between the observed and the predicted values using the training and the validation sets were 0.71 and 0.53, respectively.

Key Words: neural network, fertility, semen quality

Dairy Foods: Dairy Foods Processing/Cheese/Dairy Micro

T43 Understanding and controlling flavor and color development resulting from non-thermal browning (NTB) in cheese. A. Lopez-Hernandez*, N. Van Epps, and S. A. Rankin, *University of Wisconsin, Madison*.

Under certain conditions, some cheeses brown (i.e. parmesan, gouda) during the course of aging yielding concomitant changes in flavor and color. Very little definitive science exists to describe, define or control the reaction chemistry of non-thermal browning (NTB) in cheese from either the flavor or pigmentation perspective. There is a number of suggested pathways that attempt to explain these changes. Such pathways involve factors such as redox potential, available oxygen, the presence of α–dicarbonyl compounds, amino acid type and concentration, Mn^{2+} ions, and microbial tyrosinase activity. However, NTB still exists in the industry with no clear means of understanding or controlling its development. The aim of the present study was to define the chemical structures of flavorants and pigments generated through non-thermal browning mechanisms in cheese as an aid to understand and control its development. Our results showed that the pigments are stable to air exposure and soluble in some non-polar solvents, such as pentane and pentane-methylene chloride (2:1 v/v). The UV-Visible spectra of the extracts showed maximum absorption peaks at 400 and 450 nm. Volatile characterization revealed compounds specific to brown cheeses including lactones, ketones and pyran derivatives. The relative abundance of the compounds was found to be higher in those cheeses where browning and more intense aroma was more evident. Parallel studies in a cheese model systems demonstrated that α–dicarbonyls specific to fermentation showed little effect on the development of brown color. Extensive heat treatment of the cheese milk source also showed little effect on the development of brown pigmentation.

Key Words: non thermal browning, parmesan, maillard reaction

T44 Transcriptomic analysis of Camembert cheese fungal activity. C. Viel*, F. Boileau, A. Thériault, and S. Labrie, *Département des sciences des aliments et de nutrition, Centre de recherche en sciences et technologie du lait (STELA), Institut des neutraceutiques et des aliments fonctionnels (INAF), Université Laval, Québec, QC, Canada*.

Camembert cheeses are dynamic microbial ecosystems that evolve during ripening. The overall metabolic activity of the microbiota largely determines the quality of the cheeses. However, little is known about the genes expressed by the surface microbiota. We studied the transcriptome of a ready-to-eat Camembert cheese to gain a better understanding of the major activities of the fungi responsible for rind formation. Samples were collected from the rind of a Camembert cheese, inoculated on selective media, and three isolates were microbiologically characterized and further identified as *Penicillium camemberti*, *Geotrichum candidum*, and *Debaryomyces hansenii* by rDNA sequencing. High quality total RNA was obtained from the same rind and was analyzed by RT-PCR using a Bioanalyzer 2100. Specific primers revealed that the three species could be amplified from total RNA. mRNA was purified from the total RNA and retro-transcribed. A cDNA library was constructed. The sequence of the clones were compared to those in public databases. Putative gene functions were attributed as follows: 26% were involved in metabolism, 20% in translation, 10% in cell signaling, 4% in survival (defense, stress, and repair), 4% in ion transport, 4% in transcription, 2% in apotosis, 2% in cell transport, and 3% in other functions, while 25% had unknown functions. The results of the present study will help guide the selection of biomarker genes that can be used to monitor fungal activities during cheese ripening.

Key Words: transcriptome, fungi, camembert

T45 Comparison of Hispanic cheeses from US and country of origin manufacturers. L. A. Jimenez-Maroto[1], A. Lopez-Hernandez*[1], B. Maldonado[2], and S. A. Rankin[1], [1]*University of Wisconsin, Madison*, [2]*Tecnológico de Monterrey, Campus Querétaro, Querétaro, México*.

There is anecdotal information that US-made Hispanic cheeses are criticized by Hispanic consumers for not being authentic compared to cheeses made in their countries of origin. In order to determine what characteristics define the authenticity of Hispanic cheeses several assessments were conducted including microbial testing, sensory profiles, chemical composition, and functional character. Commercial samples of three different types of Hispanic cheeses (fresh, pasta filata, aged) were acquired from domestic (n=44) and country of origin (n=40) manufacturers from three regions (Mexico, Central America, Caribbean). Proximate analysis was conducted using standard methods. A modified melt-flow apparatus was used to ascertain the melt character. Quantitative descriptive analyses (QDA) of cheese flavor, texture, and appearance were conducted by trained panelists (n=13) and the results analyzed using principal component analysis (PCA) and canonical analysis. Consumer panels comparing US and Mexican samples were conducted for each of the cheese types studied. None of the samples tested positive for the presence of food pathogens. Country of origin cheeses had higher moisture and pH, lower salt, similar lipid and protein content. Some of the non-US cheese products contained non-dairy ingredients, such as vegetable oils. Melt character of fresh and aged cheeses showed significant differences between domestic and country of origin samples, while pasta filata cheeses showed no significant differences. There were significant differences in salt, bitter, buttery, cowy, milkfat, oxidized, unclean and rancid flavor attributes. Consumer panels showed that Mexican consumers similarly rated US-made cheeses as highly authentic when compared to the Mexican-made samples. These results provide insight into the characteristics that define the authenticity of the Hispanic cheeses analyzed. Knowledge of these characteristics will aid U.S. manufacturers to produce a Hispanic cheese with more authentic qualities that will satisfy the demands of their Hispanic consumers.

Key Words: Hispanic cheese, sensory profile, melt character

T46 Partitioning of omega-3 fatty acids in Cheddar cheese curd and whey. C. Brothersen*, D. J. McMahon, and B. Pettee, *Western Dairy Center, Utah State University, Logan*.

Full-fat Cheddar cheese was made with milk fortified with omega-3 fatty acids and the partitioning of omega-3 fatty acids into the curd

and whey was determined. Omega-3 fatty acids from both animal and plant sources in both encapsulated and free oil were used. The omega-3 fatty acid preparations were incorporated into the milk by the following methods: 1) mixing the encapsulated powder in the milk, 2) mixing oil in the milk, 3) mixing oil in cream, 4) homogenizing oil in milk, and 5) homogenizing oil in the cream. With the homogenization treatments, the homogenized milk and cream constituted ten percent of the fat in the cheese milk. Also, encapsulated omega-3 fatty acid preparations, from animal and plant sources, were added to stirred and milled curd during dry salting, and the omega-3 content of the pressed curd was determined. The recovery of omega-3 fatty acids from the animal source, averaged over all treatments was 90.5%, and was significantly higher ($\alpha\# = 0.05$) than that of the plant source at 80.3%. The average recoveries of omega-3 fatty acids from the homogenization treatments were 90.3% and 92.8% for treatments 4 and 5 respectively, and were not significantly ($\alpha\# = 0.05$) different. There was a significant difference in the average recovery of omega-3 fatty acids in curd from the homogenized treatments compared to that of the non-homogenized treatments, which were 80.2%, 79.0% and 82.8% for treatments 1-3 respectively. The recovery in the curd form the non-homogenized treatments were not significantly different from each other. When the encapsulated form of omega-3 fatty acids were added to the cheese curd during salting, the average recovery for the animal and plant sources, were significantly ($\alpha\# = 0.05$) different at 99.4% and 91.1% respectively. The average recovery of omega-3 fatty acids in the milled curd was 94.4%, and was not significantly different from that of the milled curd at 96.1%. Therefore, the most efficient method of fortifying Cheddar cheese with omega-3 fatty acids, is adding the encapsulated powder to the curd during salting.

Key Words: cheese, omega-3, fortification

T47 Microbiological quality of raw milk utilized for small scale artisan cheese production. D. J. D'Amico* and C. W. Donnelly, *University of Vermont*, *Burlington*.

This study evaluated the overall milk quality and prevalence of four target pathogens in raw milk used for small scale artisan cheesemaking and examined specific farm characteristics and practices and their effect on bacterial and somatic cell counts. Raw milk samples were collected weekly from 21 artisan cheese operations (6 organic), manufacturing raw milk cheese from cow (12), goat (5), or sheep (4) milk during the summer of 2008. Individual samples were examined for standard plate (SPC) coliform (CC), and somatic cell counts (SCC). Samples were also screened for *Listeria* spp., *Staphylococcus aureus*, *Salmonella* spp., and *Escherichia coli* O157:H7 by direct plating and PCR. Overall, 86% of samples had SPC <10,000 cfu/mL, with 42% <1000 cfu/mL. Additionally, 68% of samples tested were within pasteurized milk standards for coliform bacteria under the Pasteurized Milk Ordinance (PMO) at <10 cfu/mL. SPC and CC did not differ significantly between species. Similarly, method of sample delivery (shipped or pick up), farm type (organic or conventional) and duration of milking (year round or seasonal) did not have significant affects on farm aggregated mean SPC, CC or SCC. Strong positive correlations were observed between herd size and mean SPC and between SPC and CC as well as SCC when data from all species was combined. Although cows milk SCCs were significantly lower than those of goats and sheep, 98%, 71%, and 92% of cow, sheep, and goat milk samples were within the limits of the PMO for SCC, respectively. Fourteen of the 21 farms (67%) were positive for *S. aureus*, detected in 38% of samples at an average level of 20 cfu/mL. Neither *L. monocytogenes*, *E. coli* O157:H7 or *Salmonella* spp. were detected or recovered from any of the 101 samples tested. Our results indicate that most raw milk intended for small scale artisan cheesemaking was of high microbiological quality with no detectable target pathogens despite the repeat sampling of farms. These data will also help inform risk assessments which evaluate the microbiological safety of artisan and farmstead cheeses, particularly those manufactured from raw milk.

