

Dairy Foods: Milk, Dairy Food Chemistry and Microbiology

W66 Inactivation of *Clostridium botulinum* type A neurotoxin in milk by high pressure processing. J. E. Schlessner^{*1}, R. Gerdes², G. E. Skinner¹, N. R. Reddy¹, and B. Parisi², ¹FDA, National Center for Food Safety, Summit, IL, ²Illinois Institute of Technology, National Center for Food Safety, Summit, IL.

The threat of terrorist attacks against the nation's food supplies has created the need to study food toxins that may be potential threats to the safety and security of the food supply. There is a need for the development of prevention and mitigation technologies/strategies to reduce this potential food defense threat. Non-thermal technologies such as high pressure processing have been proposed as a potential pasteurization method for milk. No data exist regarding the high pressure resistance of *C. botulinum* type A neurotoxin. The objective of the present work was to determine the high pressure resistance of *C. botulinum* type A neurotoxin in ultra-high temperature (UHT) 2% fat pasteurized milk. Samples of UHT 2% fat pasteurized milk were spiked with acid mud preparation of *C. botulinum* type A neurotoxin at a level of 10⁶ mouse lethal dose (MLD)/ ml. Spiked samples (10 ml each) were placed in high barrier film pouches, heat sealed, and pressure-treated in a Quintus Model QFP-6 High Pressure Food Processor at 400 or 600 MPa and an initial temperature of either 4 or 25°C for holding times of 3 min. After pressure treatment, samples were stored at 4°C until analysis. High pressure treated spiked samples were diluted with gel phosphate buffer and centrifuged. Supernatants were serially diluted with skim milk buffer and analyzed for presence of type A neurotoxin levels using the DIG-ELISA. Dilutions of 3:1, 1:3 and 3:37 of the last positive DIG-ELISA sample were further confirmed for type A neurotoxin by mouse bioassay. In general, the MLD determined by mouse bioassay was 10 times more sensitive than the MLD determined by DIG-ELISA. No inactivation of type A neurotoxin was observed in the untreated spiked samples by DIG-ELISA. A 3 log₁₀ MLD inactivation of type A neurotoxin was achieved in the UHT 2% fat milk samples when they were processed at a pressure of 600 MPa, initial temperature of 25°C for 3 min. High pressure denaturation of *C. botulinum* type A neurotoxin may result in a change of the structure and the toxin's activity.

Key Words: *Clostridium botulinum*, Type A Neurotoxin, High Pressure Processing

W67 Changes in fatty acid profiles of Awassi ewe and Damascus goat colostrums during ten days postpartum. Z. Guler^{*1}, M. Keskin¹, S. Gül¹, and Y. W. Park², ¹Mustafa Kemal University, Antakya, Hatay, Turkey, ²Fort Valley State University, Fort Valley, GA.

Awassi sheep and Damascus goats are the most productive small ruminants in the Mediterranean countries including Turkey, Syria, Lebanon, and Israel. The study was conducted to characterize fatty acid profiles of colostrum milks during ten days postpartum from randomly selected 10 animals each (3-yr old) of Damascus (Shami) goat and Awassi sheep at Research Farm of Mustafa Kemal University, Hatay, Turkey. Fatty acids in colostrums were determined using GC-MS (Model 6890/5973N, Agilent, Palo Alto, CA, USA) equipped with fused silica capillary column coated with free fatty acid phase. The major fatty acids in the ewe and goat colostrums were C18:1, C16:0, C18:0, C14:0 and C10:0, which accounted for about 75 % total fatty acids. Anteiso-C15 and anteiso-C17 were the prominent branched chain fatty acids (BCFA) in both species.

C18:3 acid was found only in ewe colostrum, while trans-C18:1 was detected only in goat colostrum. For the last two days of experimental period, cis-9-octadecenoic acid was predominant in goat colostrum milk, while hexadecanoic acid was high in ewe milk. The relative concentrations of fatty acids in colostrum milks of both species were unchanged during the 8 days postpartum. The average percentages (%) of short, medium and long chain fatty acids in both species colostrums were 4.94, 15.5, and 79.4, respectively. However, for the last two days, short chain acids remained unchanged, medium chain acids decreased from 15.6 to 12.3%, and long chain acids increased from 79.4 to 83.3% in goat milk, whereas ewe milk increased in short chain acids from 4.45 to 5.86% and medium chain acids from 14.8 to 19.5%, but decreased in long chain acids from 80.7 to 74.2%. These changes in fatty acid profiles in colostrum milks during the 10 days postpartum may indicate that there are certain differences in metabolism and secretory processes between the two species.

Key Words: Damascus Goat, Colostrum, Fatty Acids Profile

W68 Interaction between β -lactoglobulin and dextran sulfate at near neutral pH and their effect on thermal stability. B. Vardhanabhuti^{*1}, E. A. Foegeding¹, U. Yucel², and J. Coupland², ¹North Carolina State University, Raleigh, ²Pennsylvania State University, University Park.

Proteins and polysaccharide interactions play a major role in the structure and stability of many foods. Understanding their interactions and the effects on thermal stability could aid processors in product development. In this study, thermal stability of β -lactoglobulin (BLG) in the presence of dextran sulfate (DS) and their interactions were investigated. Samples containing 6% w/w BLG and DS (M_w 5k to 500k) at different biopolymer weight ratios, pH (5.6-6.2), and NaCl concentrations (0-30 mM) were heated at 85°C for 15 min. Thermal stability was evaluated by turbidity measurement. Protein stability was characterized by differential scanning calorimetry. Interactions between BLG and DS were characterized by size exclusion chromatography coupled with multi angle laser light scattering (SEC-MALLS) and zeta potential measurement. Turbidity results showed that the degree of BLG aggregation increased with decreasing pH. The presence of DS at appropriate molar ratios significantly lowered the turbidity of heated BLG ($P \leq 0.01$). Low M_w DS had broader range of effective weight ratios in decreasing the turbidity than the highest M_w DS. Data showed that DS lowered the denaturation temperature of BLG. Interaction between BLG and DS was confirmed by SEC-MALLS. Higher amount of BLG-DS complex formation was found with increasing amount of DS. Addition of 30 mM NaCl inhibited complex formation and the effect of DS on inhibiting aggregation, suggesting that the interaction was electrostatic driven. Zeta potential results revealed that samples with DS were more negatively charged compared to the BLG control. These results indicated that electrostatic interactions between BLG and DS led to a decrease in denaturation temperature of BLG, formation of higher negatively charged complex, and a different type of aggregates after heating. At appropriate conditions, DS improves thermal stability of BLG. The results provide information that will facilitate the application of whey proteins and polysaccharides as functional ingredients in foods and beverages.

