it had no effect on cheese pH (5.2). After five injections, the protein matrix occupied increased area of cheese matrix (83 versus 78%). Even though citrate injection had no effect on insoluble calcium, and thus the rate and extent of cheese flow were unaffected, increased phosphate solubilization, and possibly decreased ionic calcium content, resulted in expansion of the protein matrix and increased cheese hardness.

Key Words: Calcium, Phosphate, Protein matrix

W294 Continuous manufacture of mozzarella cheese using concentrated microfiltration retentate and recovery of virgin whey proteins. A. V. Ardisson* and S.S.H. Rizvi, *North East Dairy Foods Research Center. Cornell University.*

The objective was to develop a continuous cheese-making process, which utilizes concentration factor (CF) 8-9, pH 6.0 skim milk microfiltration (MF) retentate to produce low-moisture part-skim (LMPS) Mozzarella cheese.
Pasteurized skim milk was microfiltered to a concentration factor of 8-9 at 50C using a 0.1μm nominal pore diameter microfiltration membrane unit with a total area of 0.72 m^2. The system was equipped to maintain a uniform transmembrane pressure (UTMP) in the range of 68.9 KPa to 172.4 KPa. The milk was gradually acidified during microfiltration to pH 6.0 using glucono-d-lactone (GDL) at a concentration of 1.6g/l skim milk to adjust the calcium to protein ratio in the final retentate. Experiments were conducted to test the effect of four different cross flow velocities (CFV): 2.5, 3.5, 4.5 and 5.5 m.s-1on permeate flux, which allowed the determination of fouling of the membrane. Furthermore, flux decay was evaluated at four different transmembrane pressure levels (68.9 KPa, 103.4 KPa, 137.2 KPa and 172.4 KPa). The process was scaled-up to a membrane unit with a total area of 9.1 m^2 for the continuous production of cheese. The obtained retentate was subsequently standardized with heavy cream to a casein to fat ratio of 0.85 and converted into LMPS Mozzarella cheese curd in an Alcurd continuous cheese coagulator using single strength rennet (80μl/Kg retentate). The resulting curd was then cooked and stretched. The analyses performed on skim milk, retentate, permeate and cheeses included total solids, protein (Total N, non-protein N and non-casein N), fat and ash. The fat, moisture and protein contents of the cheese produced by the process as well as its textural characteristics were within the normal ranges for LMPS.

Key Words: Microfiltration mozzarella, Microfiltration retentate cheese, Whey protein depletion of milk

W295 Lexicon development of appearance and texture descriptors for melted cheddar cheese. K. M. Asato*, I. M. Tsai, and M. R. McDaniel, *Oregon State University, Corvallis, OR.*

A lexicon to define the sensory properties of melted cheddar cheese was created using a trained descriptive panel. The lexicon characterizes appearance (surface rupture, meltedness, oiliness, and edge browning) and texture (stringiness, stretchiness, springiness, firmness, toothpull, smoothness, cohesiveness, denseness, and chewiness). The newly developed lexicon was used to evaluate seven samples consisting of three commercial brands of shredded cheddar cheese at different ages (sharp, medium and mild) in order to determine how heat treatment (oven and microwave) affected the sensory perception of melted cheese. Microwave treated cheese was higher than oven treated cheese in all descriptors except edge browning, smoothness and cohesiveness. Under the same heat treatment, melted sharp cheddar was rated higher in oiliness and lower in all texture descriptors than melted medium and mild cheddar.

