W78 Viability of free and encapsulated *Lactobacillus acidophilus* ATCC 4356 in yogurt and artificial human gastric digestion system. F. Ortakci*[1,2] and S. Sert[2], [1]*Western Dairy Center Nutrition Dietetics and Food Sciences Department, Logan, UT,* [2]*Ataturk University, Erzurum, Turkey.*

The objective of this study was to determine the effect of encapsulation on survival of probiotic *Lactobacillus acidophilus* ATCC 4356 (4356) in yogurt and during gastric digestion. 4356 was added to yogurt in calcium-alginate microencapsulated or free form at a level of 8.26 and 9.47 log cfu/g respectively, and the influences of alginate microcapsules (1.5 to 2.5 mm) on sensorial characteristics of yogurts were also investigated. Survival of 4356 in simulated gastric and bile juices included incubation in 0.08 *N* hydrochloric acid (pH 1.5) containing 0.2% NaCl and a simulated bile juice consisting of 1.2% bile salts in MRS broth. There were similar and statistically significant ($P < 0.01$) reductions (~1 log cfu/g) in both free and encapsulated 4356 during 4 wk refrigerated storage of yogurts. When incubated for 2h in gastric juice, the free 4356 did not survive (>7 log cfu/g reduction). There was, however, greater survival of encapsulated 4356 with only a 3 log cfu/g reduction occurred. Incubation in simulated bile juice (6h) did not significantly affect ($P > 0.05$) the viability of both free and encapsulated 4356 due to the natural bile resistance of the bacteria. The addition of probiotic cultures either in free or alginate encapsulated forms did not significantly ($P > 0.05$) affect appearance and color, flavor and odor of the yogurts. There were, however, significant deficiencies ($P < 0.05$) in body and texture (graininess) of encapsulated 4356 containing yogurts. It was concluded that incorporation of free and encapsulated probiotic bacteria do not substantially change the overall sensory properties of yogurts and alginate microencapsulation using extrusion method greatly enhanced the survival of probiotic bacteria against artificial human gastric digestive system.

Key Words: probiotic, microencapsulation, artificial gastric system

W79 Complete genome sequence of *Bifidobacterium animalis* subspecies *lactis* BF-6. A. Baker[1], A. Negrete-Raymond[2], K. Polzin[1], M. Souza[2], Y. Yu*[3], J. Loquasto[3], J. Amos[3], and R. Roberts[3], [1]*Cargill Texturizing Solutions, Waukesha, WI,* [2]*Cargill Biotechnology Development Center, Navarre, MN,* [3]*The Pennsylvania State University, Department of Food Science, University Park.*

The primary objective of the present work was to sequence and evaluate the complete genome of *Bifidobacterium animalis* ssp. *lactis* BF-6, a common commercial probiotic originally isolated from the feces of a healthy human, and use this information to assess relatedness to other completely sequenced strains of the same subspecies. Genomic DNA was harvested and subjected to sequencing. Shotgun sequencing using 454 GSFlx Titanium pyrosequencing resulted in 20× coverage of the genome, which was assembled using Newbler into 24 contigs. The contigs were ordered and oriented with SeqMan software using the genome of *B. animalis* ssp. *lactis* DSM 10140 as a scaffold. After ordering, primers were designed on the ends of each contig and the intervening sequence was amplified by PCR. Amplicons were Sanger sequenced to close the gaps. For repeated elements, such as rRNA operons and transposons, long-PCR was conducted and the resulting amplicons were used as templates for additional nested PCR and sequencing reactions. The final genome was 1,938,607 base pairs (bp) in length with GC content of 60.48%. Annotation of the genome using the RAST and Artemis genome browser revealed the complete genome contains 1604 genes with a coding percentage of 85.9%, 4 complete rRNA operons and 52 tRNAs. Genome wide SNP analysis revealed BF-6 was highly similar to, but distinguishable from, other fully sequenced *B. animalis* ssp. *lactis* strains. The complete genome of *B. animalis* ssp. *lactis* BF-6 reported in this study provides additional insight into the phylogenetic organization of the *B. animalis* ssp. *lactis* taxon as well as into the biological and probiotic characteristics of this microorganism.