Key Words: β -Lactoglobulin, Thermal Stability, Dextran Sulfate

W69 Using lactic acid bacteria to detect chemical substances in milk. A. AbuGhazaleh*¹ and S. Ibrahim², ¹*Southern Illinois University, Carbondale*, ²*North Carolina A&T University, Greensboro*.

The U.S. food system—from farms to processing and distribution to retail food service presents an array of vulnerable targets for terrorist attack. Intentional contamination of agricultural or food products with biological, chemical, or radiological agents could lead to potentially devastating effects on human health, as well as major economic losses to a critical sector of the economy. Therefore, there is an urgent need to develop a practical and sensitive approach to monitor food supplies, in case of a bioterrorist attack. The objective of this work was to determine the effect of cadmium and sodium cyanide on the growth of lactic acid bacteria in MRS broth. A total of 12 lactic acid bacteria were used in this study. A stock solution of each chemical was prepared and added to fresh batches of 10 mL MRS broth tubes to obtain final concentrations within the LD50 concentration assuming a 200 mL consumption (cadmium, 0.64 mg/mL; cyanide 7.0 mg/mL). MRS tubes were inoculated with individual strains, mixed and incubated at 37°C. Bacterial growth was monitored by measuring the optical density. At the end of the incubation period; the pH values of each sample were measured. Our results showed that at concentrations of 0.5 mg/mL and 1.4 mg/mL, cadmium and cyanide respectively, completely inhibited the growth of all 12 strains. The pH of those cultures that had strong inhibition remained relatively unchanged during the incubation period. These preliminary results suggest that typical dairy cultures could be a useful indicator of the presence of these chemical compounds. Further work will continue to expand the diversity of potential bioterror substances

Key Words: Lactic Acid Bacteria, Milk, Safety

W70 Development of symbiotic low fat buffalo milk yogurt. X. Han*² and M. Guo¹, ¹*University of Vermont, Burlington*, ²*Harbin Institute of Technology, Harbin, China*.

Buffalo milk represents an important animal product in South and East of Asia. As a specialty product, buffalo milk yogurt has become popular and has its own niche market in the US. In present study, symbiotic (containing both prebiotics and probiotics) low fat buffalo milk yogurt prototypes (plain and blueberry) were manufactured using 1% fat natural buffalo milk, inulin (a prebiotic ingredient), and probiotic cultures including *Bifidobacterium spp.*, *L. casei*, and *L. acidophilus* in a local commercial factory. Samples were analyzed for physicochemical and microbiological properties, and the survivability of probiotics during ten week storage. Gross composition results were: total solids 11.60 ± 0.58% and 17.12 ± 0.36%, ash 0.82 ± 0.06% and 0.78 ± 0.02, protein 4.49 ± 0.31% and 4.16 ± 0.11%, fat 0.68 ± 0.03% and 0.55 ± 0.05%, carbohydrates 5.68% and 11.38% for plain and blueberry flavors, respectively. Mineral contents were: calcium 1.97 ± 0.20 and 1.72 ± 0.06, magnesium 1.63 ± 0.02 and 1.69 ± 0.01, zinc 0.07 ± 0.01 and 0.07 ± 0.003, sodium 0.87 ± 0.15 and 0.94 ± 0.12 mg/g for the plain and blueberry flavored yogurt, respectively. The values of pH, titratable acidity and viscosity ranged from 4.34-4.01 and 4.42-3.70, 0.96-1.13 and 0.94-1.30%, 1.395-1.665 and 2.146-1.564 Pa.s for the plain and blueberry flavored yogurts, respectively. The initial population of *Bifidobacterium spp.* and *L. casei* were above 10⁸ cfu/g for both of plain and blueberry flavored yogurts. Both *bifidobacterium spp* and *L. casei* remained stable during the 10 weeks of storage (>10⁶cfu/g). However, population of *L. acidophilus* decreased sharply during the first a few

weeks and then dropped below 10⁶cfu/g. The results indicated that low fat buffalo milk may be a good vehicle for developing symbiotic yogurt. Improvement of the survivability of *L. acidophilus* in this matrix needs to be further studied.

Key Words: Buffalo Milk, Symbiotics, Yogurt

W71 Presence of geraniol in bovine milk following topical application as a natural insecticide. D. M. Watson*, J. P. Evans, R. E. Miracle, M. A. Drake, S. P. Washburn, and D. W. Watson, *North Carolina State University, Raleigh*.

