition of the product. These data revealed that heating to internal temperatures of 145°F instantaneous of 128°F for 60 minutes was sufficient to deliver a 5-6 $\log_{10}$ reduction of *E. coli* O157:H7 in pepperoni.

(192) SURVIVAL OF *ESCHERICHIA COLI* O157:H7, *LISTERIA MONOCYTOGENES* AND *SALMONELLA TYPHIMURIUM* IN GROUND BEEF JERKY

J. Harrison* and M. Harrison, University of Georgia, Cooperative Extension Service, Athens, GA 30602-4356

Home style drying procedures used for jerky made from whole meat strips may be insufficient to eliminate bacterial pathogens from jerky made from ground meat due to the possible distribution of pathogens throughout the product. The fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* during the preparation of ground beef jerky was determined. Ground beef was inoculated with these pathogens so the initial populations of each was approx. 10^6 CFU/g prior to drying. A drying method shown to reduce the population of these microorganisms by 5 logs on jerky made with beef loin strips in a home-style dehydrator maintained at 60°C (140°F) was used. Populations of each pathogen type were determined at 1, 2, 4, 6, and 8 h of drying. Internal temperature of the strips reached 60°C after 4 h of drying. At 6 h of drying, there was no reduction in the populations of any of the pathogen types. Between hours 6 and 8 of drying, there was a 3 log reduction of the population of *L. monocytogenes* and *S. typhimurium* and a population reduction of 3-4 logs of *E. coli* O157:H7. The product was judged to be slightly underdried at 6 h and slightly overdried at 8 h. Typical drying temperatures of 60°C (140°F) may be insufficient to reduce the population of these pathogens in ground beef jerky to desired levels.

(193) ACID STRESS AND DEATH IN PATHOGENIC *ESCHERICHIA COLI*

A. Castillo,* and E. Cabrera-Diaz, M. Rodriguez-Garcia, Texas A & M University, Department of Animal Science, 310 Kleberg Center, College Station, TX 77843-2471

Because of the emergence of *Escherichia coli* O157:H7 as a meat-borne pathogen, bactericidal rinses have been proposed to decontaminate carcasses. Lactic acid or acetic acid has been proven to be effective in reducing *E. coli* O157:H7 or *Salmonella*. However, in assessing efficiency of these treatments, acid-stressed cells resulting from a deficient treatment may not be detected by selective methodologies. In addition, other types of pathogenic *E. coli* may vary in sensitivity to acid. The purpose of this work was to measure the effect of contact with acetic acid on the injury or death of three types of pathogenic *E. coli*. Enterohemorrhagic *E. coli* O157:H7 (EHEC), enterotoxigenic *E. coli* (ETEC), and enteroinvasive *E. coli* (EIEC) were inoculated into flasks containing acetic acid/sodium acetate buffer (0.3 M) at pH 4.3, 4.5, and 4.7, and stored at 30°C. At different times, dilutions were plated onto violet red bile agar and standard methods agar. Mean values were compared by ANOVA to determine significance of effects of pH, type of pathogenic

E. coli and time of contact with the buffer. Sublethal injury was observed for EIEC at pH 4.3. There was no significant reduction of the pathogens at pH 4.5 or 4.7. At pH 4.3 a significant reduction of the three types of *E. coli* was attained at least after 20 min of contact with the buffer. These results are important in determining acid treatments to decontaminate meat carcasses.

(194) EFFECT OF *ESCHERICHIA COLI* O157:H7 GROWTH IN THE PRESENCE OR ABSENCE OF GLUCOSE ON ITS ACID TOLERANCE

R. Buchanan* and S. Edelson, USDA-ARS-ERRC, 600 East Mermaid Lane, Wyndmoor, PA 19038

The acquisition of acid tolerance by enteric bacteria has been generally studied by briefly exposing cells to moderately acidic conditions and then assessing resistance to subsequent exposure to harsher acidic conditions. The current study was undertaken to determine if the fermentation of glucose by *E. coli* O157:H7 could be used as a more convenient way of inducing acid tolerance. Six strains that pre-screening had indicated range from moderately to strongly acid tolerant were grown in tryptic soy broth with and without 1% glucose for 18-20 h. These media produced cultures with final pH values of 4.6-5.2 and 6.9-7.1, respectively. Acid tolerance was then assessed by inoculating brain heart infusion broth adjusted to pH 2.5 and 3.0, and incubating at 37°C. Samples were removed over 7 h and plated on BHI and McConkey agars to estimate viability and injury, respectively. There was little loss of viability at pH 3.0 or even 2.5 by glucose grown cells, whereas increased injury and inactivation was observed with some strains grown initially without glucose. In general, the enhancement of acid tolerance resulting from growth in the presence of a fermentable carbohydrate was greater with the less acid tolerant strains, suggesting that one of the characteristics of resistant strains may be that they are constitutively acid tolerant. The resistant strains also appeared better able to tolerate injury. The results support the hypothesis that one of the virulence factors responsible for the pathogenicity of this serotype is its ability to survive the acidic conditions that would be encountered in the stomach.

(195) PREVALENCE OF *ESCHERICHIA COLI* O157:H7 IN LEBANON

W. Birbari,* M. Jurdi, G. Araj and M. Mikati, Department of Environmental Health, American University of Beirut, Beirut, Lebanon

The association of *E. coli* O157:H7 infections with the consumption of contaminated meat products is well established. Three principal manifestations of illness have been attributed to *E. coli* O157:H7 infections: hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). There are several factors which might be antecedent for the prevalence of *E. coli* O157:H7 infections in Lebanon. Many popular traditional foods containing meat are consumed raw and the slaughter of livestock animals is improperly controlled. There is also scientific evidence showing contamination of drinking water with sewage. The objectives of this study are to determine whether this pathogenic bacterium is inherent to Lebanon by testing for its presence in meat products and potable water sources; to screen different kinds of *E. coli* O157:H7 from patients with hemorrhagic colitis stool samples for *E. coli* O157:H7; and to run a retrospective study, through medical records, for the prevalence of HUS and TTP in the population. Preliminary data have indicated the occurrence of HUS cases which were associated with food poisoning following consumption of raw meat dishes and the isolation of *E. coli* O157:H7 from patients with hemorrhagic colitis.

(196) PCR-BASED METHOD FOR THE DETECTION OF *E. COLI* O157:H7 FROM GROUND BEEF

W. M. Barbour,* L. D. Ecret, C. L. Sobities and S. L. Fritschel, DuPont Experiment Station, Bldg. 228, Room 310B, Wilmington, DE 19880-0228

A simple method based on the polymerase chain reaction (PCR) has been developed for the detection of *E. coli* O157:H7 from ground beef. The method consists of an overnight enrichment of sample, a simple lysate preparation, direct addition of 50 ul of the lysate to a PCR reagent tablet and thermal cycling, followed by agarose gel detection. Two commercial antibody-based test methods and the PCR method were used to analyze more than 100 spiked and unspiked ground beef samples. The spiked samples were inoculated with one of thirty O157:H7 strains or one of ten non-O157:H7 strains at less than one colony-forming unit per gram prior to overnight enrichment in modified EC + novobiocin broth or modified TSB + novobiocin broth. Results of the study indicate that the PCR-based assay represents a more sensitive and specific method than the comparison assays.

(197) A GLOBAL PERSPECTIVE OF FOODBORNE DISEASE

S. Notermans, National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 B.A. Bilthoven, The Netherlands

To be informed about foodborne disease is a basic requirement for the production of safe food. Information is also deemed necessary for a risk analysis to approach safe food production. This especially applies to identifying hazards and being informed about dose-response assessment. Establishing the patterns of disease provides a basis for legislative action, the development of intervention strategies, and the facilitation of policy decisions. It also helps set priorities in relation to research investment. In addition, new or emerging foodborne diseases need to be recognized rapidly.

There are several ways in which necessary information can be obtained: (1) analysis of reported

food-associated incidents of disease, including determination of risk factors and analysis of reported food-associated incidents of disease, (2) sentinel and population studies for specific pathogenic agents, and (3) calculation of human exposure and translating the exposure by dose-response relationship into disease.

Examining the international reports reveals that an extreme underestimation of foodborne disease exists. Nevertheless, the information available indicates that foodborne diseases are increasing worldwide, and a steady confrontation with (new) emerging problems exists. The reasons for this are the increasing globalization of food production, the application of new technologies, the attempt to meet consumer demands, and so on.

To protect the consumer, both industry and governments have a responsibility to actively collect information about foodborne disease and to make use of risk communication.

(198) MICROBIAL FOOD SAFETY ISSUES AND CONCERNS IN INTERNATIONAL TRADE: HARMONIZATION AND STANDARDS

M. van Schothorst, Nestec Ltd., Avenue Nestlé 55, Ch-1800 Vevey, Switzerland

Since the signing of the GATT Uruguay Round Agreement on Sanitary and Phytosanitary (SPS) Measures, food can move freely across borders. However, if there is a possibility that it could endanger the health of a country's population, consignments may be blocked. Decisions to block should be scientifically justified, using whenever available, standards, codes, and guidelines of the Codex Alimentarius as the yardstick.

Such scientific justification should be based on risk assessment, a methodology that is now (within the Codex system) being elaborated to deal with microbiological hazards. It should establish the rules for the determination of "unacceptable" and "acceptable" levels of potentially pathogenic microorganisms in foods.

Although the applications of HACCP, as described by Codex, will be the basis for assuring the safety of food, the rules for determining "equivalent" implementation in the importing and exporting countries must be developed. Validation, verification, and auditing are activities that need further definition and harmonization. However, certification of correct implementation of HACCP is already on the horizon, standardized or not.