Key Words: Melted cheese, Cheddar, Sensory

W296 Monitoring spores and spore-forming bacteria populations in commercial skim milk powder production plants using conventional and molecular methods. C. Murillo*[1], C. Kitts[2], and R. Jimenez-Flores[1], [1]*Cal Poly Dairy Products Technology Center,* [2]*Cal Poly Biological Sciences Department.*

The microflora of milk powder consists of a wide array of microorganisms of which special attention is given to *Bacillus spp.* spores and spore formers. *Bacillus spp.* spores survive well in all processing stages and inhabit the milk powder in the dormant state indefinitely. Upon reconstitution, spores may germinate, and through their enzymatic activity become detrimental to quality. The objectives of this study are to 1) enumerate total aerobes, mesophilic, and thermophilic spore populations in commercial, low-heat skim milk powder production plants; 2) characterize the microbial ecology of this process using Terminal Restriction Fragment Patterns (TRFPs); and 3) compare the changes in the ecology during this process. Fluid and powder skim milk was collected from 3 commercial facilities during spring, summer, and fall '01-'02. Sampling points included the raw milk silo, separator, evaporator, and spray dryer. Samples were normalized based on total solids. Every sample was evaluated for total aerobes, mesophilic, and thermophilic spores. For TRFPs community DNA was extracted, amplified by 16S PCR, and digested with *HaeIII* and *DpnII*. Spore formers are predominant in condensed and powdered milk, and tend to increase in the powder with increasing processing time. In raw milk mesophilic and thermophilic spores ranged from ¡25CFU/g to 70CFU/g and ¡25CFU/g to 10^2 CFU/g, respectively. In powder they ranged from ¡25CFU/g to 10^3 CFU/g and ¡25CFU/g to 10^6 CFU/g, respectively. Both spore counts from skim milk showed an increasing trend with run time and rendered the powder out of the 10^3 CFU/g limit. In the ecology TRF patterns successfully described microbial populations, and an overall decrease in microbial diversity between raw and powdered milk was observed. Overall, *Bacillus spp.* were found in 92important organisms included *Clostridium spp.* (57*Staphylococcus spp.* (29*Streptococcus spp.* (9*Bacillus spp.* were present in 100from all 3 plants.

Key Words: Milk powder, Terminal restriction fragment patterns, Quality

W297 Enterotoxigenic *Bacillus* spp. DNA fingerprints revealed in powdered milk products using rep-PCR. R. M. Cooper* and J. L. McKillip, *Ball State University, Muncie, IN.*

As a staple food, milk powders and other dry functional dairy ingredients must reflect strict quality control and a long shelf life. As a means of assessing the microbiological quality of a battery of dry dairy products, the technique of repetitive element palindromic polymerase chain reaction (rep-PCR) was used as a screening tool to detect DNA fingerprinting profiles from potentially enterotoxigenic *Bacillus* spp. in five industrial formulations of lecithin, soy fiber, whey protein concentrate, and nonfat dry milk powder. Following a nonselective enrichment protocol (11-13 h) in tryptone phosphate glucose yeast extract (TPGY) broth to induce spore germination and vegetative cell growth to densities of 10^6 CFU/ml, each dry product was subjected to a commercial DNA extraction procedure and rep-PCR to generate distinct amplicon banding patterns that were analyzed using agarose gel electrophoresis. A distinct 1,230bp diagnostic band consistent with that of previously characterized enterotoxigenic *Bacillus cereus* was demonstrated in rep-PCR from nonfat dry milk, lecithin, and soy powders. The identity of the diagnostic band was confirmed by restriction enzyme analysis, and in each case generated the same digest pattern as the rep-PCR amplicon from the positive control *B. cereus*. These data validate the method of rep-PCR as a viable means of screening powdered dairy ingredients (and perhaps many other foods) for enterotoxigenic *Bacillus* spp. without the need for plating and enumeration using selective and differential media.

Key Words: Enterotoxigenic *Bacillus* spp., rep-PCR, Detection

Food Safety: Food safety; Methods, prevalence and control

W298 Detection of viable Enterobacteriaceae in milk by using real-time broad-range RT-PCR. S. H. Choi* and S. B. Lee, *Sangji University, Wonju, Korea.*