Key Words: raw milk, cheese, pathogen

T48 Effect of anhydrous milk fat, milk fat globular membrane and corn oil as the fat source in the AIN93 diet on the fecal microbiota in Fisher 344 rats. R. E. Ward*[1], D. Snow[1], R. Jimenez-Flores[2], and K. J. Hintze[1], [1]*Nutrition, Dietetics and Food Sciences, Utah State University*, *Logan*, [2]*Dairy Products Technology Center, Department of Agriculture, California Polytechnic State University*, *San Luis Obispo*.

The milk fat globular membrane (MFGM) is a complex biopolymer of membrane lipids (phospholipids, sphingolipids, plasmalogens, gangliosides) and glycoproteins which coats fat globules in fresh milk. It is hypothesized that this material may contain beneficial bioactive properties, but few studies have been conducted in this area. During butter production, substantial amounts of MFGM are produced by disruption of the native fat globule, and MFGM may be recovered from the buttermilk for use as an ingredient. To better understand potential benefits of MFGM, we conducted a feeding study comparing MFGM as a dietary fat source to anhydrous milk fat (AMF) and corn oil (CO) in Fisher 344 rats. MFGM was isolated from buttermilk and proximate, mineral and comprehensive lipid analyses were conducted. MFGM was subsequently incorporated into the AIN-93 rodent diet as the sole fat source. Between the experimental diets, there was no significant effect on consumption or weight gain, and MRI analysis of whole animals indicated no significant effect on body fat (13.6% CO, 14.4% AMF, 14.1% MFGM). Fecal samples were collected from each group (n=3) and subjected to comprehensive fecal microbiota profiling using a novel pyrosequencing method. On average, 1,920 sequences were identified in each fecal sample, totaling 526 unique isolates. At the phylum level, 62% of the sequences identified were Bacteroidetes, 29% were Firmicutes, and 5% were Verrucomicrobia. Animals fed AMF had the most diversity in their fecal microbiota, which was significantly different than the MFGM and control diets at the 95% confidence level.

Key Words: globule, membrane, microbiota

T49 Beneficial effects of bovine colostrum acid protein on bone properties of ovariectomized rats. M. Du*[1], L. Zhang[1], Z. Mu[2], H. Yi[1], and X. Han[1], [1]*Harbin Institute of Technology*, *Harbin, Heilongjiang, China*, [2]*Inner Mongolia Agricultural University*, *Hohhot, Inner Mongolia, China*.

Many reports have shown that bovine basic protein components, bovine colostrum and extracts of bovine colostrum have positive effects on bone growth of humans, such as increasing bone mineral density (BMD) and promoting calcium absorption. However, the effects of bovine colostrum acid protein components (BCAP) on properties of bone have not been reported. This study investigated the effect of BCAP on the prevention of bone loss in ovariectomized (OVX) rats. Forty-eight 3-month old female Sprague-Dawley rats were ovariectomized and another 12 rats underwent a sham operation (Sham). The OVX rats were randomly separated into four groups, i.e., OVX control, OVX low-dose (2 mg/d), OVX medium-dose (10 mg/d) and OVX high-dose (50 mg/d),

and were gavaged with BCAP once per day for 12 weeks. The effects of BCAP on bone mineral content (BMC), BMD, microarchitecture and biomechanical properties were determined. We found that BCAP increased the BMC and BMD of the femur. Moreover, BCAP acted in a dose-dependent manner. Observation of the distal femur by scanning electron microscope and mechanical testing further confirmed the positive effects of BCAP on bone properties. We conclude that BCAP (2~50 mg/d) could prevent osteoporosis caused by bone loss owing to estrogen reduction in OVX rats.

Key Words: biomechanical property, histomorphometry, bone mineral density

T50 Comparison of commercially available RNA extraction methods for effective bacterial RNA isolation from milk. S. Secchi[1], A. Serrano[2], P. García-Nogales[1], S. Gutiérrez[3], and A. Arís*[2], [1]*Applied Research using OMICS Sciences*, *Barcelona, Spain*, [2]*Institut de Recerca i Tecnologia Agroalimentàries*, *Barcelona, Spain*, [3]*Centre de Recerca i Investigació de Catalunya*, *Barcelona, Spain*.

The objective of this study was to compare four commercially–available RNA extraction kits for the purification of bacterial RNA isolated from culture and milk samples: the RNeasy Protect (Qiagen), the NucliSENS miniMAG (Biomerieux), the TRIzol (Invitrogen) and the RiboPure (Ambion). We compared the efficiency in terms of quantity and quality of extracted RNA for two bacteria species, a gram negative *Escherichia coli* and a gram positive *Staphylococcus aureus*. Strains were grown on culture media and all RNA extractions were performed from 1mL of culture at the middle log phase point (OD_{550}=0.5) with or without UHT milk. The amount of colony forming units at OD_{550}=0.5 was determined for both strains as $1.4x10^8$ and $2.8x10^8$cfuxmL^{-1} for *E.coli* and *S.aureus*. RNA extraction was performed by triplicate and quantified by OD_{260}. Yield differences among the kits and between milk and culture medium were submitted to ANOVA analysis. RNA purity and integrity was analyzed using the Experion Automated Electrophoresis system (Bio-Rad). Obtained RNA yields indicated no significant differences between milk or milk-free RNA extractions either for *E.coli* or *S.aureus* although there was a tendency (*P*=0.0873) with *S.aureus* and the Biomerieux kit for greater RNA extraction from milk than from culture (30.75 µg versus 7.85 µg respectively). Biomerieux and Qiagen kits produced the best yield results from all *S.aureus* samples, and the Ambion kit resulted in the lowest amounts (0.36 µg per extraction). For *E.coli*, the Qiagen kit provided the greatest RNA yield in culture media and milk (25.26 and 38.86 µg respectively). The greatest RNA purity was obtained in all cases with the Qiagen kit. The results obtained indicate that the Qiagen kit is the most suitable commercial method for RNA extraction from culture and milk samples of *E.coli* and *S.aureus* under the conditions described in this study.

Key Words: RNA extraction, bacteria, milk

T51 Effect of carbon dioxide on microbial growth in refrigerated raw milk. P. C. B. Vianna and M. L. Gigante*, *State University of Campinas*, *Campinas, SP, Brazil*.

UHT milk represents 74% of the fluid milk consumption in Brazil and the main problem that can affect its quality is age gelation, probably caused by indigenous enzymes and extracellular enzymes produced by psychrotrophic bacteria during the refrigerated storage of raw milk. The objective of this work was to evaluate the impact of carbon dioxide (CO_2) addition and the storage temperature on the microbial growth in refrigerated raw milk. Raw milk was divided into two portions: control milk (without CO_2 addition) and treated milk (added of CO_2 until pH 6.2). Each portion was subdivided in 300 ml glass bottles fitted with metal lids and stored at 4±1°C and 7±1°C. Samples were daily analyzed to standard plate count (SPC), psychrotrophic bacteria count and *Pseudomonas* spp. count. Analyses were performed until SPC had reached 7.5×10^5 ufc/ml, which corresponds to the microbiological standard for raw milk established by the Brazilian legislation. Split-split-plot design was used and the complete experiment was replicated two times. The treatments effects on SPC were evaluated by multivariate variance analysis. Gompertz model was used to evaluate the treatments effects on the development of psychrotrophic bacteria and *Pseudomonas* spp. by lag phase, growth rate and generation time parameters. CO_2 addition, temperature and storage time significantly affected the raw milk shelf life. Milk with CO_2 stored at 4°C took approximately 14 days to reach the SPC legal upper limit for raw milk, while milk without CO_2 took 12 days. Samples stored at 7°C took 8 and 6 days, with and without CO_2 addition, respectively. Independent of the storage temperature, CO_2 extended the lag phase, increased the generation time and decreased the growth rate of psychrotrophic bacteria and *Pseudomonas* spp. The addition of CO_2 effectively increased the shelf life and delayed the bacteria growth in raw milk and could be used to prevent quality problems in dairy products caused by extracellular enzymes produced mainly by psychrotrophic bacteria.

Key Words: carbon dioxide, milk shelf life, psychrotrophic bacteria

T52 Expression profile analysis of intestinal cells effected by *Lactobacillus acidophilus* NCFM. M. Wang[1], G. Zhang[1], L. Yao[1], Y. Zhou[1], L. Han[1], and Y. Jiang*[1,2], [1]*Key Lab of Dairy Science, Ministry of Education, Northeast Agricultural University*, *Harbin, China*, [2]*National Dairy Engineering & Technical Research Center, Northeast Agricultural University*, *Harbin, China*.