Microbiological criteria will continue to be used in international trade. The Codex "principles" for their establishment are under revision. This document will be a good basis for dealing with unjustified "nontariff" barriers if they arise, even if it does not offer much guidance for setting "acceptable" levels of microorganisms.

The work of ISO and Association of Official Analytical Chemists (AOAC) has been essential, but other initiatives such as the AOAC Research Institute and MicroVal should be mentioned, especially their work on alternative, rapid method approval or validation. Standardized proficiency tests and reference materials may be needed to determine the accuracy of test results and to provide evidence of the reliability of the safety assurance system applied.

(199) MICROBIAL HAZARDS AND EMERGING ISSUES ASSOCIATED WITH PRODUCE

I. K. Wachsmuth, Food Safety and Inspection Service, U.S. Department of Agriculture, 300 12th Street, S.W., Washington, D.C. 20250

In the past two decades there has been a noticeable increase in the consumption of fresh fruits and vegetables in the United States and a noticeable increase in the globalization of the produce market. Concomitantly, public health officials have documented an increase in the number of produce-associated foodborne outbreaks in the United States. The U.S. Centers for Disease Control and Prevention reported that the number of cases more than doubled. Since 1990, there have been, for example, several large, multistate outbreaks of salmonellosis resulting from contaminated tomatoes and melons. Follow-up surveys of melons revealed that over 1% have detectable *Salmonella* spp. on rinds, and laboratory studies indicated that salmonellae could multiply on melons and tomatoes. During 1995 alone, outbreak data linked *Salmonella stanley* with alfalfa sprouts, *Salmonella hartford* with orange juice, *Shigella* spp. with lettuce and scallions, *Escherichia coli* O157:H7 with lettuce, and hepatitis A virus with tomatoes.

In response to this potential public health problem, the U.S. Food and Drug Administration asked the National Advisory Committee on Microbiological Criteria for Foods to address and better define the association of foodborne disease and microbial pathogens with fresh produce. A subcommittee formed in June 1995 is documenting relevant epidemiology, current industry practices, and laboratory data to identity potential hazards and related controls. This presentation will focus on the work and the outputs of that subcommittee.

(200) MICROBIOLOGICAL HAZARDS AND EMERGING ISSUES ASSOCIATED WITH SEAFOODS

E. S. Garrett, DOC/NOAA/National Marine Fisheries Service, National Seafood Inspection Laboratory, 3209 Frederic Street, Pascagoula, MS 39567

The international trade of seafoods is incredibly complex. A dichotomy for the United States exists since it is the world's second largest importer and exporter of seafood commodities. The U.S. seafood industry and the governmental agencies that regulate them are undergoing stressful changes. The seafood industry is experiencing increased business sophistication, particularly in the areas of raw material acquisition, processing and packaging methods, inventories, shipping, and international and national interfaces, all of which involve global product sourcing.

Legislatively, the U.S. is reexamining its approach to food regulatory controls as they concern microbiological and chemical contaminants. Today's sophisticated analytical technologies are forcing government regulatory officials to reevaluate current allowable contaminant levels in foods with the prospects of tightening those limits. This evolution will have profound effects on establishing risk levels.

In the case of seafoods, safety concerns are highly focused, well defined and limited to a very few species. These seafood safety problems are either environmental, process, distribution, or even consumer induced. With the exception of raw molluscan shellfish, cooked ready-to-eat seafoods, and hazards associated with certain process and packaging technologies, chemical and biological toxins are of greater concern than microbiological hazards.

This paper will identify where we are and where we are going regarding microbiological and chemical safety issues in seafoods. Microbiological standards of the United States, European Community, and the International Commission on Microbiological Specifications for Foods will be presented. Current chemical tolerances, guidelines, and alert levels will be discussed. Emerging issues including (1) the need for the increased use of 3-class sampling plans to determine pathogens in products, (2) identifying alternative methods for inactivation and destruction of microorganisms in seafood products, (3) development of rapid methods for identifying microorganisms and chemical contaminants, (4) increased understanding of the cause and effect of natural biotoxin outbreaks, and (5) establishment of global safety issues, etc., will be presented.

Examples of emerging seafood safety issues including water conservation, possible transgenic effects of low level contaminants, international food safety equivalency issues, biotechnology concerns, interactive risk communication techniques, and unique aquaculture safety areas will also be discussed.

(201) PARASITES IN SEAFOOD

A. M. Adams,* L. S. Leja and M. M. Wekell, Food and Drug Administration, P.O. Box 3012, Bothell, WA 98041-3012

Although parasites are naturally present in the environment, they may pose a health hazard or an aesthetic problem to the consumer. The potential for a parasitic worm to be present in a seafood product depends on many factors, including, but not limited to, the species of fish, species of parasite, geographical source of the seafood, type of seafood product, and how the product is handled by the consumer. The ability to eliminate parasites completely from seafood is not practical, but means exist to decrease the numbers and/or to kill the parasites and render them harmless to humans. This presentation will cover several species of parasitic worms that are commonly present in seafood marketed in the U.S., including the nematodes *Anisakis* and *Pseudoterranova decipiens*, the tapeworms of the genus *Diphyllobothrium*, and the trematode *Nanophyetus salmincola*.

(202) MARINE TOXINS: DETECTION AND RISK ASSESSMENT BY CELL BIOASSAY

R. L. Manger,* L. S. Leja, S. Y. Lee, J. M. Hungerford and M. M. Wekell, U.S. Food and Drug Administration, Bothell, WA 98041-3012

Marine toxins occasionally contaminate numerous finfish and shellfish species, resulting in a significant risk for human intoxication. Screening for these toxins is a formidable task with the mouse bioassay serving as the most utilized and reliable bioactivity index. Although there are distinct advantages offered by mouse bioassays, animal testing is not always feasible due to expense, lack of precision, limited sensitivity, and governmental restrictions. In contrast, cell-based assays are effective screening tools for a wide range of drugs, chemicals, and toxic compounds, thereby offering significant potential for developing effective marine toxins assays. Of the marine toxins that present a risk to the consumer, a significant number exert their toxic effects through perturbation of voltage-gated sodium channel activity on excitable cells such as neurons. This common modality has allowed the development of highly sensitive and specific *in vitro* bioassays that can be applied toward the detection of marine toxins associated with paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), and ciguatera. In a manner similar to mouse bioassays, these cell-based assays accurately reflect the relative potency of marine toxins *in vivo*. Additional advantages conferred by cell bioassays include; simplified sample preparation, tolerance of matrix constituents, enhanced assay kinetics and sensitivity, accommodation of numerous samples, and significant economic savings in comparison to animal methods. The numerous attributes of cell bioassays, for applications concerning marine toxins, suggest that these *in vitro* methods may prove to be reliable alternative or replacement to conventional animal testing.

(203) CONTROL OF BACTERIAL PATHOGENS IN SEAFOODS

M. Eklund, N.W. Fisheries Center, 18727 35 N.E., Seattle, WA 98155

Clostridium botulinum and *Listeria monocytogenes* are psychrotrophic pathogenic bacteria currently of concern in seafoods. *C. botulinum* is comprised of a group of sporeforming bacteria that differ markedly in their heat resistance, salt tolerance, and minimum temperature of growth. *L. monocytogenes* is a nonsporeforming bacterium that grows at temperatures as low as 2°C, grows in foods with high concentrations of salt and is relatively heat sensitive. An understanding of these and other characteristics of the bacteria, the characteristics of the product and the pro-

cessing parameters used in preparing different seafoods must be evaluated together in order to control these bacteria. Examples of how these characteristics have been used to increase the safety of different ready-to-eat seafood products will be discussed.

(204) RISK ASSESSMENT OF SEAFOOD IN CANADA — INITIAL STAGES

E. Todd, Health Protection Branch, Health Canada, Banting Research Centre, Ottawa, Ontario K1A OL2 Canada

The risk assessment was an initial attempt to determine the risks of all microbial pathogens, including viruses and parasites, in domestically-harvested and imported fish and shellfish, primarily from raw or lightly cooked products. At this stage two components have been identified: 1) the identification of potential hazards and their minimum infectious or toxic doses (MIDs); and 2) the estimation of risk from consuming fish and shellfish through human consumption information. The total per capita consumption increased slightly from 6.6 kg in 1980 to 7.1 kg in 1990, but there is no information on the extent of raw fish or shellfish being eaten. From the data on these pathogens, it would seem that there is more chance of being infected with viruses in shellfish than bacterial or parasitic pathogens in fish. This is in agreement with known surveillance data. Data on growth of pathogens in fish and shellfish and the probability of exposure to MIDs is not known and a number of assumptions are made, which will have to be confirmed or modified as more complete information is obtained. This study is not complete enough to be a formal risk assessment of fish and shellfish in Canada, but can be used to help determine assessments for specific situations, with appropriate risk management and risk communication strategies.

(205) EPIDEMIOLOGY AND DETECTION OF HUMAN ENTERIC VIRUSES IN SEAFOOD

L. A. Jaykus, Food Science Department, Box 7624, North Carolina State University, Raleigh, NC 27695-7624

Enteric viruses are significant human pathogens, recently ranked fifth and sixth amongst identified causes of foodborne disease in the U.S. While these agents are responsible for diseases such as gastroenteritis and hepatitis, the true scope and significance of shellfish-borne viral infection is drastically underestimated due to inadequacies in reportng and detection methods. Since the diseases caused by these agents are generally mild and self-limiting, they frequently go unrecognized and/or unreported. Furthermore, traditional methods for the detection of viruses in seafoods have relied on the ability of the viruses to infect mammalian cells in culture, a method which is time-consuming, expensive, and largely unavailable for the important foodborne viral agents. The advent of DNA amplification methods offers a sensitive and specific alternative for the detection of these previously non-detectable viral agents. The purpose of this presentation is to provide a discussion of recent shellfish-related outbreaks of enteric viral illness and describe emerging methodologies that will ultimately provide alternative rapid detection options and the basis for the development of molecular approaches to the investigation of shellfish-borne viral disease outbreaks.