This study was carried out to develop real-time broad-range RT-PCR which could detect viable Enterobacteriaceae in milk. The threshold cycle(Ct) of the RT-PCR was determined by using Multiscribe reverse transcriptase and SYBR Green PCR Master mix(Applied Biosystem) and iCycler iQ(Bio-Rad). Following the RT-PCR , the synthesized DNA was confirmed in agarose gel electrophoresis. The nucleotide sequences of primers were designed based on the ribosomal protein genes, S11

and S13, in alpha ribosomal protein operon. The RT-PCR syntesized a DNA fragment of 520 bp from the template RNA isolated from 10^7 of ten Enterobacteriaceae strains, but not from *Pseudomonas fluorescens*, *Acinetobacter calcoaceticus*, *Enterococcus faecalis*, *Listeria monocytogenes*, and *Bacillus coagulans*. The Cts to detect the Enterobacteriaceae strains ranged from 21 to 23 but the Cts to detect the other bacteria were more than 40. The limit of bacterial number to detect *E. coli* was 1000. The Ct to detect *E. coli* in milk heated at 65°C for 30 min, at 100°C for 10 min, and at 121°C for 15 min were 32.7, 37.2 and >40.0, respectively. RNase treatment of the heated *E. coli* increased Ct but not unheated *E. coli*.

Key Words: RT-PCR, Enterobacteriaceae, Milk

W299 Use of real-time polymerase chain reactions (PCR) for the detection of pathogenic microbes in bulk-tank milk. J. S. Karns*, J. S. Van Kessel, and M. L. Perdue, *USDA-ARS, Beltsville, MD*.

Recent reports suggest an increase in consumption of raw milk and products made from raw milk in the United States. Several outbreaks of food-borne disease have been associated with the consumption of these products. Traditional culture methods for detection of pathogens in foods are generally time-consuming and labor intensive, often requiring more than 96 hours for positive identification. Methods for the rapid detection of pathogenic microbes in raw milk could help to minimize risks associated with consumption of raw milk. The objective of this study was to examine the usefulness of real-time PCR for the detection of *Salmonella enterica* and *Listeria monocytogenes* in bulk-tank milk. Twenty-four milk samples identified as *Salmonella* positive by traditional culture techniques and 176 that were *Salmonella* negative based on culture techniques were chosen for PCR analysis. DNA was isolated from the same tetrathionate enrichments used for culture identification and subjected to real-time PCR analysis using a commercially available reagent kit. Fifty-three samples were identified as *Salmonella*-positive by real-time PCR analysis. Two samples that were identified as being positive for *Salmonella* via culture were identified as being *Salmonella*-negative based on real-time PCR while 23 samples originally determined to be *Salmonella*-negative were *Salmonella*-positive based on real-time PCR. Serotyping confirmed that isolates from the 2 cultures that were PCR negative were not *Salmonella* while more rigorous culture of one of the PCR-positive, culture-negative enrichments did result in isolation of *Salmonella*. Eighty-one samples of bulk-tank milk shown to be positive for *Listeria* sp. by traditional culture were chosen for analysis with a published TaqMan primer/probe set specific for *Listeria monocytogenes*. DNA was isolated from the same Modified Listeria Enrichment broth cultures used for culture identification. Of these 81 samples 42 were clearly positive by real-time PCR, 8 were tentatively positive, and 31 were shown not to contain *L. monocytogenes*, indicating that the *Listeria* isolated from them were non-pathogenic species. This study suggests that real-time PCR techniques can be used to detect pathogenic microorganisms in bulk-tank milk with a sensitivity as good or better than traditional culture methods. In addition, these methods yield results within 24 h for *Salmonella* and 48 h for *Listeria*, greatly reducing the time required for positive identification of these pathogens in raw milk.

Key Words: *Salmonella*, *Listeria*, TaqMan

W300 Survey of bulk tank milk in the United States for food-borne bacterial pathogens. J. S. Van Kessel*[1], J. S. Karns[1], B. J. McCluskey[2], and M. L. Perdue[1], [1]*USDA-ARS, Beltsville, MD*, [2]*USDA-APHIS, Fort Collins, CO*.