Lactic acid bacteria are a flock of microorganism which can make host cells healthy. However, further insights into the mechanism of action are needed to understand the rationale of their use. The objective of this study was to investigate the effect of *Lactobacillus acidophilus* NCFM, with the excellent biological characteristics and health function, on the gene expression profile of the human intestinal tract cell line Caco-2 by gene expression profile chip. Caco-2 cells were treated with *Lactobacillus acidophilus* NCFM (3×10^8cfu/ml) for 2h. The total RNA of them was extracted. Hybridization with gene expression profile chip was performed. The image scanning and data analysis were performed subsequently. The partial results of hybridization were validated by real time RT-PCR. The data showed that the expression levels of 508 genes were altered as compared with the control 473 of them were up-regulated, and 35 were down-regulated after *Lactobacillus acidophilus* NCFM treated Caco-2 cells. It was supposed that many genes in Caco-2 cells were induced, so that *Lactobacillus acidophilus* NCFM could play a part in immune response, antioxidant activity, bioadhesion, cholesterol absorption and so on. Four striking differential expression genes CCL2, PTX3, CXCR4 and TNFRSF9 which involved immune regulation system were validated by real time RT-PCR. And the results of real time RT-PCR showed the same expression trend as in gene chip. The differential expression genes in Caco-2 cell during the effect with *Lactobacillus acidophilus* NCFM can be found by gene expression profile chip. These genes in this study were likely to reveal the beneficial function and mechanism of this probiotic strain. *This work was supported by the Innovative Research Team Program of*

Northeast Agricultural University (CXT007-3-2), the Hi-Tech Research and Development Program of China (2008AA10Z311) and the Science and Technology Program of Heilongjiang Province (GB07B406).

Key Words: *Lactobacillus acidophilus* NCFM, Caco-2 cells, gene chip

T53 Development of a Multiplex-PCR detection assay for simultaneous identification of the major mastitis causing pathogens in dairy milk. B. Cressier*[1], C. Thibault[2], and N. Bissonnette[1,2], [1]*Université de Sherbrooke, Sherbrooke, QC, Canada*, [2]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada*.

Mastitis still remains the major disease affecting the dairy industry worldwide. Microbiological methods are still thought to be the gold standard for mastitis pathogen detection in Canada. Instead, using a molecular detection system would reduce the delays to identify the causative pathogen and allow proceeding with the adequate veterinary treatment. A molecular detection technique would show unambiguous results with an improved sensitivity. To detect species-specific genes, one predilection approach relies on Multiplex-PCR to amplify multiple loci each localized to a specific pathogen's genomic DNA. In this study, we challenged this method for detecting in milk the major mastitis causing bacteria in a single PCR reaction: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Mycoplasma bovis, Streptococcus uberis, Str. agalactiae, Str. dysgalactiae*. Gene specific DNA primers have been designed for each species and tested for optimal sensitivity and specificity and for the absence of primer-dimers generated by cross-reaction in a PCR reaction. The primers used are 5′-labeled with different fluorescent dyes allowing improved sensitivity by fragment analysis through a capillary electrophoresis system (Genetic Analyser). To challenge the molecular detection assay, we optimized an extraction assay for isolation of bacterial genomic DNA. The extraction assay proposed is convenient for all species and also greatly reduces carry over of milk component which could greatly inhibit molecular detection: The milk sample is clarified using a chelator, centrifuged and washed before a chelating resin is used to resuspend and boil the bacterial pellet. This technique has the benefit of being simple, quick and inexpensive; it can also be easily integrated into a high throughput scheme using 96 or 384-well plaques. Therefore, this extraction and detection protocol is then transposable for screening dairy cattle herds regularly and detecting mastitis pathogens preventively.

Key Words: mastitis, pathogen detection, multiplex PCR

T54 Nisin-inducible expression of recombinant peptides in dairy lactic acid bacteria. J. A. Renye and G. A. Somkuti*, *USDA-Agricultural Research Service, Wyndmoor, PA*.

In *Pediococcus acidilactici* a four gene operon (*papA-D*) controls expression of pediocin, a class IIa bacteriocin with antilisterial activity. Previously we confirmed that this operon functions in *Streptococcus thermophilus* and *Lactococcus lactis* and that expression could be tightly regulated using a nisin-inducible promoter. In this study, nisin-induced expression of the pediocin operon was tested in *Lactobacillus casei* C2. The vector pRSNPed was electrotransformed into *L. casei* and the presence of the plasmid was confirmed by PCR amplification of a DNA fragment containing the *nisA* promoter and *papA/B* (0.8 kb). The *L. casei* transformants produced pediocin and inhibited the growth of *Listeria monocytogenes* Scott A; however, pediocin expression was not tightly regulated as observed in *S. thermophilus* and *L. lactis*. In the absence of nisin 1,600 arbitrary units (AU) ml^{-1} of pediocin were produced, equaling the level of production observed in *L. lactis* and surpassing the level observed in *S. thermophilus* (400 AU ml^{-1}) following nisin induction. When induced with nisin, the level of production by *L. casei* increased to 3,200 AU ml^{-1} and resulted in the inhibition of a nisin-resisitant strain of *L. monocytogenes (NR30)*, which remained resistant to supernatants of the *L. lactis* and *S. thermophilus* transformants. In addition, the expression vector pRSNAHP was constructed, to test the nisin-inducible promoter for the overexpression of a recombinant *pap* operon with the pediocin structural gene replaced with a synthetic gene for a milk-derived antihypertensive (AH) peptide. The operon was designed so that the recombinant peptide would be fused in frame with the pediocin leader peptide to allow for secretion of the AH peptide. After the electrotransformation into *S. thermophilus*, *L. lactis* and *L. casei*, the presence of pRSAHP was confirmed by PCR amplification of a 0.3 kb DNA fragment containing the AH peptide gene and *papA/B*. Results of research currently in progress indicate that the *nisA* promoter may be used to regulate the expression and secretion of recombinant peptides fused to the pediocin leader peptide.

Key Words: LAB, pediocin, antihypertensive peptide

T55 Growth-promoting activities of bovine and caprine caseinomacropeptide. G. Robitaille*, R. Ioannoni, and C. Jolicoeur, *Food Research and Development Centre, Agriculture and Agri-Food Canada, St-Hyacinthe, QC, Canada*.

Caseinomacropeptide (CMP), is a 7 kDa polypeptide fragment released from κ-casein during chymosin-induced renneting of milk. Up to 50% bovine CMP (bCMP) were O-glycosylated. The extent of O-glycosylation of caprine CMP (cCMP) reaches less than 30%. N-acetyl and N-glycolyl neuraminic acid are the terminal sugars of oligosaccharides in goat specie in a proportion of 1:1, whereas N-acetyl neuraminic acid is the only amino sugar found on oligosaccharidic side chains linked to bCMP. In this study we analysed the effect of the addition of whole CMP, non-glycosylated CMP (aglyco-CMP), and glycosylated CMP (GMP) on the growth rate of 3 strains of probiotics: *L. rhamnosus* RW-9595, *L. plantarum* 299V, and *B. thermphiles* ssp *infantis* RBL 67. bCMP and cCMP preparations were fractioned by ion exchange chromatography to obtain aglyco-CMP and GMP having > 3.5 moles neuraminic acid / mole of GMP. Growth-promoting activities of CMP, aglyco-CMP, GMP, and bovine β-lactoglobulin were conducted in the basal medium described by Morishita et al. (1981) for lactobacilli, using turbidometric microplate assay under microaerophilic conditions. Supplementation of the growing medium with 0.5 to 2 mg/ml of bCMP and cCMP stimulated bacterial growth of *L. rhamnosus* RW-9595 and *B. thermphiles* ssp *infantis* RBL 67 ($P < 0.05$), compared to the medium without addition or the one supplemented with β-lactoglobulin. The growth rate improvement was related to the origin of the CMP ($P < 0.05$), about 1.3 and 1.1 times for bCMP and cCMP respectively, and was dose dependent. The addition of CMP to growth medium did not enhance *L. plantarum* 299V growth rate ($P > 0.1$) indicating that the growth-promoting effect of CMP was species specific. The growth-promoting activities of aglyco-CMP and GMP from bovine and caprine origin towards *L. rhamnosus* RW-9595 and *B. thermphiles* ssp *infantis* RBL 67 were similar ($P > 0.1$) suggesting that neither the presence of oligasaccharidic side chains nor the type of the neuraminic acid derivate was essential factors for the growth promoting activity of CMP.

Key Words: caseinomacropeptide, probiotic, bacterial growth

T56 Study of the genetic diversity of *Geotrichum candidum*. I. Alper* and S. Labrie, *Département des sciences des aliments et de nutrition, Centre de recherche en sciences et technologie du lait (STELA) – Institut des nutraceutiques et des aliments fonctionnels (INAF), Université Laval, Quebec, QC, Canada*.

Geotrichum candidum is a dimorphic yeast commonly inoculated on surface-ripened cheeses. The technological properties of *G. candidum* are strain-dependent, so analytical tools are vital for differentiating strains. The aim of the present study was to determine the genetic diversity of 16 *G. candidum* strains isolated from various environmental niches using ribotyping and random amplification of microsatellites by PCR (RAM-PCR). Ribotyping involves the sequencing of the operon coding for rDNA, especially the internal transcribed spacer regions, ITS1 and ITS2. We report, for the first time, the sequence of the rDNA operon of *G. candidum*. All the *G. candidum* strains were closely related phylogenetically and could be classified as members of de Hoog and Smith′s Group 1. While the strains could not be differentiated based solely on the sequence of the ITS1-5.8S-ITS2 region, seven could be differentiated by polymorphism analysis of the entire operon. Four RAM-PCR primers were tested, but only (GATA)4 resulted in PCR patterns that differentiated five strains. When combined, the two techniques made it possible to differentiate 10 of the 16 strains. These findings suggest that multilocus sequence typing, which is commonly used to differentiate strains of *Candida albicans*, a pathogenic yeast phylogenetically closer to *G. candidum*, may be an alternative method for studying the genetic diversity of *G. candidum* strains.