(206) AQUACULTURE

M. M. Wekell, Seafood Products Research Center, Food & Drug Administration, P.O. Box 3012, Bothell, WA 98041-3012

Aquaculture accounted for over 15% of the world seafood supply in 1993 with some farmed species representing a significant proportion of the total. Farmed shrimp comprised 30% of the total shrimp marketed worldwide; farmed salmon 25%. An increase in aquaculture-derived products is anticipated since the world's oceans are being harvested at or above sustainable levels. The benefits of aquaculture include control of product quality and availability, and elimination or control of parasites. Problems affecting aquaculture-derived species are exposure to harmful algal blooms, uptake of algal toxins, long term effects of maintaining aquaculture facilities in aquatic environments, and long term environmental effects of antibiotic use. All of these need to be understood for effective management to ensure cost effective safe products.

(207) QUALITY CONTROL ASPECTS OF WINEMAKING

A. Karumanchiri, Liquor Control of Ontario, 55 Lakeshore Blvd. E., Toronto, Ontario M5E 1A4 Canada

For quality assurance purposes the chemicals present in wine can be classified into four groups. They are:
1. **Naturally occurring components** such as alcohol, sugars, volatile acids and non-volatile acids. We have started measuring also phenolic antioxidants such as catechin, epicatechin, trans and cis resveratrol and polydatin, quercetin, hydroxy cinnamic acid and rutin. We are evaluating their health potential (clinical study) and their significance to taste quality; 2. **Food additives** — sulfur dioxide, sorbic acid and potassium ferrocyanide; 3. **Inorganic contaminants** — As, Pb, Cn, Cd and Co, and 4. **Organic contaminants** — ethyl carbamate, pesticides, fungicides and their metabolites. Through chemical analyses, the presence of all these chemicals are closely monitored and, in collaboration with the manufacturer, maintained within acceptable norms. When a contaminant reaches 70% of the acceptable norm, the manufacturer is alerted so that corrective steps can be taken. A brief description of methodologies used will also be presented.

(208) INFLUENCE OF YEAST STRAINS ON WINE QUALITY

G. C. Cone, American Yeast Co., 2233 Medallion Drive, No. 1, Union City, CA 94587

Simply stated "No yeast, no wine." Yeast are the single group of microorganisms that can turn grape juice to wine. The whole microflora of the vineyard, workers, harvesters, crushers, winery walls, pumps, valves, fermenters, barrels and other cellar equipment find their way into the must. The good and the bad are part of the microorganism soup at zero hour of the fermentation. Because of the 2.9-4.0 pH of the grape juice, referred to as "must," only a select few groups of yeast and bacteria survive. Early in the fermentation, as the oxygen is depleted and the alcohol increases, more of the organisms fall by the wayside. At the end of the fermentation, when the alcohol is between 9 and 14% alcohol, only the hardest of yeast strains and bacteria have survived. Most of the bacteria that have survived are potentially spoilage bacteria that produce a high VA, namely acetic acid. A few of the Leuconostoc oenos strains can produce subtle flavors that are greatly appreciated.

Winemakers of the past were skilled at building up mixed starter culture of yeast that minimized many of the weak, undesirable yeast strains from the indigenous population and maximizing the more desirable strains. This was still like playing enology roulette. Too much depends on luck.

The commercial production of pure strains of wine yeast has taken luck and guesswork out of the fermentation stage. Wineries and wine institutes around the world have made great strides in selecting specific strains of the *Saccharomyces* group that they feel contribute to the character of the wines of their region. Over 200 strains identified by DNA fingerprint have been selected for commercial production. Lallemand Inc. produces and markets over 180 strains. The selection process and distinctive enological characteristics of several of the yeasts will be discussed. Good fermentation practices to optimize yeast performance will be outlined.

(209) YEAST ENUMERATION AND IDENTIFICATION

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

No single medium is satisfactory for enumerating all yeasts in all foods and beverages. Dichloran rose Bengal chloramphenicol agar and acidified potato dextrose agar are suitable for enumerating wine yeasts and the majority of spoilage yeasts in grapes, grape concentrate, must, and wine. Ethanol sulfite yeast extract agar can be used to selectively enumerate ethanol-tolerant yeasts, and lysine agar can be used to select for non-*Saccharomyces* species, since lysine cannot be assimilated by *Saccharomyces*. Lin's wild yeast differential agar inhibits, or markedly restricts, the growth of culture yeasts while permitting growth of many wild yeasts. Traditional identification procedures rely heavily upon morphology of vegetative cells and type of reproduction. Standard assimilation and fermentation tests have been incorporated into identification kits and automated systems; however, the value of these identification systems is limited because databases are largely restricted to yeasts of clinical significance. Nontraditional techniques for identifying yeasts of importance to the wine industry include DNA fingerprinting, chromosome karyotyping, protein electrophoresis and cellular fatty acid analysis. The potential value of these techniques in contributing to maintenance and improvement of quality assurance programs in the wine industry is substantial.

(210) INTERACTION BETWEEN YEASTS AND MALOLACTIC BACTERIA

C. G. Edwards, Washington State University, Department of Food Science and Human Nutrition, Pullman, WA 99164-6376

Wine spoilage problems related to the growth of certain lactic acid bacteria during vinification are discussed. Three strains of bacteria, YH-15, YH-24 and YH-37, were isolated from commercial California and Washington wines undergoing slow/stuck alcoholic fermentations. In agreement with recent enological observations, inoculation of these strains greatly slowed the fermentations of Chardonnay juices catalyzed by either yeast strain Prise de Mousse or Epernay. The extent of yeast inhibition was strongest in the presence of YH-15. Believed to belong to the genus *Lactobacillus*, these spoilage organisms have not been identified to species level. Production of acetic acid by the strains appears to be only slightly inhibitory to yeast, indicating the presence of other mechanisms of yeast inhibition.

(211) PHYSIOLOGY OF THE MALOLACTIC BACTERIA

E. B. Olsen, Chateau Ste. Michelle, P.O. Box 1976, Woodinville, WA 98072

Malolactic fermentation is widely used in the wine industry for reduction of acidity in wine, and for modification of wine flavor and aroma. The malolactic fermentation is a decarboxylation reaction of malic acid to lactic acid that yields ATP via a chemiosmotic mechanism. A glucose limited chemostat operated under constant pH conditions shows an increase in cell yield upon addition of malic acid to the medium. This indicates that malolactic bacteria derive energy from the malolactic fermentation. An inducible malate transport system in *Lactobacillus plantarum* displays biphasic transport kinetics on an Eadie-Hofstee plot, indicating that two mechanisms of transport are responsible for malate uptake. The high-affinity system requires a pH gradient ($Z\Delta pH$) for malate uptake. The low affinity system operates by a facilitated diffusion mechanism. Only the low-affinity system has a high enough V_{max} to account for malolactic fermentation rates in batch culture.

Since the low-affinity uptake of malate is inhibited by an electrical potential $\Delta\Psi$ (inside negative), and since monoionic malate predominates at the optimum uptake pH of 4.5, the transport of $HMalate^-$ is electrogenic. When 20 mM L-malate is added to de-energized cells, the $\Delta\Psi$ increases to 110 mV. Gradually, $\Delta\Psi$ decreases with a corresponding increase in the ΔZpH to keep a constant Δp of 160 mV until malate is depleted. It appears that the low-affinity transport of malate is electrogenic and coupled with an electroneutral efflux of lactic acid. This mechanism creates a membrane potential that is capable of generating ATP and increasing cell yield. Malate dependent ATP synthesis has been observed in several different species of lactic acid bacteria.

(212) WINE SPOILAGE MICROORGANISMS AND THEIR CONTROL

D. F. Splittstoesser, Cornell University, Geneva, NY 14456

Wine is a semi-preserved food in that the growth of most microorganisms is inhibited by its low pH and high ethanol content. Prior to bottling, the principal spoilage organisms are acetic acid bacteria which require oxygen for their growth and metabolism. Spoilage by these organisms results in an increase in volatile acid due to the oxidation of ethanol. Bottled wines that contain 2 g/L or more fermentable sugar are subject to spoilage by yeasts and lactic acid bacteria. Growth of these organisms results in haze, gas and off flavors. Physical methods for wine preservation are filtration or pasteurization. Common chemical preservatives are sulfur dioxide and sorbic acid. Wine composition such as the concentration of ethanol influences the effectiveness of the different preservatives.