Geraniol is a plant derived monoterpene found naturally in many plants with demonstrated insect repellency properties. Geraniol is listed as a minimal risk active ingredient exempt from the Federal registration requirements and this “generally recognized as safe” (GRAS) repellent could benefit organic or other producers wishing to reduce or eliminate reliance on insecticides. However, the effect of geraniol on milk flavor was not known. This study was conducted to determine if topical application of geraniol influenced milk flavor. Dairy cows at the Center for Environmental Farming Systems in Goldsboro, NC were divided into 4 groups of 3 cows each in 2 replicates: a control group (no geraniol treatment), and 3 treatment groups of topically applied 30% geraniol (60 mL geraniol; 120 mL geraniol; 180 mL geraniol) for 6 treatments at 2-wk intervals. Milk was collected from each cow at 8 h, and at 1, 3, 7, 10, and 13 d after each treatment. Milk from cows within each treatment group at each sample period was combined into a composite sample. Milks were analyzed for total solids non-fat (w/w) and percent fat using the CEM Smart Trac System. Headspace solid phase micro-extraction with gas chromatography mass spectrometry (SPME GC-MS) was used to screen for the presence of geraniol in the milks. The sensory threshold for geraniol in whole milk was determined using an ascending forced choice (AFC) method with 35 consumers. A trained sensory panel evaluated the flavor profile of vat-pasteurized whole milks. The instrumental detection limit for geraniol was 125 ppb (signal to noise ratio of 8:1) while the orthonasal sensory threshold in milk was 333 ppb. Geraniol was not detected instrumentally in any milk samples throughout testing. Similarly, trained panel flavor profiles of milk from geraniol-treated cows were not different from milk from control cows. Geraniol did not affect milk flavor when used as a topical insect repellent and may be viable for use with organic milk production.

Key Words: Geraniol, Pesticide, Flavor

W72 Comparative study of freeze-dried milk powder to spray-dried milk powder in water adsorption and stability. M. H. Seo*, S. H. Kim, J. Ahn, and H. S. Kwak, *Sejong University, Seoul, Korea*.

The present study was carried out to compare the stability such as water adsorption isotherms and solubility between freeze-dried milk powder and the spray-dried milk powder. Water adsorption isotherm of both freeze-dried and spray-dried milk powder samples were measured at 20, 30 and 40°C using COST-90 (Cooperation in the Field of Scientific Technical System-90) modified method. Results showed that isotherm were sigmoidal in shape in both milk powders. The adsorption isotherm of moisture content (g water/100 g dry solids) increased slowly in early stage and then increased sharply thereafter at all temperatures when

water activity changed from 0 to 1. The difference of moisture content between freeze-dried and spray-dried milk powders increased with the increase of the water activity. Especially, the moisture content difference was enhanced when the temperature increased. Equilibrium monolayer moisture content was in the range of 3.0 to 3.7 g H₂O/100 g dry solids in freeze-dried milk powder, while 0.4 to 1.7 g H₂O/100 g dry solids in the spray-dried milk powder at all temperatures. The composition of the freeze-dried milk powder was 3.4% in moisture, 41.7% in fat, and 26.8% in protein, which was comparable to that of the spray-dried milk powder. The solubility index and acidity were 0.02 mL and 0.11% in freeze-dried milk powder, and 0.17 mL and 0.19% in spray-dried milk powder, respectively. There was no difference in non-casein nitrogen (NCN) and non-protein nitrogen (NPN) contents. These results showed that the adsorption isotherm of moisture content was higher in freeze-dried milk powder than that in spray-dried milk powder, which may indicate the high stability of freeze-dried milk powder.

Key Words: Adsorption Isotherm, Freeze-Dried Milk Powder, Spray-Dried Milk Powder

W73 Comparative study of reconstituted milk made by freeze-dried milk powder to control reconstituted milk. S. I. Ahn*, S. H. Kim, J. Ahn, and H. S. Kwak, *Sejong University, Seoul, Korea.*

The present study was performed to compare the physico-chemical properties and nutrients of reconstituted milk made by freeze-dried milk powder to the control or spray-dried milk powder. There were three groups as follows: 1) control, raw milk, 2) freeze-dried, the reconstituted milk made by freeze-dried milk powder and 3) spray-dried, the reconstituted milk made by spray-dried milk powder. The freeze-dried powder was produced at 85°f using PVTFD-100 and contained 13% solid. In milk composition, freeze-dried group showed the little higher fat content and little lower moisture compared with those of the control. Spray-dried group showed much less moisture content than the control and freeze-dried group. Significant difference in L-value was found between the control and both reconstituted milks at 0 day period, however, the change was decreased throughout the period times. In addition, b-value of freeze-dried milk was significantly higher than others at 0 day. Total concentration of short chain fatty acids of freeze-dried reconstituted milk was significantly higher than others at every storage period. The concentrations of most water-soluble vitamins were lower in reconstituted milk made by spray-dried milk powder than in reconstituted milk made by freeze-dried milk powder when compared with the control. The present study indicated that there was no profound difference in physico-chemical properties in reconstituted milks made by freeze-dried and spray-dried milk powders, therefore, there is possibility of the dairy product manufacture using freeze-dried milk powder.

Key Words: Reconstituted Milk, Freeze-Dried Milk Powder, Spray-Dried Milk Powder

W74 Hysteresis of buffer capacity curves of cow, goat and sheep milks. J. Li*, M. Corredig, and A. Hill, *University of Guelph, Guelph, ON, Canada.*

Acid titration and buffer capacity measurements are simple tools that may yield information about ionic equilibria in milk and associated

changes to the micelle structure with implications for dairy technology, cheese texture and melting properties. Acid and base titrations and counter titrations show buffer capacity maxima near pH 2, near pH 4, in the range of 5.0 to 6.3, and near pH 12 for all species of milk. The colloidal calcium phosphate (CCP) maximum occurred at pH 5.1 to 5.2 during acid titration from native pH. Base titration following acid titration revealed a hysteresis effect for CCP. For minimum pH values during acid titration ranging from 5.4 to 4.8, the pH values for the CCP during subsequent base titration ranged from pH 6.5- 6.2 for cow and goat milk and 6.3-5.8 for sheep milk. Because the targeted minimum pH for most rennet coagulated varieties of cheese is near pH 5.0, the CCP peak is important for process control. Similarly, the hysteresis effect during base titration subsequent to acid titration may be important to pH control during the early stages of cheese ripening.