Key Words: *Geotrichum candidum*, rDNA operon, ribotyping

T57 Effect of somatic cell count on milk composition. R. Noorbakhsh*[1], A. Mortazavi[1], F. Shahidi[1], A. F. Mehdikhani[2], M. Ahoei[2], and A. Heravi Moussavi[2], [1]*Dept of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Khorasan, Iran*, [2]*Dept of Animal Science, Ferdowsi University of Mashhad, Mashhad, Khorasan, Iran*.

The study was designed to evaluate the effect of somatic cell count (SCC) on milk composition. In total, 127 dairy farms bulk milk were used during 2007 and 2008. The farms located in east northern of Iran. Somatic cell count and milk composition were measured in the bulk milk. The SCC data was divided into five categories based on the California Mastitis Test (CMT) scores and these categories, rather than the continuous variable from which they were derived, were used for studying the effect of SCC on milk composition. The range of leukocyte levels in different CMT scores were: N, 0-200000; T, 200000-400000; 1, 400000-1200000; 2, 1200000-5000000; and 3, >5000000 per ml. Due to low number of records for the last score the data were excluded before further analysis. The model for analyzing the effect of CMT scores on milk composition also included year and season. The data were analyzed using General Linear Models. The SCC averaged 590730 ± 12353 leukocytes per ml. The median was 454000 cells and 25 and 75% quartiles were 322000 and 713000 leukocytes per ml, respectively. The distribution analysis showed that 44.3% of the data were between 250000 and 499000 leukocytes per ml. The SCC was numerically reduced in year 2008 compare with 2007 which shows an improvement in milk quality ($p=0.23$; 616614±60316 and 575043±54308 cells/ml, respectively for 2007 and 2008). The effect of season was significant and SCC was greatest in winter compare with summer and autumn ($p<0.01$). Milk fat ($p<0.01$; 3.46, 3.54, 3.56, and 3.63 ± 0.04%, respectively for N, T, 1, and 2) and protein ($p=0.04$; 3.08, 3.06, 3.05, and 3.07 ± 0.01%, respectively for N, T, 1, and 2) contents were affected by the CMT score groups. The effect of season was significant ($p<0.01$). Milk lactose and solids-not-fat contents were similar among the groups. The results demonstrated that milk fat and protein contents were affected by SCC. Results also presented a trend in milk quality improvement over the years.

Key Words: dairy cows, somatic cell count, milk composition

T58 Impact of *Lactobacillus acidophilus* NCFM surface protein expression on its binding properties toward the milk fat globule membrane. G. Brisson, H. F. Payken, E. Pettey, and R. Jimenez-Flores*, *California Polytechnic State University, San Luis Obispo*.

Dairy products are commonly used as a delivery system for the probiotic lactic acid bacteria (LAB). Recent genomics studies have revealed the importance of the milk environment in the expression of LAB probiotic functions. However, to date little is know on how the dairy products positively affect these probiotic bacteria function. The milk fat globule membrane (MFGM) contains components that are known to bind to LAB cell surface such as glycoproteins (mucins) and phospholipids. On the counterpart, proteins expressed at the surface of the lactic acid bacteria could also affect the bacterial adhesion to MFGM. This work aims to elucidate the impact *Lactobacillus acidophilus* NCFM cell surface protein on its ability to bind to the MFGM. Five mutant strains with single gene deletion on genes encoding for different surface proteins were obtained from Dr. T. Klaenhammer's laboratory (NC State University). The binding properties of these *L. acidophilus* NCFM mutant strains were tested toward the MFGM components present in buttermilk powder. The binding frequency of the different strains was determined by means of a sucrose density gradient procedure coupled to bacterial DNA quantification. The bacteria cell surface was characterized by determining their surface hydrophobicity and their surface protein profile after 5 M LiCl treatment. The results showed that the binding ability of the different strains was influenced by the bacterial surface hydrophobicity and their surface protein profile. The wild type NCFM and 4 of the mutants showed similar binding patterns, and under statistical scrutiny low significant difference. However, the deletion mutant to the S-layer protein slpA, showed a remarkable different binding pattern. This mutant bound tightly (average of 98% of cells in the assay) to buttermilk components. Verification of the differences in binding patterns were made by confocal fluorescent microscopy. Further work will focus on identifying the binding elements in the bacteria and the MFGM.

Key Words: lactobacillus, MFGM, probiotic

T59 Acid tolerance of *Lactobacillus acidophilus* LA-K as influenced by various pulsed electric field conditions. O. Cueva[1] and K. Aryana*[2,1], [1]*Louisiana State University, Baton Rouge*, [2]*Louisiana State University Agricultural Center, Baton Rouge*.

Pulsed electric field (PEF) processing involves the application of pulses of voltage for less than one second to fluid foods placed between two electrodes. *Lactobacillus acidophilus* is an important probiotic bacterium used for the production of fermented dairy products. Objective of this study was to elucidate the influence of certain PEF conditions on the acid tolerance of *Lactobacillus acidophilus* LA-K. Freshly thawed *Lactobacillus acidophilus* LA-K was suspended in sterile peptone 0.1% w/v distilled water and treated in a pilot plant PEF system. The treatments were pulse width (3, 6 and 9 µs), pulse period (10,000; 20,000 and 30,000 µs) and voltage (5, 15 and 25 kV/cm). Control was run through PEF system at 60 mL/min without receiving any pulsed electric field condition. Acid tolerance was determined at 0, 5, 10 and

15 minutes of incubation. Data were analyzed using the PROC GLM of the Statistical Analysis Systems (SAS). Differences of least square means were used to determine significant differences at $P < 0.05$. The control and the three different bipolar pulse widths studied were significantly different from each other. The acid tolerance of the control was significantly the highest, followed by the acid tolerance subjected to 3 μs and 6 μs. The acid tolerance subjected to 9 μs was the lowest. The control and the three different pulse periods studied were significantly different from each other. The acid tolerance of the control was significantly the highest, followed by the acid tolerances subjected to the pulse period of 30,000 and 20,000 us. The acid tolerance subjected to 10,000 μs was significantly the lowest. The control and the three different voltages studied were significantly different from each other. The acid tolerance of the control was significantly the highest followed by the acid tolerances subjected to 5 and 15 kV/cm. The acid tolerance subjected to 25 kV/cm was significantly the lowest. Acid tolerance of *Lactobacillus acidophilus* LA-K lowered by increasing pulse widths and voltages but lowering pulse periods.

Key Words: probiotic, PEF, lactobacillus acidophilus

T60 Growth of *Lactobacillus acidophilus* LA-K as influenced by certain pulsed electric field conditions. O. Cueva[1] and K. Aryana*[2,1], [1]*Louisiana State University, Baton Rouge*, [2]*Louisiana State University Agricultural Center, Baton Rouge*.

Pulsed electric field (PEF) processing involves the application of pulses of voltage for less than one second to fluid products placed between two electrodes. *Lactobacillus acidophilus* is an important probiotic bacterium used for the production of fermented dairy products. Objective of this study was to elucidate the influence of certain PEF conditions on the bile tolerance of *Lactobacillus acidophilus* LA-K. Freshly thawed *Lactobacillus acidophilus* LA-K was suspended in 0.1% w/v sterile peptone water and treated in a pilot plant PEF system. The treatments were pulse width (3, 6 and 9 μs), pulse period (10,000; 20,000 and 30,000 μs) and voltage (5, 15 and 25 kV/cm). Control was run through PEF system at 60 mL/min without receiving any pulsed electric field condition. Growth was determined hourly for 16 hours of anaerobic incubation at 37°C. Data were analyzed using the PROC GLM of the Statistical Analysis Systems (SAS). Differences of least square means were used to determine significant differences at $P < 0.05$. Bipolar pulse width effect had a significant ($p < 0.0001$) influence on the growth. Growth curve of the control was significantly higher than the growth of *Lactobacillus acidophilus* subjected at any of the bipolar pulse widths studied. There were no significant differences in growth among the three different bipolar pulse widths. Pulse period had a significant ($p = 0.0017$) influence on the growth. There were no significant differences among the control, 30,000 μs and 20,000 μs. The growth of *Lactobacillus acidophilus* subjected to the pulse period of 10,000 μs was significantly lower than the growth of the control and the growth when subjected to 30,000 μs. Voltage had a significant ($p < 0.0001$) influence on the growth. Growth subjected to 15 and 25 kV/cm were significantly lower than the control and 5 kV/cm. There were no significant differences between the control and 5 kV/cm. There were no significant differences between the growths when *Lactobacillus acidophilus* was subjected to 15 and 25 kV/cm.

Key Words: probiotic, growth, PEF

T61 Stability of *Bifidobacterium animalis* ssp. *lactis* BB12 in yogurt smoothie developed for use in clinical trials with children. E. Furumoto, L. Weir*, and R. Roberts, *Department of Food Science, The Pennsylvania State University, University Park*.