(213) THE DEVELOPMENT AND USE OF BACTERIOCIN-CONTAINING DAIRY INGREDIENTS TO CONTROL UNWANTED MICROORGANISMS IN FORMULATED FOODS

E. A. Zottola, Department of Food Science and Nutrition, MN-SD Dairy Foods Research Center, University of Minnesota, St. Paul, MN 55108

Over the past decade, there has been a significant amount of research directed at the isolation and identification of bacteriocins that are effective in the inhibition of pathogenic bacteria of concern in the food industry. The objectives of our research activities have been to develop methods that would utilize these bacteriocin-producing bacteria in producing fermented dairy ingredients with dual functions, that is, functionality and inhibition. These ingredients could then be used in formulated foods. To develop these products, a transconjugant lactic acid bacterium, developed in the laboratories of Dr. L. L. McKay, that produces a nisin-like bacteriocin was used. Several fermented dairy products were produced using this organism, singly or with another bacteriocin-producing lactic acid bacterium. Included in these products were Cheddar cheese, fluid milk products, fluid and dried whey and whey protein concentrate (WPC). The Cheddar cheese and the WPC were then used as ingredients in other foods and their ability to inhibit pathogenic organisms evaluated. Pasteurized process cheese spread and cold pack cheese spread was manufactured with the bacteriocin containing Cheddar cheese. The addition of the bacteriocin-containing cheese to these products inhibited the growth of the added pathogenic bacteria and effectively influenced shelf-life of the products. The WPC was used in the manufacture of ice cream inoculated with *Listeria monocytogenes* and the presence of the bacteriocin reduced the numbers of this organism from 10^4/g to less than 10/g during frozen storage. Addition of the WPC to fresh, ready-to-eat foods also reduced significantly the numbers of this organism in these products. These results indicate that manufacture and utilization of dual-function dairy ingredients is an effective method for inhibiting the growth of the pathogenic bacteria used in this study.

(214) SURVIVAL OF *E. COLI* O157:H7 IN FERMENTED DAIRY PRODUCTS

K. J. Boor, Cornell University, Department of Food Science, Ithaca, NY 14853

Yogurt has been implicated as the probable vector in a recent *Escherichia coli* O157:H7 (EC O157) outbreak in the United Kingdom. This event highlights the need to understand the behavior of this pathogen in dairy fermentation systems. Three sets of experiments were designed to assess: (i) the ability of EC O157 to survive competition with lactic acid starter cultures; (ii) EC O157 survival during yogurt production; and (iii) EC O157 survival as a post-processing contaminant in commercial dairy products. In (i), 100 ml of UHT milk were inoculated with 1% (v/v) of either thermophile, *Streptococcus thermophilus* or *Lactobacillus delbruekii* ssp. *bulgaricus*; or mesophile, *Lactococcus lactis* ssp. *lactis* or *Lactococcus lactis* ssp. *cremoris*, and low (10 CFU/ml), medium, (10^3 CFU/ml), or high (10^5 CFU/ml) levels of EC O157. The inoculated milk samples were fermented for 48 hours at 37° or 42°C (thermophiles) or at 21° or 30°C (mesophiles). EC O157 was not detectable at 48 hours in the presence of *L. delbruekii* ssp. *bulgaricus* or *L. lactis* ssp. *lactis*. With *S. thermophilus* and *L. lactis* ssp. *cremoris*, EC O157 was still detectable after 40 days, at which point the starter cultures were no longer recoverable. In (ii), yogurt was prepared from whole milk by the addition of 1.25% (v/v) of both *S. thermophilus* and *L. delbruekii* ssp. *bulgaricus* and low, medium, or high levels of EC O157. The mixes were incubated at 47°C until firm curd development, and then were held at 4°C. No EC O157 was detectable at any

point after curd formation. (iii) Commercial samples of yogurt, buttermilk, sour cream, and cottage cheese were inoculated with low, medium, and high levels of EC O157 and stored at 4°C for 60 days. At all inoculum levels, EC O157 was able to survive throughout storage. These results indicate that *L. delbruekii* ssp. *bulgaricus* or *L. lactis* ssp. *lactis* are more effective than *S. thermophilus* or *L. lactis* ssp. *cremoris* at inhibiting EC O157 and that the presence of EC O157 in commercial yogurt likely reflects post-processing contamination.

(215) MASTITIS PATHOGENS THAT CAUSE PUBLIC HEALTH CONCERN

J. S. Cullor, Dairy Food Safety Laboratory, Veterinary Medicine Teaching and Research Center, 18830 Road 112, Tulare, CA 93274

On-farm food safety has taken on new meaning in the '90's and has become the modern contract between the consumer, producer, processor, and veterinarian. Managing the dairy for animal health and well-being, public health, and environmental health/medical ecology requires that everyone re-evaluate current practices on the production unit. Bacterial isolates from mammary gland secretions that may be considered hazardous to human health will be discussed. Their potential reservoirs and possible methods of pathogen reduction will be presented.

(216) PATHOGEN SURVIVAL AND BOTULINUM TOXIN PRODUCTION IN NON-FAT AND REDUCED-FAT CHEDDAR CHEESE AND PROCESS CHEESE

E. A. Johnson, Food Research Institute, University of Wisconsin-Madison, Madison, WI, 53706

Reduced-fat cheeses, dessert foods, and other products hold considerable economic potential for the dairy industry. Given this potential, it is important to determine if removal of fat or use of fat-replacers will impact on product stability and safety. In this study, survival of *Salmonella* sp., and of *Listeria monocytogenes*, and toxin formation by *Clostridium botulinum*, was determined in reduced-fat Cheddar and its full-fat counterpart. Botulinal toxin formation was also determined in non-fat and reduced-fat process cheese. Overall, reduced-fat Cheddar was less permissive to survival of *Salmonella* and *L. monocytogenes*. Botulinal toxin formation was also delayed in the reduced-fat cheese and non-fat and reduced-fat process cheese. Of several antimicrobials tested in reduced-fat Cheddar, monolaurin, and nisin showed inhibitory activity against *L. monocytogenes* and *C. botulinum* in reduced-fat Cheddar, whereas only monolaurin was active against *Salmonella*. These results suggest that removal of fat imparts a more hostile environment for the pathogens tested than full-fat product, and that safety can be enhanced by certain antimicrobials.

(217) HACCP MODEL PROGRAM FOR THE DAIRY INDUSTRY

R. Bishop, Wisconsin Center for Dairy Research, 1605 Linden Drive, Madison, WI 53706

The International Dairy Foods Association and the Wisconsin Center for Dairy Research have developed 15 model HACCP programs specifically for the dairy industry. The programs involve Pre-requisites/GMPs, Critical Control Points, flow diagrams, and a computer program for the following products: fluid milk, vanilla ice cream, Cheddar cheese, Mozzarella cheese, cottage cheese, pasteurized process cheese, plain yogurt, frozen yogurt, butter, condensed milk, evaporated milk, dry milk, whey products (condensed, dry, WPC, WPI), lactose, and dairy product solids.

(218) THE APPLICATION OF RISK ANALYSIS METHODOLOGIES TO MICROBIAL FOOD SAFETY

D. Vose, DVRAS, 16 Mill Street, Wincanton, Somerset BA9 9AP United Kingdom

There are essentially three generic methodologies for the assessment of risks: qualitative, semi-quantitative, and quantitative. When applied to microbial food safety, qualitative risk analysis attempts to describe the possible chains of circumstances that result in human consumption of infected food and the subsequent effect on the consumer. Quantitative risk analysis (QRA), usually using Monte Carlo simulation modeling, seeks to estimate both the probability of the human health exposure and the possible resultant health impacts. It can help producers, distributors, and government regulators design efficient procedures that will reduce risks to acceptable limits. However, QRA demands a specific set of skills from the analyst that is not yet commonly available. It is also time consuming, difficult to verify by a non-expert and poses a variety of non-intuitive traps that can easily invalidate any results.

Furthermore, QRA can only be performed where it is possible to design a model that reasonably encompasses all of the factors relating to a specific risk issue and where each variable within the model can sensibly be estimated. Unfortunately, these conditions are often not met. Semi-quantitative risk analysis may help to fill the gap between qualitative and quantitative risk analyses. It could provide an intuitively appealing means for developing a consistent and efficient risk management policy and enable an analysis to include possible political, social, and environmental impacts and other factors that are not comfortably converted to numerical measurements.

(219) QUANTITATIVE MICROBIAL RISK ASSESSMENT ACTIVITIES BY THE NATIONAL ADVISORY COMMITTEE FOR MICROBIOLOGICAL CRITERIA FOR FOODS

R. L. Buchanan, Agriculture Research Service, Eastern Regional Research Center, U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, PA 19038

The U.S. Department of Agriculture/Health and Human Services National Advisory Committee for Microbiological Criteria for Foods was asked by the sponsoring agencies to consider how quantitative risk assessment techniques could be applied to microbial food safety concerns. The committee quickly concluded that, despite successful application of risk assessment methods to chemical food safety concerns, there have been few attempts at assessing quantitatively the risks associated with the transmission of biological agents in foods. Further, the inherent differences in the characteristics of biological and chemical agents limit the direct application of assumptions and techniques previously developed for chemical risks. In particular, the likelihood that the numbers of microorganisms can change drastically in a short period and the wide range of susceptibilities to specific biological agents were both identified as key factors that would require classical risk assessment approaches to be modified before they could be used to address microbiological concerns. As an initial step the committee has drafted a "general principles" document that discusses the unique attributes associated with biological agents, proposed modified definitions of terms used in risk assessment to better incorporate microbiological concerns, and described the types and potential sources of information needed to successfully conduct an effective quantitative microbial risk assessment.