Key Words: Buffering Capacity, Milk, Hysteresis Effect

W75 Effects of yogurt fermentation bacteria on milk-based bioactive peptides. M. Paul and G. Somkuti*, *USDA-ARS-ERRC, Wyndmoor, PA.*

The incorporation of bioactive materials to increase the nutraceutical value of yogurt requires that these compounds survive all of the production processes, including proteolysis by endogenous enzymes. Since strains of the yogurt starter cultures *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) are known to have cell-associated peptidases involved in breaking down proteins to peptides transportable across cell membranes, this research focused on the sensitivity of selected bioactive peptides to ST and LB cells. The 11mer antimicrobial and 12mer hypotensive milk-protein based peptides were incubated with mid-log ST and LB cells at pH 4.5 and 7.0 to monitor the extent of proteolysis by membrane-bound peptidases, the presence of which was demonstrated by PCR analysis. Samples were removed at various time points and analyzed by reverse phase-high performance liquid chromatography (RP-HPLC). Results showed that the peptides were rapidly and extensively degraded by LB strains at both pH 4.5 and 7.0, while they remained intact at pH 4.5 in the presence of ST strains. The results suggested that the optimum time for the addition of these bioactive peptides is near the end of the yogurt making process when pH levels have dropped to 4.5 or lower, thereby limiting the extent of peptide loss caused by peptidase digestion.

Key Words: Lactic Acid Bacteria, Bioactive Peptides, Bacterial Peptidases

W76 Simplified petrifilm assay for lactococcus phage. Y. C. Tseng* and C. L. Hicks, *University of Kentucky, Lexington.*

Simplified procedures were developed to determine lactococcal phage plaques on specially prepared Petrifilm. To test this system *Lactococcus lactis* ssp. *lactis* C2 and c2 phages were used as a test culture/phage system and all films were compared to top/bottom gels in Petri plates. Phage plaques were viewed after 6 to 8 h of incubation at 30°C. M17 medium containing 1.0% fast hydrating cold set gelling agent, 0.01% FD&C Blue #1 dye and 15 mM CaCO₃ was prepared and weighed into vials (2.8 ml in each vial). In another vial 0.1 ml of *Lactococcus lactis* culture was combined with the appropriate dilution of phage (0.1 ml).

The two vials were mixed together and glucono- δ -lactone was added (final conc. 30 mM). The mixture then was poured into the well of the Petrifilm. Two types of Petrifilm prepared by 3M Corporation were tested. The first film was prepared by securing a clear film flap onto a second film. A 45mm ID o-ring (3 mm thick) was laid on the first film, and the gelling mixture was poured into the center of the o-ring. The well of second Petrifilm was prepared by stacking two expanded styrene films (2 mm thick) onto a clear film. The expanded styrene contained a 50 mm hole that formed the well, thus the well was 4 mm deep. After the gelling mixture was poured into the well, the top plastic sheet was lowered to seal the well. The development of large defined plaques required the use of a gelling agent that would form a soft non-weeping gel adhered well to the plastic film similar to the qualities of top agar. Soft gels allowed phage movement within the gel and the development of large plaques. A head space of 0.5 mm above the gel surface was required to prevent the top film from resting on the gel. If the film rested on the gel and the phage diffused across the surface of the gel, the whole lawn on the gel would plaque out. The use of a dye enhanced the contrast between the bacterial lawn and the clear plaque. Plaque size and counts were comparable to those determined using a standard top/bottom M17 medium agars in a Petri dish system.

Key Words: Simplified Assay, *Lactococcus*, Phage

W77 Construction of an integrative vector for recombinant gene expression in *Streptococcus thermophilus*. J. A. Renye* and G. A. Somkuti, *USDA-ARS-ERRC*.

Streptococcus thermophilus (ST) is an essential starter culture in yogurt and cheese production. Its “generally recognized as safe” (GRAS) status makes this organism an attractive candidate for the expression of gene products to improve the safety and nutritional value of dairy foods. The antibiotic resistance marker genes traditionally used in plasmid vectors may be eliminated by inserting heterologous genes into the bacterial chromosome. This pINTRS integrative vector was constructed with a temperature sensitive origin of replication and an erythromycin resistance marker gene (Erm) for initial selection in *S. thermophilus*. The green fluorescent protein (GFP) gene was transcriptionally controlled by a plasmid borne hsp promoter and cloned into pINTRS. The GFP gene was flanked by sequences homologous to an inactive ST pseudogene to facilitate integration within the locus. The pINTRS-GFP vector in *E. coli* DH5 α showed strong GFP expression at 32°C. In *S. thermophilus* the plasmid was maintained at 32°C but a temperature shift to either 37°C or 42°C resulted in plasmid loss. Weak GFP fluorescence was observed microscopically in individual cells of cultures grown at both low and high temperatures. Plasmid from cells grown at 32°C was back-transformed into *E. coli* yielding strong fluorescing colonies, suggesting that limited GFP expression in *S. thermophilus* is caused by low gene copy number. PCR analysis showed the *hsp-gfp* construct in colonies grown at 32°C and 37°C, but cells grown at the high temperatures were negative for the Erm gene suggesting that proper integration had occurred. The vector is also useful in testing the biological activity of recombinant products when expressed from single copy of a gene on the chromosome.

Key Words: *Streptococcus thermophilus*, Integrative Vector, GFP

W78 Fresh style panela cheese as a vehicle for probiotics and resistant starch. M. C. Escobar-Ramirez*¹, S. L. Amaya-Llano¹, M. Singh², and M. J. Miller³, ¹*PROPAC, Universidad Autónoma de Querétaro, Querétaro, Qro, Mexico*, ²*National Center for Agricultural Utilization Research, Peoria, IL*, ³*University of Illinois, Urbana*.