The objectives of this work were to develop a yogurt-based smoothie capable of delivering 10^{10} cfu of *Bifidobacterium animalis* ssp. *lactis* BB12/100 ml serving and to evaluate survival of BB12 during refrigerated storage at 5°C. Yogurt smoothie was manufactured using a two step process. In step one, a yogurt mix was pasteurized, homogenized, heat-treated for 30 minutes at 85°C, cooled to 42°C, inoculated with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, incubated quiescently to a final pH of 4.6 then cooled to 25°C. In step two, a "stabilizer slurry" containing pectin, sugar and 36DE CSS was prepared, heated to 85°C, held for 30 minutes then cooled to 40°C. Yogurt, stabilizer slurry and strawberry puree were blended, homogenized, packaged and cooled. To determine the population of BB12 in the product, samples were diluted in sterile peptone water and pour plated using modified MRS agar containing dicloxacillin, LiCl, and cysteine hydrochloride followed by anaerobic incubation at 37°C for 48 h. Representative colonies counted as BB12 were picked and evaluated by PCR using *B. animalis* ssp. *lactis* specific primers to verify the selectivity of the plating method. In preliminary experiments two procedures for addition of BB12 (10^9 cfu/ml) to the yogurt smoothie were evaluated. In one procedure BB12 was added at the same time as the lactic starter culture (i.e. prior to fermentation) and in the second procedure BB12 was added after fermentation and cooling to 25°C. When BB12 was added prior to fermentation a pronounced "acetic acid flavor", probably due to metabolic activity of the bifidobacteria, was observed in the product which was deemed undesirable. Therefore the second method was chosen for manufacture of the intervention for use in clinical trials. Stability data for intervention manufactured monthly for 12 months revealed the population of BB12 declined slowly (average D-value 47.4±12 days) over storage at 5°C but remained at acceptable levels i.e. above 10^8 cfu/ml (10^{10} cfu per serving) for at least 30 days with no appreciable changes in viscosity, pH or flavor.

Key Words: *Bifidobacterium*, yogurt, probiotic

T62 Bile tolerance of *Lactobacillus acidophilus* LA-K as influenced by certain pulsed electric field conditions. O. Cueva[1] and K. Aryana*[2,1], [1]*Louisiana State University, Baton Rouge*, [2]*Louisiana State University Agricultural Center, Baton Rouge*.

Pulsed electric field (PEF) processing involves the application of pulses of voltage for less than one second to fluid products placed between two electrodes. *Lactobacillus acidophilus* is an important probiotic bacterium used for the production of fermented dairy products. Objective of this study was to elucidate the influence of certain PEF conditions on the bile tolerance of *Lactobacillus acidophilus* LA-K. Freshly thawed *Lactobacillus acidophilus* LA-K was suspended in 0.1% w/v sterile peptone water and treated in a pilot plant PEF system. The treatments were pulse width (3, 6 and 9 μs), pulse period (10,000; 20,000 and 30,000 μs) and voltage (5, 15 and 25 kV/cm). Control was run through PEF system at 60 mL/min without receiving any pulsed electric field condition. Bile tolerance was determined hourly for 16 hours. Data were analyzed using the PROC GLM of the Statistical Analysis Systems (SAS). Differences of least square means were used to determine significant differences at $P < 0.05$. Bipolar pulse width effect had a significant ($p < 0.0001$) influence on the bile tolerance. Bile tolerance of the control was significantly higher than the bile tolerance subjected to any of the bipolar pulse widths studied. There were no significant differences among the three different bipolar pulse widths. Pulse period had a significant ($p < 0.0001$) influence on the bile tolerance. The control

and the three different pulse periods studied were significantly different from each other. The bile tolerance of the control was significantly the highest, followed by the bile tolerances subjected to 30,000 μs and 20,000 μs respectively. The bile tolerance subjected to 10,000 μs was significantly the lowest. Voltage had a significant ($p < 0.0001$) influence on the bile tolerance. Bile tolerance of the control and bile tolerance of *Lactobacillus acidophilus* LA-K subjected to 5 kV/cm were significantly the highest while the bile tolerance when subjected to 25 kV/cm was significantly the lowest.

Key Words: probiotic, bile, PEF

T63 European Union Decision 2073/2005: A comparison between 3M Petrifilm *Enterobacteriaceae* and ISO 21528:2 in a milk powder production chain. M. Ferraz*, M. Cerqueira, and M. Souza, *Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil*.

The experiment was carried out in a dairy industry located in Minas Gerais, Brazil. The objectives were to evaluate the presence of *Enterobacteriaceae* in the milk powder production chain (raw milk, pasteurized milk, milk powder, handler and equipments swabs) and also to verify the influence of the pre-enrichment of samples for microbiological recovery. The analyses were made in seven periods, during three months. The pre-enrichment of the samples yield in a significant variation in the initial population of *Enterobacteriaceae* only in raw milk ($p < 0.05$). It was concluded that there was an equivalence between methodologies International Organization for Standardization (ISO) 21528:2 and 3M Petrifilm *Enterobacteriaceae* for all the samples, with and without pre-enrichment ($p > 0.05$). There was a significant difference in the counting of *Enterobacteriaceae* in the raw milk compared to the other points ($p < 0.001$). When comparing the samples between them, except for raw milk, there was statistical equivalence in the counting of *Enterobacteriaceae* ($p > 0.01$). This indicates that raw milk is the main source of *Enterobacteriaceae* and also that the process of this company is efficient in its reduction. The average counting in raw milk was 5,68log and 5.83log and in the milk powder 0.45log and 0.97log, respectively to 3M Petrifilm *Enterobacteriaceae* and ISO 21528:2. The results obtained in this study indicate that the 3M Petrifilm may be an alternative method to the ISO 21528:2 for enumeration of *Enterobacteriaceae*. Another conclusion is that the industry used in this study can be considered a potential exporter of milk powder to the European Union in terms of *Enterobacteriaceae* counting.

Key Words: *Enterobacteriaceae*, milk powder, Petrifilm

T64 Environmental scanning of bacteria with the potential to produce ropy milk in a farm. A. Laubscher*[1], K. White[1], A. Cano[1], R. Cano[2], and R. Jimenez-Flores[1], [1]*Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo*, [2]*Biological Sciences Department, California Polytechnic State University, San Luis Obispo*.

Prevention of microbial contamination in raw milk is an important objective in farms where value added is tied with quality. Recent reports in some regions of ropy milk have made us aware again of the reoccurring problem. Ropy milk is characterized by its viscosity and tendency to form a slimy thread, which causes rejection. The viscous character of the milk is produced by a complex oligosaccharide present in the capsule of different microorganisms. Over 250 raw milk samples were received from plants throughout the southern states. Isolates from 160 positive "ropy tests" were found to belong to the enterobacteracea family. Using API biochemical identification tests, over 80% of the positives are in the *Klebsiella* species. This result gives us the belief that the presence of ropy milk can be correlated to coliform counts. The objectives were to identify high-risk areas for contamination of the responsible bacteria, the correlation with coliform and *Escherichia coli* (E/C) counts, and development of a subjective or quantitative method to evaluate the risk of finding ropy milk producing bacteria. Ten locations were examined on the Cal Poly Dairy Farm, with bedding having the highest E/C counts and the most probable source for ropy-causing bacteria contamination on the farm. The threshold for the enumeration of ropy-causing bacteria was determined to be only 2.5 CFU/10 ml in a sterile milk sample was enough to turn the milk ropy. The threshold of the ropy-causing bacteria is much higher in the presence of typical raw milk microorganisms, suggesting a poor competitive nature. Our results indicate that "ropyness" is a result of poor-competing bacteria and poor sanitary conditions, in particular those associated with biofilm formation.

Key Words: milk quality, food safety, exopolysaccharide

T65 Influence of growth medium composition on survival and storage stability and viability of lactobacilli during freeze-drying. M. I. Tudor, E. P. Cuesta-Alonso*, and S. E. Gilliland, *Oklahoma State University, Stillwater*.

Lactic acid bacteria (LAB) play important role as starter and probiotic cultures in the production of fermented foods. Preservation technologies, like freeze drying, are required to guarantee long-term viability and functional activity of the cultures. However undesirable effects are observed since some starter cultures are not resistant to freezing and are not stable during storage. The objectives of this study are to evaluate the effect of growth medium composition and pH of growth of probiotic strains of *Lactobacillus* on survival after freeze-drying and subsequent refrigerated storage. Four strains of *Lactobacillus acidophilus* (O-16, 381-IL-28, L-1, L-23), two strains of *L. casei* (E-5 and E-10) and *L. reuteri* X-18 were grown in MRS broth supplemented with different concentrations of Tween 80 (0, 0.1, 0.2, 0.3, 0.4, and 0.5). In the study of influence of growth pH, the cultures were grown in a fermentor with controlled pH (4.5, 5.0, 5.5, and 6.0). The cultures were harvested by centrifugation and resuspended in 10% milk. The cultures were freeze dried, sealed under vacuum, and stored at 4°C. The bacterial counts before and after freeze-drying and during storage of 1, 7, 14 and 21 days were determined. Strains of *Lactobacillus* varied in viability after freeze-drying and refrigerated storage up to 21 days with viable counts of up to 1010 CFU/mL. Tween 80 improved survival rate in some but not in all strains after freeze-drying. Survival rate of 99.2% was obtained from *L. acidophilus* O-16 grown in 0.2% Tween 80. Higher concentrations (0.4 and 0.5) of Tween 80 resulted in decreased survival of L. casei. In general, survival rates of cultures were lower when grown in pH 4.5. The results suggest that Tween 80 and pH influenced survival of some LAB during freeze-drying and that it is possible to grow LAB with a higher number of viable cells to be used for the development of stable probiotic freeze-dried cultures.