(220) AN OVERVIEW OF MICROBIAL RISK ASSESSMENT IN THE AGRI-FOOD INDUSTRY: APPROACHES TO IDENTIFYING INTERVENTION STRATEGIES FOR RISK REDUCTION

A. M. Lammerding, Food Safety Risk Assessment Unit, Health of Animals Laboratory, Agriculture and Agri-Food Canada, 110 Stone Road West, Guelph, Ontario N1G 3W4 Canada

Risk assessment is a systematic methodology used to organize and analyze scientific information to estimate the probability and severity of an adverse event. Applied to microbial food safety, the risk assessment process can also help identify those stages in the manufacture, production, processing, distribution, handling, and consumption of foods that contribute most significantly to the risk of foodborne pathogens, and can help focus resources and efforts in areas that most effectively reduce the risk to human health. Risk assessment is a process that can be used to identify important data gaps and prioritize areas of research. Ideally, quantitative data defining exposure, and the human health consequences upon exposure to a foodborne pathogen, should be used to derive accurate estimations of risk. In the absence of sufficient data, qualitative information, expert opinion, and consensus can be used for initial risk assessments within a framework that allows the incorporation of quantitative data as they become available.

(221) CONCEPTUAL FRAMEWORK FOR MICROBIAL WATER SAFETY RISK ASSESSMENT: REPORT OF THE ILSI RISK SCIENCE INSTITUTE WORKING GROUP ON MICROBIAL RISK ASSESSMENT

J. Seed, ILSI Risk Science Institute, 1126 Sixteenth Street, N.W., Washington, D.C. 20036-4810

The process of quantitative risk assessment has been a valuable tool for assessing the human health effects associated with exposure to chemicals. This information has been invaluable to decision makers responsible for developing regulatory standards, assessing treatment requirements, and conducting risk-benefit analyses. However, the development of an approach for assessing the human health effects associated with exposure to pathogens has received far less attention. The assessments that have been conducted for viruses and for *Giardia* in drinking water have utilized the conceptual framework that was developed for chemical risk assessment. This process consists of four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization. As these risk assessments have been developed, many unique complexities have been noted. These include, but are not limited to, an assessment of pathogen-host interactions, consideration of secondary spread, consideration of the severity of the disease, quantification of the pathogen, and an assessment of the conditions that allow the pathogen to propagate. Given the many complexities, the question has arisen as to whether the conceptual framework outlined for chemical risk assessment is the most appropriate for the assessment of risks of human disease following exposure to pathogens.

To address this concern, the ILSI Risk Science Institute in cooperation with the U.S. EPA Office of Water convened a working group to develop a conceptual framework for assessing risks of human disease associated with waterborne pathogenic microorganisms. This working group was not asked to critically evaluate or develop specific analytical methods, but rather to take the opportunity to step back and broadly consider the entire process of risk assessment as applied to waterborne pathogens. Discussions among the participants led to the development of a conceptual framework, which will be described.

(222) DEVELOPMENT OF RISK ASSESSMENT GUIDELINES FOR FOODS OF ANIMAL ORIGIN IN INTERNATIONAL TRADE

S. Hathaway, Ministry of Agriculture and Fisheries Regulatory Authority (Meat and Seafood), P.O. Box 646, Gisborne, New Zealand

International debates over the role of science in designing and applying food control programs have increasingly focused the attention of the Codex Alimentarius Commission (CAC) on risk analysis in the elaboration of standards and guidelines for the international trade in food. These standards and guidelines are especially important in terms of the future multilateral trade work of the World Trade Organization.

There is a marked increase in the desire for quantitative data on the risks associated with the consumption of different classes of foods of animal origin, and traditional GMP-based food hygiene requirements are coming under increasing challenge. The principles of food safety risk assessment will be increasingly applied in establishing critical limits for HACCP plans and in elaborating sanitary measures for trade. However, this may prove problematic where specific industry sectors and regulators do not have control/jurisdiction over the entire food chain from the production of raw materials through to consumption. Developing international guidelines for risk management by the CAC arguably presents the greatest challenge in establishing and maintaining sanitary measures for food in international trade, and in judging their equivalence.

(223) PRACTICAL APPROACHES TO RISK ASSESSMENT

M. van Schothorst, Nestec Ltd., Avenue Nestlé 55, Ch-1800 Vevey, Switzerland

In this paper, risk means the chance that harm will be done. Risk assessment is a necessary step in decision making. The decision can be easy, for example, whether or not to eat a certain food or meal, or it can be difficult, for example, whether or not to recall a product with a minor defect. Risk assessment is also applied in deciding how many samples to analyze for the presence of pathogens in an imported lot of food. The pragmatic ICMSF (International Commission on Microbiological Specifications for Foods) approach, based on estimating the severity of a hazard and the likelihood of its occurrence, is still regarded as giving practical guidance. As a consequence of the World Trade Organization's Agreement on Sanitary and Phytosanitary Measures to ensure fairness in trade, there is renewed interest in protocols for setting microbiological criteria. In this context, the Codex Alimentarius standards, codes, guidelines, etc., will be used to deal with food safety matters.

As a consequence, the HACCP concept has been changed from a voluntary management tool in food processing to a mandatory system to assure food safety. The determination of hazards and critical control points and critical limits is now decisive for the acceptability of food in (international) trade. However, risk assessment in HACCP is not well developed. Questions such as, "Is unacceptable contamination at this step in the process possible, probable, or likely?" are difficult to answer. Quantification of risk (chance) is even more problematic. Normally, a safe product is delivered, but at what point does a deviation from normality become unacceptable? Answers to these questions determine the "residual" risk, or exposure, governments have to deal with in risk management. Even if the food industry, caterers, and all other food handlers could reduce microbiological food safety risks to acceptable ones, we still would have to deal with the inherent risks of unprocessed foods as well as the voluntary risks people take in eating certain raw products.

(224) *SALMONELLA ENTERITIDIS* SURVEILLANCE IN NEW YORK STATE

J. J. Guzewich* and M. J. Cambridge, New York State Department of Health, 2 University Place, Room 404, Albany, NY 12203-3399

This paper will describe *Salmonella* serotype *enteritidis* surveillance in New York State for the period 1980 - 1995. There were 1,807 reported foodborne disease outbreaks in New York State between 1980 and 1994 involving 39,214 cases of illness, 1,329 hospitalizations and 44 deaths. *Salmonella* was the agent in 291 of these outbreaks with 9,082 cases of illness, 715 hospitalizations and 32 deaths. *Salmonella enteritidis* was the agent in 132 outbreaks involving 4,617 cases, 361 hospitalizations and 18 deaths in the period 1980 - 1995. Eggs were associated with 86 of these outbreaks. Reported *Salmonella enteritidis* sporadic cases and laboratory isolations from patients will be described. Contributing factors, methods of preparation, significant ingredients, vehicles, places of contamination and egg sources in *Salmonella enteritidis* outbreaks will also be discussed.

(225) *E. COLI* O157:H7: SURVEILLANCE, OUTBREAKS, AND CONTROL STRATEGY IN THE NORTHWESTERN UNITED STATES

C. A. Bartleson, Washington State Department of Health, P.O. Box 47826, Olympia, WA 98504-7826

The Northwestern states (WA, OR, ID, MT) have had the longest experience with *E. coli* O157:H7 illnesses of any part of the U.S. The first recorded outbreak occurred in Medford, Oregon, in 1982. Since that time many more outbreaks have been detected and investigated in the Northwest. Washington was the first state in the nation to require reporting of *E. coli* O157:H7 in 1987, with Oregon following in 1990.

Outbreaks have not only been linked to ground beef and raw milk but also to "new food sources" including lettuce, fermented salami and venison jerky.

Surveillance activities in these four states show that the incidence rate for reported cases of *E. coli* O157:H7 has declined since 1993. Possible reasons for this decline include adequate cooking of ground beef by food workers and consumers, better beef slaughter methods and more food safety knowledge available to help prevent illness.

(226) FOODBORNE DISEASE — A NATIONAL PERSPECTIVE

E. C. D. Todd, Bureau of Microbial Hazards, Health Protection Branch, Health Canada, Banting Research Centre, Ottawa, Ontario K1A OL2 Canada

Collection of foodborne disease date for collation, interpretation and dissemination is done in several European countries, Canada, the United States and Japan. In addition, other countries have produced periodic reports but not on an annual basis. Such surveillance is pertinent to the design of risk assessments, HACCP, and research projects, and affects of international trade. However, the definitions and methodologies used are not the same. There needs to be a coordinated approach to the type of data to be collected and how it should be presented. The information provided from the local, regional or provincial/state level needs to be verified through accepted criteria and presented in a similar format, e.g., a line listing with at least the date of occurrence, locality, implicated etiological agent and food(s), clinical information, number ill, and place of mishandling. Factors contributing to outbreaks, significant food ingredients in specific meal items, the type of food processing or preparation, and economic analysis should also be included to assist in long-term strategies for control. Data should be entered into a computerized format that allows for a uniform approach, but includes modules for specific data, e.g., lot numbers and company information for processed foods, or costs for cost-benefit analysis. This computerization should allow for best possible interpretation of data with the use of tables and graphs. Dissemination of the final reports also should be on an electronic format for those involved with public health decision, to those who provided the data, and international coordinating centers.

(227) FOODBORNE DISEASES SURVEILLANCE IN LATIN AMERICA AND THE CARRIBEAN: AN INTERNATIONAL PERSPECTIVE

P. Arambulo III, Pan American Health Organization, 525 23rd Street, NW, Washington, DC 20037

By mandate of the countries of the Americas, the Pan American Health Organization coordinates a regional plan of Action for Technical Cooperation in Food Protection. The plan encompasses four components 1) Organization of Integrated programs; 2) Development and strengthening of inspection and analytical services; 3) Epidemiological surveillance and 4) Control of foodborne diseases, and 5) Promotion of food protection through community participation. The plan places special emphasis on the establishment of a national system for epidemiological surveillance of foodborne diseases. The systems are coordinated through the PAHQ/Pan American Institute for Food Protection and Zoonoses (INPPAZ) located in Buenos Aires, Argentina.

