623 Impact of NaCl substitution with KCl on cell-wall extract and cell-free supernatant proteinase activities of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* and *Lactobacillus acidophilus* and *Lactobacillus casei* at different pH and salt levels. M. M. Ayyash[1], F. Sherkat[2], and N. P. Shah*[1,3], *[1]Victoria University, Melbourne, Vic, Australia, [2]RMIT University, Melbourne, Vic, Australia, [3]The University of Hong Kong, Pokfulam, Hong Kong.*

To reduce salt concentration in cheeses by substitution with KCl, it was important to study the effect of salt substitution on starter culture proteinases which play a vital role in ripening and texture profile of cheeses. The objective of this study was to examine the effect of NaCl substitution with KCl at different pH levels (6.0, 5.5, and 5.0) and salt concentrations on proteinases activity of cell-wall and supernatant of *Lactobacillus delbrueckii* ssp. *bulgaricus* 11824, *Streptococcus thermophilus* MS as well as of *Lactobacillus acidophilus* and *Lactobacillus casei* was investigated. MRS broths were mixed with 4 salt substitutions at 2 different concentrations (5% and 10%) and incubated individually at 37°C for 22 h. The collected cell pellets were used to prepare cell-wall proteinases and the cell-free supernatants were used as source of supernatant proteinases. The proteolytic activities and protein contents of both portions were determined. The supernatants after incubation of both portions with 3 milk caseins (α, β, kappa-casein) were subjected to ACE-inhibitory activity and proteolytic activity by OPA method. Significant differences were observed in ACE-inhibitory activity and OPA between salt substitutions of cell-wall extract and cell-free supernatant of LB and ST at same salt concentration and same pH level. The conclusion is that there were significant effects of pH and salt substitution on all measured variables.

Key Words: salt substitution, proteinase activity, ACE-inhibitory activity

624 Survival of microencapsulated probiotic *Lactobacillus paracasei* LBC-1e during manufacture of Mozzarella cheese and simulated gastric digestion. F. Ortakci,* J. R. Broadbent, W. R. McManus, and D. J. McMahon, *Western Dairy Center Department of Nutrition, Dietetics, and Food Science, Utah State University, Logan.*

An erythromycin resistant strain of probiotic *Lactobacillus paracasei* ssp. *paracasei* LBC-1 (LBC-1e) was added to part skim Mozzarella cheese in alginate microencapsulated or free form at a level of 10^8 and 10^7 cfu/g respectively. The objective of this study was to investigate the survival of LBC-1e and total lactic acid bacteria through the pasta filata process of cheese making where the cheese curd was heated to 55°C and stretched in 70°C-hot brine, followed by storage at 4°C for 6 wk and subjected to simulated gastric and intestinal digestion. This included incubation in 0.1 *M* and 0.01 *M* hydrochloric acid, 0.9 *M* phosphoric acid and a simulated intestinal juice consisting of pancreatin and bile salts in a pH 7.4 phosphate buffer. There were some reductions in both free and encapsulated LBC-1e during heating and stretching with encapsulated LBC-1e surviving slightly better. Changes in total lactic acid bacteria losses during heating and stretching did not reach statistical significance. During storage there was a decrease in total lactic acid bacteria but no statistically significant decrease in LBC-1e. Survival during gastric digestion in HCl was dependent on extent of neutralization of HCl by the cheese with more survival in the weaker acid in which pH increased to 4.4 after cheese addition. The alginate microcapsules did not provide any protection against the HCl. Interestingly, there was greater survival of the encapsulated LBC-1e during incubation in H_3PO_4. Proper selection of simulated gastric digestion media is important for predicting delivery of probiotic bacteria into the human intestinal tract. Neither free nor encapsulated LBC-1e was affected by incubation in pancreatin/bile solution. It was concluded that based on survival during simulated gastric digestion, the level of probiotic bacteria that would need to be added to cheese to provide a beneficial health benefit is lower than is generally assumed for other fermented dairy foods or when consumed as supplements.