The importance of probiotic-containing products for maintaining health and well-being is becoming a key factor affecting consumer choice. In particular, there is interest in developing new probiotic containing products. Panela cheese, a Mexican fresh cheese, is a staple food in Mexico yet it lacks any probiotic microorganisms. As a potential vehicle for probiotics, panela has several advantages over yogurt including: 1) higher pH; 2) higher fat content; and 3) solid matrix. In addition, Panela cheese may also be a vehicle for resistant starch (RS) addition due to the potential for RS to complement the texture of traditional Panela cheese. The aim of this investigation was to manufacture and evaluate a Mexican fresh cheese (Panela) made with probiotic bacteria and resistant starch (RS). *Lactobacillus* spp. and *Bifidobacteria* spp. from our culture collection were screened to identify strains that could ferment RS from faba beans (*Vicia Faba* L.). None of the twenty *Lactobacillus* spp. examined were able to ferment RS. Of the 8 *Bifidobacterium* spp. examined, only *B. breve* ATCC 15700 were able to ferment RS. *B. breve* ATCC 15700 and *L. rhamnosus* GG ATCC 53103 were selected for use as probiotics. Two types of fresh cheese (with and without 3% RS) were made. Four combinations of probiotics was added to each of these cheeses: 1) no added cultures; 2) *L. rhamnosus* GG only; 3) *B. breve* only; and 4) both *L. rhamnosus* and *B. breve*. Viability analysis during 4 weeks of storage at 4°C demonstrated that the addition of RS had minimal effect on the viability of either probiotic culture. Scanning electron microscopy revealed that Panela cheese with RS has a more closed structure than traditional panela cheese. The addition of RS produced a greater softness in penetration test for cheeses samples. Panela cheese is an effective vehicle for delivery of probiotic microorganisms.

Key Words: Panela, Resistant Starch, Probiotic

W79 α -Ketoglutarate biosynthesis in *Lactococcus lactis* strains isolated from specific natural niches. N. Gutiérrez-Méndez, E. Valenzuela-Soto, A. F. González-Córdova, and B. Vallejo-Cordoba*, *Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD, A.C.), Hermosillo, Sonora, México*.

There has been an increased interest in the exploration of new strains of *L. lactis*, particularly those isolated from artisanal raw milk dairy products or vegetables since wild strains may produce large amounts of flavor compounds in dairy products. These compounds produced by *L. lactis* mainly derive from the amino acid catabolism, where the limiting factor is the α -ketoglutarate (α -KG) required in the transamination step. There are two main pathways in α -KG biosynthesis. One is the citrate-isocitrate pathway which needs the action of two key enzymes, the aconitase (AS) and the isocitrate dehydrogenase (IDH). The second pathway requires the enzyme glutamate dehydrogenase (GDH). Thus, the objective of this work was to explore both pathways involved in the biosynthesis of α -KG in strains of *L. lactis* isolated from different natural niches previously characterized for their capacity to produce aroma. The enzymatic activities (IDH, AS and GDH) of eighteen strains of *Lactococcus lactis* isolated from raw-milk cheeses, vegetables and commercial dairy starter cultures (DSC) were studied. Strains did not

present isocitrate dehydrogenase (IDH) and aconitase (AS) activities. Thus, it was concluded that *L. lactis* strains were not able to biosynthesize α -KG by the citrate-isocitrate pathway. On the other hand, half of the strains confirmed glutamate dehydrogenase (GDH) activity. Thus, the ability of *L. lactis* to synthesize α -KG via GDH was confirmed. Additionally, NADP-GDH activity was mainly found in strains isolated from vegetables, whereas NAD-GDH activity was mainly found in strains isolated from dairy products. These enzymatic activities may be related to the flavor production capacity of the different strains.

Key Words: *L. lactis*, Flavor Production, α -Ketoglutarate Biosynthesis

W80 Characterization of *Streptococcus thermophilus* isolates from traditional Turkish yogurts. N. Altay^{*1,2}, G. C. Gurakan¹, and J. L. Steele³, ¹Middle East Technical University, Ankara, Turkey, ²Selcuk University, Konya, Turkey, ³University of Wisconsin, Madison.

The objective of this study was to phenotypically and genotypically characterize *S. thermophilus* isolates from traditionally produced Turkish yogurts. By plating on LM17 agar, sixty putative *S. thermophilus* isolates were obtained from ten yogurts. The species designations of the Turkish isolates and four commercial isolates were confirmed by 16S sequencing. Fifty-six out of sixty Turkish isolates and all 4 commercial isolates were identified as *S. thermophilus*. Technological properties of the isolates were screened in sterile reconstituted skim milk. To determine the rate and extent of acid production, acidification profiles were examined. The twenty-six isolates having acidification rates comparable to commercial isolates (Δ pH at 4h \geq 1.3) were also tested for their final pH after 24 h incubation, acetaldehyde production and proteolytic activity. The final pH after 24 h incubation at 42°C was within the range of 3.76-4.09, which was comparable to the commercial isolates. The acetaldehyde production by the Turkish isolates was also comparable to commercial isolates and measured between 3.96-7.02 μ g acetaldehyde/ml using an acetaldehyde determination kit. Proteolytic activities of the isolates were examined by an OPA-based colorimetric assay and Δ Abs at 340nm were between 0.042-0.065. For genotypic characterization, CRISPR 1 locus was amplified and both leader and trailer ends were sequenced. Sixteen distinct amplicons were identified based upon amplicon size within the Turkish isolates. Based upon the sequence of the leader and trailer ends of the CRISPR 1 locus, the Turkish isolates were divided into 10 and 9 groups according to the first three spacers, respectively. The results demonstrate that significant phenotypic and genotypic diversity is present within *S. thermophilus* isolates from traditional Turkish yogurts and that these isolates may have commercial potential.

Key Words: *S. thermophilus*, Technological Properties, CRISPR

W81 Influence of encapsulated probiotic bacteria on the characteristics of plain yogurt. E. Noland and K. Aryana^{*}, Louisiana State University, Baton Rouge.