To assist in the organization of national systems, a document was published on the Guidelines for the Establishment of Systems for the Epidemiolgical Surveillance of Foodborne Diseases and Investigation of Outbreaks of Foodborne Diseases. Training courses on foodborne surveillance have been organized in several countries and national authorities have been appointed focal points to coordinate the system at the national level and send information to INPPAZ for the publication of quarterly reports. Countries started to report in 1994 and up to now there are 10 countries actively participating in the system. In addition, to strengthen control and inspection services, training courses have been initiated on Hazard Analysis and Critical Control Points.

The implementation of national surveillance systems is considered fundamental to measure the effectiveness of the national food protection program and to promote actions to prevent and control foodborne diseases.

(228) ON-SITE INVESTIGATION OF WATERBORNE DISEASE OUTBREAKS

K. R. Fox, U.S. Environmental Protection Agency, 26 West MLK Drive, Cincinatti, OH 45268

During the past decade, there have been several waterborne disease outbreaks in the United States including one (Milwaukee) where more than 400,000 people became ill. Most disease outbreak investigations begin with the epidemiology studies. Once drinking water becomes suspect, engineering investigations are often necessary. This talk will focus on the engineering aspects of waterborne disease investigation. The presentation will summarize how several investigations proceeded and techniques used to pinpoint where and how the disease agent got into the drinking water.

(229) PROCEDURES TO INVESTIGATE WATERBORNE ILLNESS — THE IAMFES APPROACH

F. L. Bryan, Food Safety Consultation and Training, Lithonia, GA 30058

In response to some large waterborne outbreaks in the United States and elsewhere and floods that subjected water supplies to contamination, the manual "Procedures to Investigate Waterborne Illness" was revised and published in 1996. This second edition contains guidance for (a) developing waterborne disease surveillance and emergency operations program, (b) acting on notification of illness, (c) preparing for investigations, (d) taking steps to verify diagnosis, (e) developing case definitions, (f) making epidemiologic

associations, (g) expanding the investigation, (h) seeking sources and modes of contamination and ways by which contaminants survived treatment, (i) analyzing data calculating economic costs of outbreaks, (j) submitting reports, and (k) using outbreak data for prevention. It contains 22 forms and 11 tables to aid investigators. Instruction and interpretation for statistical calculation and interpretation of laboratory results of water samples are given. Procedures for field investigation are expanded over that in the previous edition. The disease listings and data bank were expanded to cover waterborne diseases acquired by ingestion and by contact and by those transmitted via aerosols generated from water. Highlights of these are described and illustrated.

(231) STRATEGIES FOR CONTROLLING *SALMONELLA ENTERITIDIS* IN EGG-LAYING CHICKENS

R. K. Gast, USDA-ARS, South Atlantic Area-Southeast Poutry Research Laboratory, 934 College Station Road, Athens, GA 30605

Based on the number of isolates submitted to CDC, *Salmonella enteritidis* (SE) apparently caused illness in humans in the U.S. more often than any other *Salmonella* serotype in 1994. In recent years, human SE infections in the U.S. have been associated more frequently with the consumption of contaminated eggs or egg-containing foods than with any other single source. In several epidemiological investigations, contaminated eggs implicated as responsible for human SE outbreaks have been traced back to flocks of infected laying hens. Reducing the incidence of SE infections in egg-laying flocks has accordingly become a very significant public health and economic priority. Unlike most other *Salmonella* serotypes, SE is often highly invasive for mature chickens. After colonizing the intestinal tract, many SE strains can disseminate widely to various internal tissues and are thereby sometimes deposited in the contents of eggs prior to oviposition. Restricting intestinal colonization, therefore, is only one component in strategies for controlling SE in breeding and laying flocks. Flock testing programs, vaccination, and stringent sanitation and biosecurity practices (including cleaning and disinfection of poultry houses and rodent control) have all played significant roles in efforts to address the SE problem in poultry.

(232) ECOLOGICAL CONCEPTS FOR DEVELOPING DEFINED COMPETITIVE EXCLUSION CULTURES TO ENHANCE COLONIZATION RESISTANCE AGAINST ENTERIC PATHOGENS IN FOOD ANIMALS

D. J. Nisbet,* D. E. Corrier, S. C. Ricke, P. J. Fedorka-Cray and J. R. DeLoach, USDA-ARS, Food Animal Protection Research Laboratory, College Station, TX 77845

Previously in our laboratory it was shown that 10-day-old broiler and layer chicks that were fed diets containing 5-10% lactose from day-of-hatch were significantly protected against *Salmonella typhimurium,* and *S. enteritidis* cecal colonization, and resistance against salmonellae cecal colonization was further enhanced in treatment groups provided dietary lactose in combination with an undefined mixture of anaerobic bacteria. In order to make a defined competitive exclusion culture that was efficacious in enhancing colonization resistance against salmonellae, we cultured cecal contents obtained from adult broilers (maintained on a diet containing 5% lactose) in a continuous-flow (CF) culture apparatus, that was maintained at parameters that would best represent the cecal environment. Two different CF-cultures were developed (CF1 and CF2) that were efficacious in enhancing colonization resistance against salmonellae in broilers and in layers when used in combination with diets containing lactose, however production economics dictated that a defined culture be developed that was efficacious in controlling salmonellae cecal colonization in the absence of dietary lactose. A third CF-culture was developed from cecal contents of adult broilers (not fed lactose) using the same CF-culture technique. This culture (CF3) contains 29 different bacterial isolates and has been shown under experimental conditions to significantly protect newly hatched chicks against *S. typhimurium* and *S. enteritidis* colonization, and in a large commercial field trial to significantly reduce salmonellae colonization in adult market-age broilers. Other research with CF3 has shown that chicks provided the culture at day-of-hatch have greater than a 100-fold increase in cecal anaerobic CFU at three-days-of-age compared to untreated controls. Additionally it has been shown by electron microscopy that a large proportion of this increase occurs on the cecal mucosal epithelium. The result of this rapid establishment of a cecal bacterial ecosystem is a significant increase in cecal total volatile fatty acids, most notably propionic acid. Over several studies the correlation between the level of cecal propionic acid in three-day-old chicks and salmonellae colonization has been extremely high and we now use this physiological response as an indicator of CF3 culture establishment and a predictor of efficacy in laboratory experiments. Currently using the CF-culture technique we are developing a defined CF-culture to reduce salmonellae colonization in swine. This research is being conducted in cooperation with the USDA/ARS swine research unit at Ames, Iowa. Presently this culture is being tested in laboratory experiments to determine if this type of pathogen intervention strategy can also be used in the swine industry to aide in the reduction of enteric pathogen colonization in the swine gut.

(233) COLONIC BIOTA AS A HOST DEFENSE

K. H. Wilson, VA Medical Center/Duke University, 508 Fulton Street, Durham, NC 27705

The colonic biota is a complex ecosystem comprised mostly of anaerobic bacteria. Hundreds of species colonize the human colon, many falling into

Bacteroides and *Bifidobacterium* and into phylogenetic groups related to *Clostridium coccoides* and *C. leptum*. This ecosystem functions as a host defense by preventing many harmful species of bacteria from colonizing the gut. Although most enteric pathogens have specific mechanisms to circumvent this colonization resistance, none is capable of competing directly with the colonic biota within the lumen of the gut. The complexity of this ecosystem appears to be an important factor in its function. The major mechanisms of colonization resistance demonstrated so far reveal a system of redundant controls. In essence, the biota transforms the colonic milieu into a formidable environment characterized by low redox potential and the presence of growth retarding chemicals such as H_2S and volatile fatty acids. Nutrients are sparse and there is a lack of adhesion sites for bacteria that are not community members. An optimal method utilizing our present knowledge to establish colonization resistance in a host with an inadequate biota would be complex but workable.

(234) RESEARCH STRATEGIES FOR UNDERSTANDING FOODBORNE PATHOGEN COMPETITIVENESS UNDER STRICT ANAEROBIC AND GASTROINTESTINAL CONDITIONS

S. C. Ricke,* D. J. Nisbet, D. E. Corrier and J. R. DeLoach, Texas A & M University, Kleberg Center, Room 101, College Station, TX 77843-2472

Despite considerable evidence that establishment of indigenous microflora and their continued presence in the gastrointestinal tract are essential for preventing pathogen colonization, little is known about the mechanism(s) involved. This is partly because knowledge of *Salmonella* spp. metabolism and physiology is largely based on studies conducted under aerobic conditions. Consequently, there is considerable information about environmental signals that control growth and pathogenesis during and after invasion of the intestinal tract but little is known about the ecology of *Salmonella* spp. in the gastrointestinal tract prior to attachment and invasion. Utilizing strict anaerobic methodology for metabolism and physiology studies should provide a better understanding of salmonellae competitiveness under these ecological conditions. This approach, when combined with the wealth of molecular information and powerful techniques already available for this organism, will yield considerable insight into the capabilities of salmonellae establishment and growth in the gastrointestinal tract. Accomplishing this research agenda will aid the elucidation of the mechanism(s) involved in the successful antagonism of competitive exclusion cultures against pathogens.