Key Words: microencapsulation, probiotic, Mozzarella

625 Characterization of *Lactobacillus* sp. GF103 as potential probiotics in vitro. X. L. Dong[1], Q. Y. Diao*[1], N. F. Zhang[1], Y. Tu[1], M. Zhou[1,2], L. H. Zhao[1], and X. H. Gao[1], *[1]Key Laboratory of Feed Biotechnology of Ministry of Agriculture/Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Animal Science Xinjiang Agricultural University, Urumqi, China.*

Probiotics are living microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance. A potentially probiotic strain is expected to have several desirable properties to exert its beneficial effects. The purpose of this study was to characterize the strain *Lactobacillus* sp. GF103 as potential probiotics. The strain was identified as *Lactobacillus planturm* by 16S rRNA sequence analysis (GenBank accession number: JQ411248). It was further characterized for acid and bile salt resistance, simulated gastrointestinal fluid tolerance and antibacterial activity. Results showed that *Lactobacillus* sp. GF103 can fully resist to pH 3 and 0.3% bile salts. The percentage of bacteria viable count was 85% during 3 h under the conditions of the simulated gastric fluid. Viability was not affected during 3-h culture in the simulated intestinal fluid. Antibacterial activity found the strain has ability to inhibit the growth of 2 pathogens, *Escherichia coli* K88 and *Staphylococcus aureus*. The results suggest that the strain *Lactobacillus* sp. GF103 has potential probiotics properties. It can survive and propagate in animal intestinal tract as probiotics additives.

Key Words: probiotics, 16S rRNA, characterization

626 Microbial safety assessment of Juustoleipa cheese manufacture. B. Ganesan,* D. Irish, and C. Brothersen, *Western Dairy Center, Utah State University, Logan.*

Juustoleipa cheese is manufactured by direct acidification of milk and stored either refrigerated or frozen. The cheese has a shelf life ranging from 2 weeks to over several months depending on mode of storage. The cheese is cooked (either toasted or mildly sautéed) before use in foods, which is a post-manufacture step that helps destroy unwanted bacteria. However, even such heat treatments do not exclusively limit bacterial load. To understand microbial quality, spoilage-, and safety-related issues arising from Juustoleipa cheese-making, we hypothesized that survival of unwanted bacteria in Juustoleipa cheese is reduced by frozen storage, thereby altering its shelf life, quality and safety. The objective of the study was to understand how storage conditions and pre-storage baking of Juustoleipa cheese affect its microbial quality. Juustoleipa cheese was made in 3 replicates. *Escherichia coli* and *Pseudomonas fluorescens* were added to separate cheese portions at an initial level of 10^4 cfu/g cheese along with a control (bacteria not added). Bacterial addition was performed 30 min after salting and cheeses were further

pressed for 1.5 h and stored refrigerated or frozen with or without pre-storage cooking in a pizza oven (internal temperature of 82°C). We compared the survival of these bacteria by plate counts on selective media in refrigerated and frozen storage conditions that were sampled at a) 0, 14, and 28 d for refrigerated cheese and b) 0, 30, and 90 d for frozen cheese. Bacterial survival was reduced ($P < 0.05$) by 1,000 to 10,000-fold when samples were stored frozen, justifying our hypothesis. Pre-storage cooking of cheese reduced *E. coli* and *Pseudomonas* below detection by plate counts, but aerobic thermoduric bacteria that were incident during manufacture survived cooking at 82°C. As expected, microbial counts in cheeses that were not cooked were higher ($P < 0.05$) by 10- to 1000-fold compared with those in cooked samples. Characterization of the thermoduric bacteria is underway to comprehend their survival mechanisms. We concluded that pre-storage cooking and frozen storage reduce survival of but do not fully eliminate thermoduric bacteria in Juustoleipa cheese, which might affect its shelf life and microbial quality.

Key Words: Juustoleipa cheese, *E. coli*, *Pseudomonas*

627 Viability of probiotic bacteria and yeasts in traditional and commercial kefir following frozen storage. K. V. O'Brien,* C. A. Boeneke, K. J. Aryana, and W. Prinyawiwatkul, *Louisiana State University, Baton Rouge.*