Key Words: lactic acid bacteria, *Lactobacillus*, freeze drying

T66 Development of a sequence-based molecular subtyping method for *Bacillus cereus* dairy isolates. D. Miller*, S. Doores, and R. Roberts, *Pennsylvania State University, University Park*.

Bacillus cereus is recognized as a foodborne pathogen and as a spoilage microorganism associated with development of quality defects limiting the shelf life of pasteurized milk. When trying to determine routes of contamination by *B. cereus* during manufacture of dairy products it is important to be able to subtype strains obtained at the farm, in raw milk silos and in the finished product. Multilocus sequence typing (MLST) is a DNA sequence-based approach to subtyping that produces unequivocal and highly portable data. However, the cost associated with sequencing seven housekeeping genes, as is done in many MLST schemes, may limit the utility of this subtyping method for large-scale tracking studies. To reduce the number of genes that must be examined to discriminate between strains, hypervariable genes, such as virulence genes may be employed in a MLST scheme. The objective of this work was to develop a two- or three-gene MLST scheme for subtyping *B. cereus* dairy isolates based on a combination of housekeeping genes and virulence genes. To select virulence genes for inclusion in the MLST scheme, the incidence of nine virulence genes (*entFM*, *hblA*, *hblB*, *hblC*, *hblD*, *nheA*, *nheB*, *nheC*, and *sph*) was evaluated in 13 *B. cereus* reference strains and milk isolates Two virulence genes, *entFM* and *nheC*, were detected in all strains evaluated The number of sequence types obtained using various combinations of housekeeping and virulence gene sequences was evaluated and was found to range from five (*ccpA* and *pyrE*, *adk* or *recF* and *sucC*) to eight (any housekeeping gene and *nheC*, or *entFM* and *nheC*) for the two-gene MLST schemes. The addition of a third gene to the MLST scheme did not increase the number of sequence types. The two-gene MLST schemes could be used in studies to identify sources of *B. cereus* contamination during milk production and processing.

Key Words: *Bacillus cereus*, multilocus sequence typing, subtyping

T67 Confirmation of *Bacillus cereus* milk isolates using traditional microbiological and a recently developed molecular method. D. Miller*, S. Doores, and R. Roberts, *Pennsylvania State University*, *University Park*.

Bacillus cereus, recognized as a foodborne pathogen and as a spoilage microorganism associated with development of quality defects limiting the shelf life of pasteurized milk, is a member of the *Bacillus cereus* group which is composed of a cluster of six closely related species. Mannitol-egg yolk-polymyxin (MYP) agar is the AOAC-approved medium for isolation of *B. cereus* from milk; however other members of the *Bacillus cereus* group exhibit similar reactions on MYP agar. Thus, *B. cereus* must be further differentiated from these closely related species. The objective of this study was to evaluate application of a recently developed PCR-based method for confirmation of *B. cereus* on a set of 58 presumptive-positive *B. cereus* group members and 315 presumptive-negative colonies isolated on MYP agar as part of a broader survey evaluating the microbiological quality of retail pasteurized milk. Samples were characterized using both traditional microbiological and PCR-based methods. Using PCR-based methods, 54 of 58 presumptive *B. cereus* isolates (93%) were confirmed as members of the *B. cereus* group but only 17 (32%) were further confirmed as *B. cereus* using the PCR-based confirmation method. All of these isolates were also confirmed as *B. cereus* with traditional microbiological methods. Isolates confirmed as *B. cereus* group members but not *B. cereus* exhibited growth at 7°C and may have been *B. weihenstephanensis*. Four known *B. weihenstephanensis* strains were evaluated with the PCR-based confirmation method, and none yielded a positive reaction. When the 315 non-presumptive *B. cereus* group isolates recovered on MYP agar were screened using the *B. cereus* group-specific PCR-based method, all yielded negative results, and none were confirmed as members of the *B. cereus* group. The findings of this study suggest the PCR-based method developed by Choo et al. (2007) can be used as a reliable means for confirmation of *B. cereus* dairy isolates.

Key Words: *Bacillus cereus*, *Bacillus cereus* group, polymerase chain reaction

T68 Influence of the sample pre-heating and time for reanalysis in the Total Bacteria Count of milk by flow cytometry. L. Clementino[1,2], F. A. Pinto[1,2], L. M. Fonseca[1,2], J. F. Castro[1], R. Rodrigues[1,2], M. M. O. P. Cerqueira*[1,2], M. O. Leite[1,2], C. S. P. Fonseca[1], C. F. A. M. Penna[1,2], and M. R. Souza[1,2], [1]*Federal University of Minas Gerais (UFMG), School of Veterinary Medicine, Department of Food Technology and Inspection*, *Belo Horizonte, MG, Brazil*, [2]*Laboratory of Milk Quality Analysis*, *Belo Horizonte, MG, Brazil*.

Flow cytometry is a technique that is becoming widely used to measure microbial counting for milk quality evaluation. In Brazil, legal requirements approved the use of these equipments for measurement of microbial contamination in raw milk. It is necessary to know the factors that can affect the efficiency of this technique. The objective of this research was to evaluate the effects of sample temperature and the viability of sample reanalysis when carry-over contamination from a previous sample demands a new analysis. The experiment was done in the Laboratory of Milk Quality Analysis (School of Veterinary Medicine, Federal University of Minas Gerais, Brazil). For Total Bacteria Count the equipment Bactocount IBC (Bentley®) was used. To verify the effect of sample pre-heating, 126 samples of raw milk were randomly obtained from bulk tank milk in farms close to Belo Horizonte-MG, collected in 250mL sterilized flasks containing Azidiol(conservante), and sent to the Laboratory under adequate conditions. The samples were analyzed in experimental groups of 20, with the 19th and 20th samples used as control of respectively, temperature, and bacteria counting. Each sample was divided in four aliquots, each pre-heated at temperatures of 5°C, 15°C, 20°C or 40°C for 15 minutes. The statistical analysis was done by general linear model, after logarithmic transformation of the colony forming units (CFU). Comparison between treatments was made by Fisher Test. The results showed that there was no statistical difference ($p \geq 0.05$) between the experimental groups. To evaluate the feasibility of sample reanalysis when high carry over contamination occurs, 440 samples of raw milk were obtained and processed as above indicated. Each sample was divided in 50 mL aliquots and analyzed after 20, 40, and 60 minutes of storage at 25°C, with a total of 1,760 samples. The statistical analysis (Tukey Test) showed that the time of storage for samples which demand reanalysis did not affect the bacteria counting up to 60 minutes after storage at 25°C, since preserved by azidiol. *Acknowledgements: FUNDEP/UFMG; FAPEMIG; CNPq; CAPES.*

Key Words: Azidiol, flow cytometry, total bacteria count

T69 Methodology for differentiation of lactic acid bacteria in cheese made with probiotic adjunct cultures. C. J. Oberg*[1], L. Moyes[1], C. Brothersen[2], and D. J. McMahon[2], [1]*Microbiology Department, Weber State University*, *Ogden, UT*, [2]*Western Dairy Center, Utah State University*, *Logan*.

Many media have been developed to facilitate enumeration of lactic acid bacteria (LAB) with some media containing compounds or chemical adjustments. Defined strains of *Lactococcus*, *Lactobacillus*, and *Bifidobacterium* were screened on selective media designed to prevent growth

of competing LAB or to promote growth of certain LAB over others. Specific media were selected that gave the highest differential results, M17-Lactose for starter lactococci, MRS with added sorbitol for total LAB, either MRS or reinforced *Clostridium* agar (plus bromocresol green) with vancomycin for *Lb. casei* and *Lb. paracasei*, MRS with sorbitol as the sole sugar for *Lb. acidophilus*, and MRS supplemented with cysteine and an antibiotic cocktail (neomycin sulfate, nalidixic acid, lithium chloride, and paromomycin sulfate) for *Bifidobacterium*. Lactococci media was incubated aerobically at 30°C, *Lb. acidophilus* media was incubated at 45°C in a gaspak, with all other media incubated at 37°C in a gaspak. MRS plus sorbitol agar gave higher bacterial counts at all test periods even above *Lb. casei* specific media in aged cheese suggesting it could be used to obtain the total LAB count for cheese. Lactococci media must be analyzed at 24 h while all other media were analyzed at 48 h. Some strains of *Lb. casei* and *Lb. paracasei* appear able to grow on *Bifidobacterium* media making colony size exclusion an important consideration. Incubation time and temperature along with antibiotic concentrations are important when trying to suppress nonstarter (NS) LAB counts in aged cheese analysis. Even with these exact parameters, after 90 d, NSLABs appear in cheese flora on some selective media. It is important to test starter and adjunct cultures on selective media prior to cheese making trials as some lactobacilli can grow on other exclusionary media. Comparison of *Lb. casei* media to media for *Bifidobacterium* and *Lb. acidophilus* media during cheese aging can provide an indication of probiotic adjunct survival versus NSLAB growth. These media can provide an indication of probiotic adjunct survival in cheese but care must be taken in interpreting results particularly as cheese ages.