(235) IMMUNOBASED METHODOLOGY FOR DETECTION OF MICROORGANISMS IN COMPETITIVE EXCLUSION CULTURES

L. H. Stanker,* C. R. Young, D. J. Nisbet and J. R. DeLoach, USDA-ARS Food Animal Protection Research Laboratory, College Station, TX 77843

We previously described an anaerobic culture system (CF3) containing 29 bacterial species that were obtained from adult chickens. The administration of CF3 cultures to newly hatched chicks results in exclusion of *Salmonella* and is known as competitive exclusion. In order to evaluate the fate of these bacteria in the bird we have generated a panel of monoclonal antibodies against the constituent microorganisms of the mixed microflora CF3 culture. Presently, we have developed monoclonal antibodies to the following bacteria: *Fusobacterium* spp.; *Bifidobacterium* spp.; *Enterococcus avium*; *Proprionobacteria granulosum*; *Eubacteria* spp.; and *Veillonella* spp. from the CF3 culture. Data on the preparation and characterization of these monoclonal antibodies will be presented in this study. Five to ten individual monoclonal antibodies to each bacterial species listed above have been isolated. Isotype determination revealed antibodies of the following classes IgA, IgG1, IgG2a, IgG2b, IgG3, and IgM, and both kappa and lambda light chains. All of the antibodies were able to detect between 10^2 and 10^3 CFU. All displayed a high degree of specificity as determined by cross-reactivity to a panel of heterologous bacteria. Using these monoclonal antibodies we have developed a rapid method for detecting and quantifying the number of specific bacteria in the ceca of chickens and in the chemostats used to produce the CF3 material. The assay is formatted as a competition enzyme-linked immunosorbent assays (cELISA). Measurements of bacterial population in both the chemostat and in the ceca from chickens will be discussed.

(236) THE UTILITY OF MOLECULAR ASSAYS FOR UNDERSTANDING MICROBIAL GENE EXPRESSION IN GASTROINTESTINAL TRACTS

S. D. Pillai, Texas A & M University Research Center, 1380 A & M Circle, El Paso, TX 79927

Some of the primary difficulties in obtaining accurate data on the ecology of the gastrointestinal microflora are due to limitations associated with their sampling and cultivation. To delineate the precise roles of the microflora within this complex ecosytem, it is imperative that methods to both identify the individual components and monitor their activities be developed. Significant advances have occurred in molecular methods that have applicability in studying microbial diversity and activity within gastrointestinal tracts. The main advantage of using molecular tools is that the genetic potential of the microflora as

well as their gene activity data are obtainable, both at the community level and at the single isolate level. Methods are currently available that permit studying fluxes in community diversity, the differential expression of genes, and horizontal transfer of genes. These methods involve the utilization of nucleic acid probes, mRNA analyses and genomic fingerprinting.

(237) OCCURRENCE, TOXICITY AND FATE OF FUMONISINS AND DEOXYNIVALENOL IN PROCESSED FOODS

L. Bullerman, Department of Food Science and Technology, University of Nebraska, Lincoln, NE 68583

Fumonisins and deoxynivalenol are mycotoxins produced by several species of *Fusarium* molds. *Fusarium moniliforme* and *Fusarium proliferatum* commonly occur in corn and produce fumonisins. Low levels of fumonisins are routinely found in corn-based food products. *Fusarium graminearum* and related species cause gibberella ear rots in corn and fusarium head blight in wheat and barley, and produce deoxynivalenol. Consequently, corn, wheat and barley can also be contaminated with deoxynivalenol, which may find its way into processed cereal-based foods. Fumonisins and deoxynivalenol appear to be fairly heat stable, but apparent reductions in the levels of the toxins are observed during thermal processing. Problems with analytical methodologies, however, raise questions about whether these are actual losses or binding to food matrices, resulting in poor recovery. So the actual fate of these toxins in processed foods needs to be assessed. Fumonisins have been linked to esophageal cancer in humans and deoxynivalenol has been associated with several human illnesses and adverse effects on immune systems. Other *Fusarium* toxins may also be significant as contaminants of the food supply, and potential food safety hazards. These toxins, including moiliformin, fusaric acid, beauvaricin and zearalenone, may become toxins of emerging significance in the future.

(238) ALTERNATIVE METHODS FOR ISOLATION, CULTURE AND IDENTIFICATION OF FUNGI IN FOODS

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

Ideal media for isolating and enumerating yeasts and molds in foods should totally suppress bacterial growth, be nutritionally adequate to support relatively fastidious species and, in the case of selection for a specific genus, species or group of molds or yeasts, promote growth at the expense of background microflora. Advances in development of media and techniques to achieve this goal are being made. Selective media for mycotoxigenic molds, xerotolerant fungi, acid-resistant yeasts, fungi with specific enzymatic capabilities and fungi associated with specific food groups are under development. Rapid techniques for enumeration and identification of foodborne yeasts and molds are receiving increased attention. Simplification of procedures for identifying yeasts associated with food spoilage has been achieved. An overview of advances and alternatives in food mycology methodology will be presented.

(239) IMMUNOLOGICAL AND GENETIC METHODS TO RAPIDLY DETECT FUNGI IN FOODS

M. A. Cousin, Food Science Department, 1160 Smith Hall, Purdue University, West Lafayette, IN 47907

Molds are important in foods because they cause food spoilage, produce mycotoxins, are used in fermentations, and may produce allergic reactions in susceptible people. Analysis of foods for molds can give information on the ingredient history, the success of processing, the sanitation of processing, and the potential shelf-life. Current methods used to detect and enumerate molds in foods are neither rapid nor able to be used on line. New methods for mold detection and taxonomic identification being researched world-wide use the same molecular techniques that have been used successfully with bacterial and viral detection. The three main areas of research are: (1) the detection of chemical metabolites, enzymes, cellular components or other compounds produced by molds but not by other organisms; (2) the identification, selection and characterization of genetic determinants; and (3) the development of antibodies to mold cellular material, metabolites, enzymes or other chemicals specific for molds. Chemical that are specific for molds can be used to either detect or enumerate molds in foods because their presence or amount can be correlated to mold growth. The genetic material can be used for both taxonomy and detection because this material would be unique for specific genera or species depending on the application. Antibodies, defense proteins against a foreign substance, can be produced to molds that will recognize antigens of molds in foods. Although most of these methods are still just done in research laboratories, in the future they could be as common in food companies as are the rapid methods for bacterial detection.

(240) BIOCONTROL OF MOLD GROWTH AND MYCOTOXIN PRODUCTION

H. Gourama, Penn State University, Berks Campus, Tulpehocken Road, P.O. Box 7009, Reading, PA 19610

The prevention and control of mold growth and mycotoxin production can be accomplished through various practices including management of crop residues, crop rotation, development of resistant varieties, drying, moisture control, use of antifungal agents,

processing and manufacturing, detoxification and physical methods of separation. In addition to the above measures the development of biocontrol and biocompetitive agents for toxigenic molds seems to have good potential. Many microorganisms were reported to affect mold growth and inhibit or degrade mycotoxins, particularly aflatoxins. *Flavobacterium aurantiacum* has been reported to remove aflatoxin from various food products. The cause of this inhibition was linked not only to low pH, hydrogen peroxide and depletion of nutrients, but also to other lactic acid bacteria metabolites. *Bacillus subtilis* was found to inhibit mold growth and aflatoxin production. This inhibition was related to the production of peptidolipid compounds. Other biocompetitive organisms include *Nannocystis exedens, Aspergillus niger* and other microbial species. An update and review of these bicompetitive agents will be presented.

(241) HEAT RESISTANT MOLDS AND PRESERVATIVE RESISTANT YEASTS

A. D. King, Jr., USDA, Western Regional Research Lab, Albany, CA 94710

Because of their unique heat resistance or ability to grow in the presence of preservatives, heat resistant molds and preservative resistant yeast can cause unpredictable spoilage in preserved foods. *Byssochlamys fulva*, *Talaromyces flavus* and *Neosartorya fisheri* are the most common heat resistant molds that cause spoilage although there are others. The yeast *Zygosaccharomyces bailii* and *Saccharomyces bisporus* can cause spoilage in foods treated with preservatives. *Saccharomyces rouxii* causes spoilage in low pH and low water activity products but is not known for its resistance to preservatives. These sets of microorganisms can cause spoilage when only a few cells are present. The presence of any *Z. bailii* can cause spoilage in preservative treated fruit juice. The heat resistant molds can grow in the amount of oxygen entrapped in fruit juice and the head space of a sealed container. Heat-resistant mold ascospores are many times more heat resistant than other mold ascospores, or the more common conidiospores. To detect heat resistant ascopsores a portion of the product is heated to 75°C for 30 min and the product incubated and observed for growth or gas production. Preservative resistant yeasts can be detected by growth on Malt Extract Agar plus 0.5% acetic acid. Other yeasts will not grow on this medium. Preservative resistant yeast can metabolize sorbic and benzoic acid and can grow in acetic acid and SO_2 concentrations that normally protect foods. Control measures include heat, sanitation, or sterile filtration as is appropriate.