Kefir is a fermented milk traditionally made from a unique starter culture, which consists of numerous bacteria and yeast species bound together in an exopolysaccharide matrix produced by certain lactic acid bacteria. Many health benefits are associated with traditionally produced kefir; however, bulging and leaking packaging, caused by secondary yeast fermentation during storage, has limited large-scale manufacture of traditionally produced kefir. Commercial kefir products have been designed to reduce these effects by using a pure starter culture consisting of a mixture of bacteria and yeast species that give a flavor similar to traditional kefir; however, some health benefits may be lost in commercial production such as reduced microbial diversity and lack of beneficial exopolysaccharides. The objective of this study was to examine the effects of various periods of frozen storage on the survival of probiotic species in traditional and commercial kefir. The traditional kefir was prepared by inoculating 1 L of pasteurized whole goat milk with approximately 30 g of kefir grains. Commercial kefir was prepared by inoculating 1 gallon of full fat, pasteurized goat milk with a commercial kefir starter (Lifeway Foods, Morton Grove, IL). The milk was allowed to ferment at room temperature (24–28°C) until pH 4.6 was reached. Samples were frozen (−8 to −14°C.) immediately following the completion of fermentation and were thawed and plated for lactobacilli, lactococci and yeasts on d 0, d 7, d 14 and d 30 of frozen storage. Statistical analysis was performed using the variance analysis (ANOVA) F-test ($P < 0.05$). Means were compared by the least squares difference (LSD) test. Lactobacilli, lactococci and yeasts were significantly reduced in number during frozen storage ($P < 0.05$); however, the traditionally produced kefir was shown to have significantly higher counts of bacteria and yeast at each sampling ($P < 0.05$). It was concluded that the development of frozen kefir products would eliminate most packaging concerns associated with large-scale production of traditionally produced kefir, resulting in increased manufacture and marketability of this healthful product.

Key Words: kefir, probiotics, frozen dairy

628 Probing the foaming characteristics of milk proteins. J. A. Stankey*[1] and J. A. Lucey[1,2], *[1]University of Wisconsin-Madison, Department of Food Science, Madison, [2]Wisconsin Center for Dairy Research, Madison.*

The strength of protein-protein interactions in foams gas-liquid interface dictate stability and formation of the dispersed gas phase. Molecular properties of proteins determine its overall foamability. Intermolecular interactions within and between casein can be modulated through the addition of salts, which affect the hydrophobic interactions. The Hofmeister salt (HS) series modifies hydrophobic interactions by strengthening (Na_2SO_4) or weakening (NaSCN) protein-protein interactions due to their preferential interactions with water or protein. Comparisons were made with the Hofmeister-neutral NaCl. This study evaluates the importance of hydrophobic interactions in milk protein foams by modifying casein interactions with HS. Mechanisms responsible for foam structure are of practical interest within the food industry. Foams were made from milk protein concentrate solutions (5% protein, w/w; pH 6.6) and HS were added (0.05–1.0 *M*) at 22°C. Protein solutions were whipped in a KitchenAid mixer for 5, 10, 15, or 20 min at the highest speed. Yield stress of foams was measured immediately after 20 min whipping using a rheometer with a vane attachment. Foam overrun and stability were measured at each time point. Foams made with high levels (≥0.5 *M*) HS exhibited salt-specific effects. Foams made with HS that decreased hydrophobic interactions (≥0.05 *M* NaSCN) formed quicker and were more voluminous, less stable, and weaker (yield stress) foams than the other treatments ($P < 0.05$). Foams made with HS that increased hydrophobic interactions (0.05–0.75 *M* Na_2SO_4) required longer time to foam, and produced denser, stronger, and overall more stable foams ($P < 0.05$). At high levels (1.0 *M*) of Na_2SO_4, foams formed slowly due to the high ionic strength and its salting-out effect on proteins. Overrun of foams made with NaCl decreased with increasing concentrations but stability and yield stress increased. Fluorescence microscopy was performed after whipping 20 min. Foams with 0.5 *M* NaSCN had fewer, larger air cells compared with foams made with 0.5 *M* NaCl or Na_2SO_4, which had smaller, more numerous air cells. Hydrophobic interactions between milk proteins at the gas-liquid play an important role in the strength and stability of foams.