Encapsulation is a method of providing probiotic living cells with a physical barrier against adverse environmental conditions. *Lactobacillus acidophilus* is one of the very effective forms of probiotic bacteria and

is commercially available as pure culture (non encapsulated) and in an encapsulated form. In an attempt to use yogurt as a vehicle to successfully deliver large number of viable cells of *L. acidophilus*, it is not clear whether the use of encapsulated *L. acidophilus* will result in yogurt of a better quality compared to *L. acidophilus* in pure (non-encapsulated) form. The objective was to determine whether encapsulated *Lactobacillus acidophilus* R0052 altered the physico-chemical, microbiological and sensory characteristics of plain yogurt. Yogurt mixes were pasteurized, cooled to 37°C and inoculated with *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and encapsulated *L. acidophilus* R0052 or non encapsulated *L. acidophilus* R0052. Yogurt manufacture was replicated three times. Yogurts with encapsulated *L. acidophilus* R0052 had significantly ($p < 0.05$) higher flavor scores, significantly ($p < 0.05$) lower body and texture scores and significantly ($p < 0.05$) lower appearance scores compared to yogurts with non-encapsulated *L. acidophilus* R0052. Apparent viscosity, pH, syneresis, lactobacilli counts and L*, a* and b* values of the yogurts with encapsulated *L. acidophilus* R0052 were not significantly ($p < 0.05$) different from those of yogurts with non-encapsulated *L. acidophilus* R0052. Use of encapsulated *L. acidophilus* R0052 resulted in better tasting yogurts.

Key Words: Yogurt, Probiotic, Encapsulated

W82 Evaluation of buffering capacity of amino acid and milk protein ingredients in acidic conditions. V. Harrison^{*}, D Song, F. O. Uruakpa, C. W. Seo, and S. A. Ibrahim, North Carolina A&T State University, Greensboro.

The buffering capacity of amino acids and protein is an important physico-chemical characteristic that corresponds to the ability of the food products to be acidified or alkalinized. There is a considerable amount of data available concerning the buffering capacity (BC) of several substances, but very little pertaining to the BC of amino acids and milk protein ingredients. The objective of this study was to determine the BC of amino acids and various milk proteins ingredients in acidic conditions. Nineteen amino acids (asparagine, methionine, proline, cysteine, alanine, arginine, serine, glycine, valine, threonine, glutamic acid, isoleucine, tyrosine, leucine, phenylalanine, aspartic acid, tryptophan, and histidine) and seven different milk protein ingredients were tested for their ability to resist pH change when titrated with 0.1 M HCl. Testing solution was prepared by mixing 1.0 g of amino acid or milk protein ingredient powder in 100 ml deionized water. The pH of the solution was then adjusted to 7 using NaOH in solution. A standard solution of 0.1 M HCl was used to titrate the amino acid solution at 1.0 ml intervals using a bottle top burette until pH 2.0 was reached. The buffering capacity was calculated as the amount of acid required to reach pH 2.0 divided by the overall change in pH. Our results showed that protein ingredients such as hydrolyzed milk had high BC (>50). Glycine and aspartic acid had the highest BC values (33.3 and 34.5) while tyrosine had the lowest BC value of 5.0. It is evident that proteins and amino acids could be used in several food systems to maintain pH value. In addition, including such ingredients in food products with controlled buffering capacity would be desirable to maintain stable food characteristics.

Key Words: Buffering Capacity, Milk Proteins

W83 Use of beta-cyclodextrin to lower level of cholesterol in milk and its influence on activity of probiotic bacteria. L. Alonso^{1,2}, P. Cuesta^{*1}, J. Fontecha³, M. Juarez³, and S. E. Gilliland¹, ¹Oklahoma State University, Stillwater, ²Instituto de Productos Lacteos. CSIS, Asturias, Spain, ³Instituto del Frio. CSIC, Madrid, Spain.

While dairy products in general have the image of healthy foods, this image is often not perceived for products with a high fat content like butter, cream and certain type of cheeses. The World Health Organization, the American Heart Association and others have recommended that consumers reduce their consumption of saturated fatty acids and cholesterol as a deterrent to coronary heart disease. Thus there is a growing interest in the manufacture of cholesterol reduced dairy products. Currently, the most effective method for reducing cholesterol content in milk is by using beta-cyclodextrin (BCD). One objective of this study was to find the optimum conditions for cholesterol removal from raw milk at 4°C on an industrial scale by adding BCD in a specially designed bulk mixer tank for use in continuous recycling of the milk from a storage tank. Optimum conditions found for removing cholesterol was treating the milk with 0.6% BCD for 20 min followed by holding it 6 h. Profiles of triglycerides and fatty acids in control and treated milk did not show significant differences. Related experiments revealed the BCD does not adversely affect the growth of probiotic cultures of lactobacilli in a broth medium but did enhance their deconjugation of bile acid. Thus in addition to being able to remove cholesterol from milk the BCD may enhance control of serum cholesterol by consumption of a selected probiotic culture since bile salt deconjugation is one of the proposed mechanisms whereby the culture provides this benefit.

Key Words: Beta Cyclodextrin, Cholesterol, Lactobacilli

W84 Effect of prebiotics on probiotic growth curves and resulting pH changes in skim milk and a model system. D. Olson* and K. Aryana, Louisiana State University, Baton Rouge.

The effect of Raftiline[®] GR, Raftiline[®] HP-Gel, and Raftilose[®] P95 prebiotics on growth curves of the probiotics *Lactobacillus casei*-01 and *Lactobacillus acidophilus* LA-K and resulting pH changes in skim milk and a model system consisting of 0.1% peptone was investigated. Skim milk and 0.1% peptone each containing 1% Raftiline[®] GR, Raftiline[®] HP-Gel, or Raftilose[®] P95 prebiotics were autoclaved and then inoculated with 13.21 µL of either *Lactobacillus casei*-01 or *Lactobacillus acidophilus* LA-K per 100 mL of skim milk and 0.1% peptone. Controls containing the probiotics without the prebiotics and controls containing neither prebiotics nor probiotics were also prepared. Both the growth of each probiotic and the changes in pH were followed for 16 h at 37°C. The growth was determined by measuring the absorbance at 650 nm. The 0.1% peptone proved to be a more suitable medium than skim milk for preparing growth curves because of the high absorbance of the skim milk blanks. Absorbance readings for the 0.1% peptone controls containing neither prebiotics nor probiotics were very stable as they varied by less than 0.002 absorbance units in 16 h. The 0.1% peptone containing prebiotics had less change in absorbance over 16 h than their probiotic containing controls for *L. casei* but greater change than the corresponding controls for *L. acidophilus* suggesting that prebiotics stimulated growth of *L. acidophilus* but not *L. casei*. Skim milk was a better medium than 0.1% peptone for following pH changes due to greater buffering capacity of skim milk. The increase in the change in pH over 16 h for skim milk containing prebiotics compared with the

probiotic containing skim milk control was greater when using *L. acidophilus* instead of *L. casei* implying that prebiotics had a greater effect on *L. acidophilus* than on *L. casei*. Absorbance data obtained for 0.1% peptone and pH data obtained for skim milk implied that the prebiotics stimulated the growth of *L. acidophilus* more than *L. casei*.