Key Words: probiotic, cheese, methodology

T70 Use of supercritical fluid extraction to remove non-polar lipids from whey buttermilk powder. M. R. Costa*[1,2], M. L. Gigante[2], and R. Jiménez-Flores[3], [1]*Universidade Norte do Paraná, Londrina, Paraná, Brazil*, [2]*Universidade Estadual de Campinas, Campinas, São Paulo, Brazil*, [3]*California Polytechnic State University, San Luis Obispo*.

The presence of glycoproteins and phospholipids in buttermilk makes this product unique in its function and reported health benefits. However, industrial production of ingredients containing high concentrations of milk fat globule membrane (MFGM) material is normally associated with high triglyceride concentration. Supercritical fluid extraction (SFE) has been utilized to extract lipids from foods, such as nuts and spices. More recently, our research group among others has used this technology on dairy products. The objective of this work was to evaluate the efficiency of the supercritical fluid extraction to remove the non-polar lipids from a whey buttermilk powder. A whey buttermilk powder was produced by ultrafiltration/diafiltration of whey buttermilk (membrane of 10 KDa molecular weight cutoff, at 25 °C) followed by spray-drying of the final retentate. The whey buttermilk powder was submitted to supercritical extraction (350 bar, 50 °C) using carbon dioxide. The powders, prior and after the supercritical extraction, had their gross composition, lipid profile by Thin Layer Chromatography (TLC), and phospholipids content by High Performance Liquid Chromatography (HPLC) evaluated. All the experiments were done in triplicate. The whey buttermilk powder prior the SFE had, in dry matter basis, 47.4, 47.3, 7.2, 3.0 and 2.3% of proteins, lipids, phospholipids, lactose and ash, respectively. The supercritical extraction removed approximately 34 g of lipids from each 100 g of sample, using 97 g of carbon dioxide to extract each gram of this fat. This extraction represented a reduction of 73% in the quantity of total lipids present originally in the powder sample. The process removed exclusively triglycerides from the powder matrix, as seen on the extracted lipids TLC profile. The proteins, lipids, phospholipids, lactose and ash contents in the final powder were 72.7, 20.6, 12.0, 3.5 and 3.2%, respectively.

Key Words: supercritical carbon dioxide, lipid profile, phospholipids

T71 Effect of pH and ionic strength on heat-induced deposition of whey proteins at the surface of fat droplets in oil-in-water emulsions. M. Britten* and S. Lamothe, *Food Research and Development Centre, Agriculture and Agri-Food Canada, St-Hyacinthe, QC, Canada*.

The increasing demand for food products with improved nutritional qualities stimulates the food industry to develop new techniques to encapsulate and protect bioactive ingredients. Whey proteins are commonly used in food products as emulsifiers to form a protecting membrane around fat droplets during emulsification process. Controlling the amount of protein adsorbed at the droplets surface (protein load) could then modulate the properties of the emulsion, such as oxidative stability, kinetics of flavor release or texture of rennet and acid gels. The objective of this study was to increase whey protein load of emulsions using heat-induced protein deposition. The effect of pH and ionic strength during thermal treatment was studied. An emulsion was prepared by mixing 20% w/w sunflower oil with whey protein dispersion (2.75%) adjusted to 6.8. Homogenization was performed at 3000 psi (2 passes) and 500 psi (1 pass). These conditions were chosen to produce fine emulsion with droplet size of ~300 nm. Emulsion were diluted (1:1, w/w) in phosphate buffer 30 mM to the desired pH (6.8 to 6.0) and ionic strength (0 or 25mM NaCl) and thermal treatment (80°C, 60 min) was applied. Protein load and physical stability measurements were performed. Heat treatment had only a slight effect on protein load, which increased from 2.4 to 2.6 mg/m^2. However, protein loads higher than 4.0 mg/m^2 were obtained with appropriate adjustment of pH and ionic strength. This corresponds to the adsorption at the droplets surface of more than 90% of the proteins in the initial dispersion. Heat-induced protein deposition was irreversible since the increase of the pH to the initial pH of 6.8 did not cause a significant protein load decline. Protein deposition slightly increased the mean diameter of emulsions (from 300 to 345 nm) and polydispersity index remained lower than 0.3, indicating that the treatment did not induce flocculation. These results suggested that appropriate adjustement of pH and ionic strength during heat treatment can be used to control whey protein deposition at the surface of fat droplets.

Key Words: whey proteins, protein load, emulsion

T72 The impact of antioxidant addition on flavor stability of Cheddar whey and whey protein. I. W. Liaw*[1], H. Eshpari[2], P. S. Tong[2], and M. A. Drake[1], [1]*North Carolina State University, Raleigh*, [2]*CalPoly University, San Luis Obispo, CA*.

Whey protein ingredients are widely used in food formulations. Off-flavors in whey products can carry through into ingredient applications and negatively affect consumer acceptance. The objectives of this study were to evaluate the impact of antioxidant addition in prevention of flavor deterioration of fluid whey and flavor of whey protein concentrate (WPC). Cheddar cheeses were manufactured in triplicate. Fresh whey was collected and pasteurized followed by fat separation. Three treatments, 0.05% w/w ascorbic acid, 0.5% w/w whey protein hydrolysate (WPH), and nitrogen flushing, were administered to the pasteurized whey. A control with no antioxidant addition was also

evaluated. Wheys were stored at 4C and evaluated after 0, 2, 4, 6, and 8 days. Whey flavors were documented by descriptive sensory analysis. Volatile components were evaluated by solid phase micro-extraction with gas chromatography mass spectrometry. Selected treatments were subsequently incorporated into liquid whey and processed into spray dried WPC. Cardboard flavors increased in fluid wheys with storage. Liquid wheys with ascorbic acid, WPH, or nitrogen flushing had lower cardboard flavor across storage compared to control whey. Lipid oxidation products, such as hexanal, heptanal, and nonanal, increased in liquid whey during storage, but liquid whey with added ascorbic acid, WPH, or nitrogen flushing had lower concentrations of these products. WPC with added ascorbic acid or WPH had lower cardboard flavor and lower concentrations of hexanal, heptanal, and nonanal compared to control WPC. WPC with added WPH, however, had a distinct potato flavor by sensory analysis which was absent in control WPC or WPC with added ascorbic acid. These results suggest that addition of an antioxidant to liquid whey prior to further processing may be beneficial to flavor of spray dried whey protein ingredients.

Key Words: whey protein, flavor, antioxidant

T73 Comparison of composition, sensory and volatile components of 80% whey protein and serum protein concentrates. J. P. Evans*[1], J. Zulewska[2], M. Newbold[2], D. M. Barbano[2], and M. A. Drake[1], [1]*North Carolina State University*, *Raleigh*, [2]*Cornell University*, *Ithaca, NY*.

Serum or 'native' whey protein concentrates (SPC) are whey proteins that are removed from milk prior to cheese-making. Since SPC are not exposed to the cheese make-process, enzymatic and/or chemical reactions that can lead to off-flavors are reduced. The objectives of this study were to identify and compare the composition, flavor, and volatile components of 80% protein SPC and WPC (SPC80, WPC80). SPC80 and WPC80 were manufactured in triplicate with each pair of serum and traditional whey protein manufactured from the same lot of milk. At each replication, spray-dried (SD) product from each protein source was collected. Commercial WPC80 were also collected for sensory and volatile analyses. A trained sensory panel documented the sensory profiles of the rehydrated powders. Volatile components were extracted by solid phase micro-extraction (SPME) with gas chromatography-mass spectrometry. Consumer acceptance testing was conducted with 6% protein acidic beverages made with SPC80 and WPC80, as well as commercial WPC80. SPC80 was lower in fat and had a higher pH than pilot plant manufactured WPC80 and commercial WPC80 ($p<0.05$). Few sensory differences were documented between the directly rehydrated SPC80 and WPC80 manufactured in this study, but their flavor profiles were distinct from flavor of rehydrated commercial WPC80 ($p<0.05$). WPC80 manufactured in this study generally had higher concentrations of lipid oxidation products than SPC80, and concentrations of lipid oxidation products in commercial WPC80 were generally higher than those concentrations ($p<0.05$). Trained panelists documented protein-associated flavors in acidic beverages that were not detected in reconstituted neutral pH protein solutions. Protein beverages made with SPC80 were not liked as well as protein beverages made with WPC80 manufactured in this study or one commercial WPC80 ($p<0.05$). These results suggest that composition, physical properties and volatile compound composition of SPC80 are distinct from WPC80. These differences may contribute to differences in flavor in low pH ingredient applications.

Key Words: serum protein, whey protein, flavor

T74 Production efficiency of a 95% serum protein (SP) reduced micellar casein concentrate (MCC) produced with ceramic microfiltration (MF) membranes. E. E. Hurt*[1], J. Zulewska[2], M. W. Newbold[1], and D. M. Barbano[1], [1]*Cornell University*, *Ithaca, NY*, [2]*University of Warmia and Mazury*, *Olsztyn, Poland*.