Key Words: milk protein concentrate, foaming, Hofmeister series

629 The influence of Bactoscan total bacteria counting (TBC) and preliminary incubation (PI) counting on subsequent infrared milk component results. K. L. Wojciechowski and D. M. Barbano,* *Cornell University, Department of Food Science, Northeast Dairy Foods Research Center, Ithaca, NY.*

Our objective was to determine if sampling and sample handling for TBC and PI counting using a Bactoscan FC influences milk component test results by mid-infrared (IR) milk analysis. Raw milk was gravity separated for 22 h at 4°C. After 22 h, the lower 90% of milk was removed from the cone bottom gravity separation tank and then the cream layer was collected in 6 sequential fractions. The cream from the upper most fraction (highest bacteria count) was used to blend with a portion of the lower 90% (gravity skim about 2% fat) to produce milks with 3, 4, and 5% fat in addition to the gravity separated milk with no added cream. Milk at each fat concentration was mixed and split into 60 vials. If the removal of a test portion from a sample container by the auto-sampler of the Bactoscan when the milk was cold removed a non-representative portion of fat, then the effect of sampling on IR results would be expected to increase progressively with increasing fat concentration. The TBC count is done on fresh cold milk while the sample for the PI is incubated for 18 h at 14°C before testing. Within each fat level, 20 vials of milk

were tested by IR only, 20 vials by Bactoscan TBC followed by IR, and 20 vials by Bactoscan TBC and PI, followed by IR. This was replicated 3 times with a new batch of milk and the samples were tested using a different Bactoscan FC in a different laboratory in each replicate. Running TBC with a Bactoscan first followed by IR milk analysis had minimal effect (<0.01%) on milk component tests when milk bacteria counts were within Pasteurized Milk Ordinance limits <100,000 cfu/mL. For milks with bacteria counts of > 500,000 cfu/mL, there was an effect on the fat test results. Running PI counts had an effect ($P < 0.05$) on IR component tests. The effect on component testing by IR was due to microbial growth in the samples, not the physical process of sampling by the Bactoscan. The effect of microbial growth was the largest on fat tests. The direction and magnitude of change in component tests will vary depending on the bacteria count, the type of bacteria that grow and the compounds they product in the milk.

Key Words: milk payment testing, bacteria testing, Bactoscan

630 Protective action of serum amyloid A3 against *Salmonella* Dublin infection. A. Domènech*[1], A. ArÍs[1], A. Bach[1,2], and A. Serrano[1], *[1]Institut de Recerca i Tecnologia Agroalimentària (IRTA), Caldes de Montbui, Barcelona, Spain, [2]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.*

Milk is an important source of antimicrobial compounds. Serum amyloid A3 (SAA3) is an acute phase protein expressed at high levels in colostrum and may be an important candidate for the protection of calves against intestinal infections. Previous studies have explored its protective role in the gastrointestinal tract indicating that it inhibits the binding of enteropathogenic bacteria in cell cultures. However, the SAA3 potential at regulating the intestinal immune response has been poorly explored. The objective of this study was to evaluate the SAA3 effects in infected and non-infected tissues ex vivo, quantifying the associated immune response and bacterial internalization. A total of 28 punches of 8-mm diameter from jejunal bovine Peyer patches were cultured in 24-well plates with Krebs media and assigned to 2 different treatments: with or without recombinant SAA3 (n = 14 per treatment). Tissues were incubated for 3 h at 37°C and 5% CO2. After the first hour of incubation, jejune explants were challenged with 10^2 cfu/well of *Salmonella* Dublin (n = 20), according to previous optimization of the ex vivo enteropathogenic model, or incubated with media (n = 8). For cytokine quantification, total RNA was extracted from the tissue and IL-8 and INF-γ levels were quantified by qPCR. To determine internalized bacteria, tissue fragments were homogenized and viable cell counts were enumerated by colony counting after serial dilution plating. Data were analyzed using an ANOVA test. In non-infected tissues, pro-inflammatory IL-8 response increased ($P < 0.05$) with the presence of SAA3. Moreover, the SAA3 treatment tended ($P = 0.08$) to double the expression of IL-8, and increased ($P < 0.05$) the expression of INF-γ in infected tissues. Furthermore, SAA3 clearly decreased ($P < 0.05$) *Salmonella* Dublin infection ($5.1 \times 10^3 \pm 1.3 \times 10^3$ cfu/mL versus $1.6 \times 10^3 \pm 0.46 \times 10^3$ cfu/mL with SAA3). In conclusion, SAA3 enhances inflammatory immune response in the gastrointestinal tract and suggests a direct protective effect against intestinal infections such as that caused by *Salmonella* Dublin.

Key Words: SAA3, intestine, *Salmonella* Dublin