Key Words: Prebiotics, Probiotic, Growth

W85 Evaluation of the effect of bovine colostrum on gene expression in *E. coli*. M. Worku*, Z. Liu, and S. Oh, North Carolina A&T State University, Greensboro.

Bovine colostrum-based immune milk products have proven efficacy in prophylaxis and treatment against various infectious diseases caused by pathogens such as *E. coli*. A proline-rich polypeptide (PRP) in colostrum has a regulatory effect on the immune response. The objective of this study was to determine the effect of commercial nutraceutical grade colostrum (high in PRP), on expression of *E. coli* K-12 heat shock protein genes using microarray analysis. Two *E. coli* K12 Starter V2 array chips, consisting of 2 identical grids, with a total of 192 spots (MWG Biotech, High Point) were used for expression profiling of *E. coli* and to conduct dye swap experiments. Data were acquired using Jaguar analysis software and preliminary analysis was conducted using the MicroArray Genome Imaging and Clustering Tool Version 1.0. Data were log transformed and the value for the negative control was subtracted. Expression ratios were used to evaluate the effect of colostrum treatment compared to a negative control on gene expression. The highest expressed genes on two slides have been selected for further characterization and validation. These genes are listed in the Kyoto Encyclopedia of Genes and Genomes, and include 1: Fused mannose-specific PTS enzymes: IIA component/IIB component [EC:2.7.1.69]; 2:50S ribosomal subunit protein L30 Signal Recognition Particle (SRP) component with 4.5S RNA (ffs); 3:F1 sector of membrane-bound ATP synthase, delta subunit [EC:3.6.3.14]; 4: conserved protein with nucleoside triphosphate hydrolase domain; 5: D-tagatose 1,6-bisphosphate aldolase 2, catalytic subunit [EC:4.1.2.40]; 6: NADH:ubiquinone oxidoreductase, membrane subunit L [EC:1.6.5.3]; 7:UDP-N-acetylmuramate-L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase [EC:6.3.2.-]. Further validation and characterization of the effect of colostrum components on these genes may provide keys to understanding *E. coli* pathogenesis and control.

Key Words: Colostrum, *E. coli*, Gene Expression

W86 Binding characterization between lactic acid bacteria and milk fat globule membrane in different dairy products. G. Brisson* and R. Jimenez-Flores, California Polytechnic State University, San Luis Obispo.

We have developed a combination of methods to characterize the binding between four different strains of lactic acid bacteria (LAB) and four products with high content of milk fat globule membrane (MFGM). This binding is important because probiotic bacteria are mostly consumed in dairy products and because there is genomic evidence that the interactions between these bacteria and milk fat globules are essential for turning on some genes involved with preservation and delivery of benefit to humans. However, more objective methods are necessary for evaluation of processes and strains that can better describe the relation-

ship between genomics and function in our dairy products. The method we have developed allowed assessing different LAB phenotypes and their interaction with MFGM-containing dairy products by means of a sugar gradient ultracentrifugation (SGU) fractionation step, fluorescent microscopy observations, and protein profiles. The dairy products we tested include: raw and pasteurized creams, buttermilk, and buttermilk powder. The DPTC culture strains (NCFM *L. acidophilus*; 33199 *L. gallinarum*; 1063-S *L. reuteri* and 53103 *L. rhamnosus*) were grown in normal MRS media. Harvested cells were washed 3 times with PBS, then left to contact the dairy product for 5 minutes prior to loading them on top of the ultracentrifuge tube with the sugar gradient. The sugar gradient was optimized between 25 and 50% sugar (w/v), and the ultracentrifugation conditions were 54,000g for 4 hrs at 4 °C. Different fractions were taken for microscopic observations. Also, characterization of the LAB surface proteins was achieved after using a 5M LiCl treatment and the extracts were analyzed by SDS-PAGE. The results of these analysis and the combination of microscopic observations and SDS-PAGE analysis indicate that genes regulating S-layer protein components in the surface of the LAB are the major determinant in binding frequency with the MFGM related dairy product.

Key Words: Lactic Acid Bacteria, MFGM, Binding

W87 Biophysical analysis of the milk fat globule membrane.

C. Cabral, T. Nelson, D. Rios, D. Gragson, and R. Jimenez-Flores*, *California Polytechnic State University, San Luis Obispo.*

We have used several modern surface characterization techniques to elucidate physicochemical properties of monolayer films derived from the components of the milk fat globule membrane (MFGM). Our work to date has focused on comparing the physicochemical behavior of the whole lipid fraction (polar and non-polar) with the non-polar lipid fraction of the MFGM extracted from commercial bovine buttermilk powder. We have gained information about film stability and elasticity using a Langmuir-Blodgett film balance, about film morphology on widely varying length scales using fluorescence microscopy and atomic force microscopy (AFM), and most recently about the formation and stability of bilayers with a quartz crystal microbalance (QCM). Additionally we have obtained mass spectral data of the polar lipid fraction detailing composition at the level of fatty acid chain length and saturation. Our results indicate that lipid domains form under appropriate temperatures and film pressures in monolayer films composed of MFGM lipid components analogous to lipid rafts in cell membranes. Further, we find that sphingomyelin and phosphatidylcholine both play a crucial role in forming these lipid domains. Our analysis give us great insight into the spatial distribution of lipids in MFGM monolayers and thus provide insight into the role each component plays in the native MFGM.