The consumption of raw milk and raw milk products has led to periodic disease outbreaks in the United States and more information is needed to assess the incidence of food-borne pathogens in bulk tank milk. The objective of this study was to determine the prevalence of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* in bulk tank milk in the United States. As part of the NAHMS Dairy 2002 survey, 861 bulk tank milk samples were collected from farms in 21 states and, when possible, shipped overnight on ice to the USDA-ARS laboratories in Beltsville, MD. Milk was directly plated on selective agars (MacConkey, Sorbitol MacConkey, XLT4, and Modified Oxford media) for direct bacterial enumeration and was enriched in selective broths (EC, tetrathionate, and modified Listeria enrichment broth) to increase detection sensitivity. After enrichment, cultures were streaked on selective media as above. Coliforms are often used as a general indicator of fecal contamination and coliforms were detected in 798 (92.7%) of the milk samples. Twenty two samples (2.6%) were culture-positive for *Salmonella*. When the *Salmonella enterica* isolates were serotyped, nine different serotypes were represented. The most common serotype was Montevideo which was found in seven milk samples. *Salmonella enterica* Newport was isolated from four samples, *S. enterica* Muenster, *S. enterica* Meleagridis, *S. enterica* Cerro, and *Salmonella* 44:Z36, (Z38) were identified in each of two milk samples, and *S. enterica* Dublin, *S. enterica* Anatum and *Salmonella* Sal9,12:nonmotile were identified in one milk sample each. *Listeria* was detected in 90 milk samples (10.4%) and, based on hemolysis, approximately 50% of these were *Listeria monocytogenes*, the only species of *Listeria* pathogenic to humans. The results of this survey demonstrate that *Listeria* and *Salmonella* contamination of bulk milk is relatively low and infrequent. Although their presence presents a risk to consumers of raw milk and raw milk products, pasteurization kills each of these species.

Key Words: *Listeria*, *Salmonella*, Milk

W301 Efficacy of lactic acid to prevent rapid *Salmonella* infection in market weight swine. M. D. Howard*[1], H. S. Hurd[2], and J. K. Gailey[2], [1]*National Swine Research and Information Center*, [2]*National Animal Disease Center, Ames, IA*.

Swine can become rapidly infected with *Salmonella* during lairage at the abattoir and during transport; suggesting a need for intervention. The goal of this research was to determine the efficacy of lactic acid to reduce rapid *Salmonella* infection. Thirty six market weight swine were randomly assigned to one of four treatments: 1) 0% lactic acid and 0 g Tylan®; 2) 0% lactic acid and 20 g Tylan®/907 kg of feed; 3) 0.44% lactic acid and 0 g Tylan®; 4) 0.44% lactic acid and 20 g Tylan®/907 kg of feed. Lactic acid was administered through drinking water. Lactic acid and Tylan® were available to pigs for 7 d prior to euthansia. Animals were placed in a pen contaminated with nalidixic-acid resistant strain of *Salmonella typhimurium* χ4232 and remained in the pen a minimum of 2 h and a maximum of 4 h prior to euthanasia. Samples of stomach fluid, ileal tissue, ileocecal lymph nodes, cecal contents, and distal colonic contents were analyzed for the presence of *S. typhimurium* χ4232. Among the four treatments no differences ($P \geq 0.05$) were detected in *Salmonella* prevalence from stomach fluid, ileal tissue, ileocecal lymph nodes, cecal contents, and distal colonic contents. Although not statistically significant some indications of treatment effects were noted. Cecal contents of pigs drinking water free of lactic acid tended ($P=0.27$) to have lower *Salmonella* prevalence than pigs consuming lactic acid. Distal colonic contents of Tylan®-free pigs tended ($P=0.21$) to have lower *Salmonella* prevalence than pigs consuming Tylan®. Ileal tissue of pigs drinking lactic acid water tended ($P=0.28$) to have lower *Salmonella* prevalence than pigs drinking untreated water. No treatments differences were detected in stomach-fluid concentrations of total lactic acid, dissociated, and undissociated lactic acid ions. This research suggests lactic acid was ineffective in reducing *Salmonella* prevalence; lack of lactic acid concentration differences in stomach-fluid suggests orally consumed lactic acid was either rapidly absorbed or administered at a level that was inadequate to raise it above physiological values.

Key Words: Lactic acid, *Salmonella typhimurium*, Market swine