Four lots of pasteurized skim milk (72ºC, 15s) were microfiltered at 50°C [about 3X concentration] using a uniform-transmembrane-pressure bleed-and-feed process on a 0.1µm ceramic membrane. This was followed by 2 diafiltration steps, where the retentate was diluted 1:2 with reverse osmosis water. The MCC after the 3-stage process had a total solids of 10.8%, crude protein of 9.12% and fat of 0.37%. The L value (whiteness) of skim milk is typically about 74. Retentate whiteness increased by MF stage from 78.3 to 79.1 to 80.1. Whiteness of MCC from the 3rd stage was equivalent to milk containing 1.5% fat. The amount of SP removed from skim milk was determined by 2 methods. The 1st method used the mass of SP [(TN-NPN)*6.38] in permeate divided by the mass of SP [(NCN-NPN)*6.38] in skim milk where SP was determined using Kjeldahl. The 2nd method (SDS-PAGE) used SP (sum of the relative area of SP bands) in skim milk minus the same bands in retentate for each stage divided by the relative area of SP in skim milk. Theoretical (coefficient of rejection equals 0) SP removal is 68, 22 and 7% for the 1st, 2nd and 3rd stages, respectively with a total SP removal of 97% for a 3-stage 3X process. Based on the Kjeldahl method 67.15, 24.92, 12.24% of the SP was removed in the 1st, 2nd and 3rd stages, respectively for a total SP reduction of 104.31% in the present study. In a separate study, casein content of MF permeate was found to be as high as 0.03% and this would cause an overestimation of SP [(TN-NPN)*6.38] removal in the present study. If the amount of casein in the permeate was 0.01, 0.02 or 0.03%, then total SP reduction by the process described above would be 101.29, 98.26 and 95.21% respectively. According to SDS-PAGE analysis the SP removed was 68.5, 18.4 and 2.8% from the 1st, 2nd and 3rd stages, respectively with a total SP reduction of 89.7%. The 3-stage process using the conditions described above produced a 90 to 95% SP reduced MCC.

Key Words: microfiltration, micellar casein concentrate

T75 Functionality characterization of 65% and 95% serum protein (SP) reduced micellar casein concentrates (MCC): Concentration and drying effects. C. M. Beliciu*[1], J. Zulewska[2], M. Newbold[1], C. I. Moraru[1], and D. M. Barbano[1], [1]*Cornell University*, *Ithaca, NY*, [2]*University of Warmia and Mazury*, *Olsztyn, Poland*.

This work evaluated the influence of SP removal and drying method on the functionality of 65% and 95% SP reduced MCC obtained by microfiltration. For powders, bulk density, solubility, wettability, emulsification and foaming properties were determined. For liquid preparations (fresh retentates and reconstituted powders), rheological and coagulation behavior were evaluated. Drying methods had no impact on bulk density of 65% SP reduced MCC powders, but affected wettability, solubility, emulsion and foaming. The degree of SP removal had no influence on wettability, emulsification and foaming, but did impact bulk density and solubility for both MCC types. The drying method and degree of SP removal had no significant influence on the rheological properties of reconstituted MCC. Viscosity and yield stress (at 20°C) of liquid MCC increased exponentially with casein (CN) concentration. Above 10% CN, the liquid MCC became shear thinning. The viscosity of liquid MCC increased exponentially as temperature decreased, with temperature effects being more pronounced at high CN concentration. To evaluate the coagulation behavior of liquid MCC, rennet was added at a constant rennet:CN ratio. Coagulation dynamics was monitored

by the change in elastic modulus (G′). Maximum G′ was used as a measure of coagulum firmness. Coagulation rate was evaluated using the time constant of the Scott-Blair model. For both fresh retentates and reconstituted MCC the degree of SP removal did not affect coagulum firmness. Fresh retentates had significantly higher G′max (1351±75Pa) than reconstituted MCC (1062±83Pa). Coagulation rate was higher for 95% SP reduced as compared to 65% SP reduced MCC. Reconstituted 95% SP reduced MCC formed weaker coagulum than 65% SP reduced MCC, possibly due to a lower calcium content of the 95% SP reduced caused by the use of diafiltration in the manufacturing process. This study provides processors with useful information for the processing, storage and use of MCC.

Key Words: casein concentrate, rheology, coagulation

Food Safety

T76 A modeling system to predict *S.aureus* growth and SEA production in milk. F. Zhao, X. Qu, X. Lv, L. Xiang, B. Yan, and Y. Jiang*, *Northeast Agricultural University*, *Harbin, China*.

Staphylococcus aureus food poisoning often breaks out among patients who ingest dairy products. The main cause of this food poisoning was staphylococcal enterotoxin A (SEA). So it is an importance to predict *S.aureus* growth and SEA production in contaminated milk. The objective of this study is to provide a modeling system to predict the growth and SEA production of *S.aureus* in milk. Growth and SEA production of *S.aureus* 13565 in milk were studied at constant temperatures of 10-30 centi-degree. At each temperature, pure culture of strain 13565 was added to bacteriologically negative and SEA-free sterilized liquid milk to obtain an initial inoculum of 10^2-10^3 cfu/ml. The inoculated milk was then dispensed to sterile tubes and placed in a controlled temperature incubator. The incubation time was determined by temperature. After each incubation period, duplicate sample tubes were removed from incubator. Viable cell counts of samples were determined with the spread plate method (three plates per dilution). Averages and standard deviations of the transformed values were then calculated. SEA in samples was measured by VIDAS Staph Enterotoxin Test. The SEA concentration was determined by a standard curve developed using purified SEA in milk. The averages of two measurements were calculated for each data point. Growth curves can be described with the modified logistic model and the modified Gompertz model at high temperatures, such as 20-30 centi-degree, but the former described more accurately than the latter model. The amount of toxin in milk increased linearly with time from the time the cell population reached about $10^{6.4}$ cfu/ml. And the rate of toxin production increased linearly at these temperatures. The modeling system for *S.aureus* growth and SEA production has been roughly established in the study. The predictions of the system can be used as an indication, and also as reminder. *This work was supported by the National Key Technology R&D Program of China (2006BAD04A08-13) and the Key Project of Chinese Ministry of Education (208036). Corresponding Author: Dr. Yujun Jiang.*

Key Words: *Staphylococcus aureus*, enterotoxin, modeling system

T77 *Salmonella* serotype shift during an endemic dairy infection. J. Van Kessel* and J. Karns, *USDA-ARS*, *Beltsville, MD*.

Dairy farms are known reservoirs for *Salmonella* spp. and control of this organism is challenging. Salmonellae have been shown to be endemic in herds in part because they are easily spread between animals and throughout the farm environment. The impact of the infection on the herd is variable and dependent, in part, on the serotype. More than 2500 serotypes are known and animal carriers can be difficult to identify because they are often asymptomatic. As part of a multi-herd study, a dairy herd with an endemic, asymptomatic *Salmonella* infection was monitored extensively for 4 years. Bulk milk and in-line milk filters were collected weekly, and individual fecal samples were collected from all adult animals every 6 to 8 weeks. *Salmonella* detection was based on traditional culture methods. Prevalence of fecal *Salmonella* shedding in the 105 cow herd averaged 56% (range 10-95%) during this time. Although several *Salmonella* serotypes were identified over the course of the study, the initial dominant (>99% of isolates) serotype was Cerro. After 1.5 years into the outbreak, the serotype Kentucky began to gradually increase in prevalence, and at 2 years 39% of the fecal isolates were Kentucky. Over the next six months Kentucky became the dominant serotype representing >85% of fecal isolates. The serotype conversion from Cerro to Kentucky was first detected via analysis of the weekly milk filters. However, while monitoring of the milk filters was useful for detecting major shifts in serotype dominance, serotype prevalence in the individual milk filters was not predictive of the concurrent fecal serotype prevalence. This is the first detailed description of an endemic *Salmonella* infection in a dairy herd that undergoes a gradual shift from one serotype to another.

Key Words: *Salmonella*, dairy, serotype

T78 Determination of the mechanism(s) by which direct-fed microbials control *Escherichia coli* O157:H7 in cattle. L. M. Guillen*, S. McCoy, M. R. Bible, L. O. Burciaga-Robles, M. M. James, C. R. Krehbiel, and S. E. Gilliland, *Oklahoma State University*, *Stillwater*.

The objective of this experiment was to determine if immune enhancement may be responsible for the success of the direct-fed microbials, *Lactobacillus acidophilus* and *Propionibacterium freudenreichii*, in reducing the carriage of *Escherichia coli* O157:H7 in live cattle. To examine this, 10 steers were fed a pelleted growing diet ad libitum. Five steers received the direct-fed microbials (1 x 10^9 cfu) while the control group received only lactose (carrier). Day 0 baseline weights, fecal samples, rectal swabs, and blood samples were taken prior to the initiation of treatments. Treatments were fed once daily for 14 days after which they were transported to a bovine BSL-2 barn for *E. coli* O157:H7 inoculation and for the rest of the trial. All 10 steers were rectally inoculated with *E. coli* O157:H7 ATCC 43894 (10 ml of 2×10^7 cfu/ml) and housed in separate pens. Feeding and treatments were continued as before. After inoculation the previously mentioned samples were taken every 12 hours for 48 hours, then daily until 7 days post-inoculation, and then weekly until day 42. After the day 14 samples were taken the animals were reinoculated with a higher dose of *E. coli* O157:H7 (10 ml of 1×10^9 cfu/ml). Performance, immunological, and microbial plating data were analyzed using Proc Mixed ANOVA, repeated measures ANOVA, and Fischers exact test respectively. The dry matter intake was higher in the control group at weeks 4 ($p=0.03$) and 6 ($p=0.0004$). The feed:gain was higher in the control group at weeks 4 ($p=0.03$) and 6 ($p=0.0002$). Average daily gain for the control group was higher at week 4 ($p=0.04$). The immunological results of the trial showed higher levels of serum IgA, granulocytes, and monocytes as a percentage of