Key Words: MFGM, Phospholipids, Sphingomyelin

W88 Methods for screening ropy milk producers in raw milk samples. A. Cano, A. Laubscher*, and R. Jimenez-Flores, *California Polytechnic State University, San Luis Obispo.*

A recent concern about the presence of bacteria that can cause “ropy” milk has caused the examination of old testing methods to detect milk that can develop “ropyness”. Ropy milk is characterized by its viscosity

and its tendency to adhere to surfaces and form a slimy thread when the surfaces to which it is attached are pulled apart. The viscous character of the milk is produced by a complex oligosaccharide normally present in the form of a capsule around the bacteria. The objectives of this study were to survey the commercial supply of milk presenting the ropy milk defect with various traditional and molecular microbiological methods, and to give information on which microorganism or population of microorganisms are responsible for this defect. Over 200 raw milk samples were received from plants throughout the southern states. Samples are plated on TPC agar and incubated at 25°C for 36 hours to identify 5 types and obtain isolates. Isolated strains are tested for RM production by inoculation in sterile and UHT milk, which was then incubated at 25°C for 36 hours. The API method was used for bacterial identification of isolated strains which is based on a series of tests for sugar utilization that discern the genus and species. The “ropy test” proved to be successful with ropy isolates producing 100% reproducibility ropy milk and non-ropy isolates producing 100% non-ropy milk. We have established a database that classifies the isolated bacteria from milk on the basis of their gram stain, morphology, and exopolysaccharide capsule. Identification based on detailed biochemical analysis has indicated that the strains isolated belong to the coliform group of bacteria, with genus *Klebsiella* (*Klebsiella pneumoniae* and *Klebsiella oxitoca*).

Key Words: Milk Quality

W89 Effect of pH and reconstitution methods on heat stability of milk protein concentrate. V. Sikand*, B. L. Golden, and P. S. Tong, *Dairy Products Technology Center, San Luis Obispo, CA.*

The objective of the research was to determine the effect of pH and reconstitution methods on heat stability (HS) of 80% milk protein concentrate (MPC80). In the first series of experiments, we determined the effects of solvent type (water or permeate) used to reconstitute MPC80 on HS. MPC80 was reconstituted (RC) to 3.5% protein content with either water or permeate powder (PP) from skim milk ultrafiltration. To study the impact of homogenization, a second series of experiments were conducted in a pilot plant as previously described and subjected to homogenization treatments at 2000 and 500 psi in the first and second stages, respectively. The pH of the RC MPC80 in either series of experiments was adjusted to a pH varying from 6.6 to 7.0 and samples were tested for HS. Each experimental unit was replicated three times. The HS, defined as the heat-coagulation time (HCT), was measured as the time required for visible coagulation of the sample immersed in a 140°C oil bath. In the first experiments, HCT for MPC80 RC with water showed a steady increase from pH 6.6 to 7.0. HCT for MPC80 RC with permeate did not change significantly from pH 6.6 to 7.0. The least squares mean of HCT for MPC80 RC containing 3.5% protein with water was 7.96(± 0.64) minutes and with permeate was 5.06(± 0.78) minutes. In the second experiment, HCT for MPC80 RC with water showed a steady increase from pH 6.6 to 7.0. MPC80 RC with permeate first increased with increase in pH from 6.6 to 6.8 and then decreased with increase in pH of 6.9 - 7.0. MPC80 homogenized with permeate showed a significant increase in HS ($p < .001$). The least squares mean of HCT for homogenized MPC80 containing 3.5% protein RC with water was 7.48 (± 0.55) minutes and with permeate was 13.82(± 0.64) minutes. It was observed that permeate RC homogenized MPC samples are very similar to HCT- pH curves of skim milk sample. We suggest that the milk permeate can be exploited to improve HS (compared to water) of beverages containing MPC80 between natural pH of 6.7–6.8 when milk

components can be dispersed effectively (i.e. use of homogenization).

Key Words: MPC, Permeate, pH

W90 The concentration of lactoferrin in the bovine colostrum and immune milk. J. B. Cheng¹, J. Q. Wang*¹, D. P. Bu¹, G. L. Liu¹, C. G. Zhang^{1,2}, X. L. Dong^{1,2}, H. Y. Wei¹, L. Y. Zhou¹, and K. L. Liu¹, ¹*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China*, ²*College of Animal Science and Technology of Yangzhou University, Yangzhou, China*.

Lactoferrin (LF) is a key element in the host innate defense system, and the level of protection mainly depends on the concentration of LF in milk. In this study, changes of LF level in the immune milk were monitored and compared with that of colostrums. The colostrum from thirty-six Holstein cows was collected in this study. The concentration of LF in the milk was detected by Sandwich enzyme-linked immuno-

sorbent assay (ELISA). The relative concentration of them in colostrum was analyzed by Dodecyl sodium sulfate-gel electrophoresis of proteins (SDS-PAGE). The immune milk used in this study was made by implanted Antigen Release Devise. The data was analyzed with FIXED MODEL by SAS 9.0. It was indicated that the concentration of LF in colostrum had a dramatic change, especially in the first 24 hours after parturition, from 1.315 ± 1.086 mg/mL to 0.655 ± 0.377 mg/mL ($P < 0.05$), then another sharply decrease occurred on day 4, with the level of 0.264 ± 0.098 mg/mL, which had no difference to the LF concentration in the normal milk (0.209 ± 0.071 mg/mL, $P > 0.05$). SDS-PAGE results resembled the aforementioned trends. In addition, implanted Antigen Release Devise could increase the concentration of LF in the milk. On day 7 after implantation, the concentration of LF in the milk (0.313 ± 0.133 mg/mL) could be equal to the level of colostrum on day 3. This result might indicate that the concentration of LF in the colostrum and milk is influenced by parturition and immune stress, indicating LT might be an important part of bovine protection system.

Acknowledgement: Research funded by Ministry of Science and Technology (2006BAD12B03).

Key Words: Lactoferrin, Colostrum, Sandwich ELISA