(242) PROBIOTICS

N. A. Cox,* J. S. Bailey, N. J. Stern, J. E. Line and S. E. Craven, USDA-ARS, Russell Research Center, Athens, GA 30613

Food safety associated with fresh poultry is complex because of multiple stages (steps) involved in production and processing. Multiple entry points exist for human enteropathogens, such as salmonellae and campylobacters. As a consequence, multifaceted intervention approaches will likely be required during the various phases of poultry grow-out. A probiotic is defined as a live microbial feed substance which beneficially affects the host animal by improving its intestinal microbial balance. Most probiotic preparations comprise one, or at the most a few species that are mainly within the genera *Bacillus, Enterococcus* and *Lactobacillus*. The beneficial effects of probiotics can be explained by the provision of (a) nutrients or digestive enzymes, (b) antibacterial substances antagonistic to harmful bacteria, and (c) live bacteria which metabolize *in vivo* and provide nutrients, enzymes or antibacterial substances. Previous laboratory results with simple mixtures of treatment bacteria suggest that it is unlikely that probiotics as presently formulated, will have a worthwhile role in the control of salmonellae in poultry. A two-step treatment of broiler chicks with a mucosal starter culture (MSC) developed at Russell Research Center was tested in large scale commercial field trials. The MSC was first sprayed on the chicks in the hatchery followed by administration in the first drinking water. In Puerto Rico, the salmonellae prevalence in processed carcasses was reduced from 41% in control flocks to 10% in the treated flocks and in Georgia was reduced from 9.1% in control groups to 4.5% in the treated groups. These field trial studies show the treatment of chickens in a commercial setting with MSC can serve as a useful means to reduce salmonellae contamination. To produce salmonellae-free poultry, one should consider a multifaceted approach such as rapidly applying the best possible chemical disinfection to the freshly laid fertile egg, combined with disinfecting the circulating air in hatching cabinets, plus the application of a yeast treatment to remove any salmonellae originating from breeder flocks or hatcheries prior to the application of an effective MSC.

(243) SLAUGHTER

J. Riemann, Excel Corporation, 2901 North Mead, P.O. Box 8183, Wichita, KS 67208

Conversion of meat animals to safe meat products is a process of many challenges. It starts with the slaughter process in which workers must be very careful to avoid contaminating the carcass surface while removing the feet, hide and viscera. It is easy to tell when visible contaminants (hair, dirt, fecal, ingesta) get on the carcass but impossible to visually detect microbial contamination. The invisible contaminants may only accelerate spoilage of the meat or they may be pathogenic. The desire to eliminate pathogens and significantly reduce bacteria on carcasses has led to development of different intervention strategies.

Visible contamination has been removed traditionally by knife trimming. Knife trimming increased since initiation of the USDA Zero Tolerance program. Recently steam vacuum spot cleaners were developed to remove sensible contaminants and pos-

sibly reduce knife trimming. The spot cleaners effectively remove usable contaminants plus reduce significantly surface bacteria due to the steam used in the cleaning process.

Antimicrobial treatments developed for the slaughter process have included hot water (180°F) carcass wash, organic acid rinses or sprays, trisodium phosphate spray and most recently steam pasteurization. Difficulties of maintaining water temperature, incomplete coverage of the carcass surfaces' equipment maintenance and high energy cost have resulted in slow acceptance of hot water carcass washing by slaughterers. Organic acids (lactic, acetic) may be used as a pre-evisceration rinse and/or following final carcass wash. Some organic acids are selective in antimicrobial effect, and all accelerate corrosion of equipment. Trisodium phosphate (TSP) spray is used after the carcass wash and has had limited success in reducing bacteria. Application requires specialized equipment (mixing and spraying) and additional processing of waste water is necessary to remove added phosphate. All intervention treatments applied as a spray have their effectiveness reduced because of shadowing or inability of the spray equipment to get the chemical into carcass areas difficult to access, such as behind the kidney fat or under the diaphragm muscle. Costs of the chemical applications when used on beef carcasses are difficult to determine but are estimated at $.08 to $1 $.15/head for organic acids and $.50 to $.70/head for TSP.

Steam pasteurization is a new and very effective antimicrobial treatment that reduced pathogenic bacteria 99.9% in pilot plant tests and food spoilage bacteria approximately 90% in pilot plant and production plant tests. Steam, above atmospheric pressure, thoroughly blankets the carcass heating the surface to 195°F or higher for a minimum of six seconds. Six to ten second treatment times produce no carcass surface discoloration but longer pasteurization times may cause some lean discoloration. No chemicals are used and the treatment is estimated to cost $.03 to $.05/head.

(244) PROBIOTICS CHEMICAL TREATMENTS/ BACTERIOCINS

B. Sheldon, N. C. State University, Raleigh, NC 27696

An overview will be presented of past and ongoing studies to evaluate the inhibitory activity of various chemical treatments and biopeptides (i.e., bacteriocins, magainins) to reduce the level of bacterial pathogens and spoilage microorganisms associated with muscle food products. Summaries of both bench-top and commercial field trial studies will be presented on the inhibitory activities of a new oxyhalogen inorganic disinfectant (Salmide), phosphates (TSP), chloride dioxide, and organic acid rinses on specific pathogen reductions associated with muscle foods (*Salmonella*, *Campylobacter*, and *Listeria* species). The lethality of several naturally occurring biopeptides including bacteriocins, magainins (amphibian derived), and cecropins (insect-derived) against selected foodborne bacterial pathogens associated with meat and poultry products will be presented. Specifically, the application of nisin, a food-grade bacteriocin produced by the dairy fermentation microorganism *Lactococcus lactis* subspecies *lactis* to reduce bacterial pathogens on the surfaces of poultry products will be highlighted. Their modes of action, spectrum of activity against microorganisms, influence of environmental factors, and various modes for applying these peptides such as incorporation into primary packaging materials (i.e., over-wraps, shrink-wraps, vacuum packaged, drip pads) and edible films (i.e., carbohydrate, proteins) will be discussed. Advantages and disadvantages of these inhibitors will be considered relative to their efficacy, stability, safety, cost, and impact on product quality.

(245) IRRADIATION

J. S. Dickson, Department of Microbiology, Immunology and Preventive Medicine, Iowa State University, Ames, IA 50011

The impact of irradiation on the microflora of foods has been well documented over the years. Relatively low doses of radiation, in the two to three kilo-Gray (kGy) range, are sufficient to eliminate most vegetative bacterial pathogens at the levels that are likely to occur in foods. For example, a 2 kGy dose would eliminate approximately 3 $\log_{10}$ cycles of *Salmonella* or *Listeria* under most processing conditions, and even greater reductions would be seen with *Campylobacter* and *Escherichia coli* O157:H7 Clearly, food irradiation is a significant technology in improving the microbiological safety of meats. This technology has applications at many stages of meat production. Irradiation can be used to improve the microbiological safety of animal feeds and concentrates by eliminating salmonellae in the feed. Irradiation of products after slaughter, either of intact muscle or ground products, can significantly reduce the risk associated with raw meats. In addition, since irradiation can be applied to packaged products, the risk of recontamination after processing can be minimized. While the microbiological benefits of this process are documented in the scientific literature, the ultimate acceptance of the technology will rest with the consumer.

(246) RESTAURANTS — THE INTEGRATION OF FOOD SAFETY SYSTEMS FUNCTIONAL CONTROL THROUGH HACCP BASED SYSTEMS

D. Theno, Foodmaker, 9330 Balboa Avenue, San Diego, CA 92123-1516

The science technology and strategic approach to the management of food safety has evolved a great deal in the last decade. Major food/water-borne outbreaks of the last five years have further accelerated the pace of change.

Leading the evolution in food safety is the concept of vertical integration of the individual participants involved in the farm to table food supply continuum. The transformation of plants and animals to human food is a complex multi-step process. Within each element of the chain, a certain level of control is realistically attainable. To maximize the effectiveness of the individual control programs throughout the food production chain, the use of Hazard Analysis and Critical Control Point (HACCP) based programs has been demonstrated to be the most effective control system.

Historically the individual sub-components of the food continuum designed and operated food safety systems were either mandated by a regulatory agency and/or were designed from the manufacturer's own perspective. This situation resulted in gaps within the food safety chain between the different sub-categories. Without a total system of risk control and management, threats could and did pass into segments of the food chain where interventive measures were not available or anticipated.

The concept of farm to table control of food safety has been touted as a goal for the last decade but has only recently become a reality. Unprecedented partnerships have been formed. Data and information are being shared between all members of the food supply chain at levels never before envisioned.

The increased demand for food safety accountability will only serve to further this directional trend. Results to date indicate that as food safety partnerships strengthen, the control level becomes greater. Once again the key structural element supporting this system is HACCP. A future in food safety where the free market drives food safety improvement may not be too far off. Legal liability/accountability issues will continue to propel those that provide food products to consumers toward greater levels of control. The best effective methodology currently available to promote control throughout the food supply continuum is HACCP. Control of threats is typically most effective at their source of introduction into the system. Intervention steps can also help to improve the margin of food safety but are generally limited by the effectiveness of the original source control. The food safety systems of the future are easy to envision. The question remains, How do we get there from here? Vertical integration through HACCP is the tool we will use; we only need to agree on the process.

(247) RETAIL

G. Prince, The Kroger Co., 1014 Vine Street, Cincinnatti, OH 45202

Consumers express their confidence in the safety of meat at the retail meat display case. Consumer research indicates the selection of the store where they shop is based upon their confidence in the quality and safety of the perishables offered by the retailer. The perishable department is the cornerstone of the modern successful supermarket. The retailer must earn this reputation by the quality of product they offer.

Fulfillment of consumer expectation starts with product specifications that will be used to procure the quality of meat the consumer will demand. This, coupled with operating practices, will deliver to the display case meat with eye appeal. Product quality is perceived by the consumer's eye and proven at the dinner table. Food safety is the Invisible Challenge that must be met many times every day in each and every package.

Food safety begins with the live meat animal prior to slaughter and ends when the product is consumed. From the moment of slaughter to the point of consumption, time and temperature are the critical control points in maintaining food safety. Retail food store operating strategies must have built in operating measures to assure that critical control points are followed. A key safety strategy is employee training on their responsibilities in food handling. Food safety strategies must include the consumer handling and preparation.