Key Words: *Bifidobacterium animalis* ssp. *lactis*, genome, SNP

W80 Growth of yogurt culture bacteria in the presence of two antimicrobials. M. Vives[1,2] and K. Aryana*[2,1], [1]*Louisiana State University,* [2]*Louisiana State University Agricultural Center.*

Antimicrobials such as potassium sorbate are added in the manufacture of dairy products such as flavored yogurts and process cheeses. Potassium nitrite and potassium metabisulfite have been reported to have an antimicrobial effect on pathogenic microorganisms such as *Clostridium botulinum, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* and *Pseudomonas fluorescens*. Potassium nitrite is commonly used in cured meats, canned cured meats, vacuum-packaged and fermented meats, bacon, cheese and seafood, while potassium metabisulfite is used in beverages and fruits. Yogurt is known for its health benefits, mainly due to the presence of its cultured bacteria. potassium nitrite and potassium metabisulfite are not commonly used in the dairy industry. How well the yogurt bacteria grow in the presence of 2 antimicrobials (potassium metabisulfite and potassium nitrite) at different concentrations is not well known. The objective was to study the influence of potassium nitrite and potassium metabisulfite at various concentrations, on the growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Different concentrations of potassium metabisulfite and potassium nitrite (100, 1000, 10,000, 100,000, and 1,000,000 ppm) were added to MRS broth previously inoculated with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* separately. Peptone water 0.1% w/v was inoculated with 1% (v/v) of freshly thawed *Lactobacillus bulgaricus* and *Streptococcus thermophilus* previously exposed to the different antimicrobial concentrations. The control did not have any concentration of either antimicrobial. Growth was determined by plating the different treatments and the control at 0, 24, 48 and 72 h of incubation of both microorganisms. Data were analyzed using Proc Mixed model of Statistical Analysis System with a repeated measures design. Three replications were conducted. All concentrations for both antimicrobials where significant for the growth of *Lactobacillus bulgaricus* LB-12 and *Streptococcus thermophilus* ST- M5 at 0, 24, 48 and 72 h ($P < 0.0001$). The highest concentration of potassium metabisulfite and potassium nitrite (1,000,000ppm) was significantly different from the rest of the lower concentrations, having the highest counts of bacterial populations for *Streptococcus thermophilus*. The effect of the antimicrobial at the highest concentration did not inhibit the growth of *Streptococcus thermophilus* at 72 h, but showed an exponential growth at this time period. Potassium metabisulfite and potassium nitrite did not negatively affect the growth of yogurt bacteria.

Key Words: yogurt, culture, antimicrobial

W81 Acquired resistance of yogurt culture bacteria to two different antimicrobials. M. Vives[1,2] and K. Aryana*[2,1], [1]*Louisiana State University,* [2]*Louisiana State University Agricultural Center.*

A bacterial strain can said to be resistant if it can survive and multiply itself in the presence of an antimicrobial agent that would normally inhibit or kill this particular kind of microorganism. Potassium nitrite and potassium metabisulfite have been reported to have an antimicrobial effect on pathogenic microorganisms such as *Clostridium botulinum, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* and *Pseudomonas fluorescens*. Potassium nitrite is commonly used in cured meats, canned cured meats, vacuum-packaged and fermented meats, bacon, cheese and seafood, while potassium metabisulfite is used in beverages and fruits. Antimicrobials such as potassium sorbate are added in the manufacturing of several products such as flavored yogurts and process cheeses. Antimicrobials such as potassium nitrite and potassium metabisulfite are not commonly used in the dairy industry. Yogurt is known for its health benefits, mainly due to the presence of its cultured bacteria. Whether yogurt bacteria can acquire resistance to 2 different antimicrobials (potassium metabisulfite and potassium nitrite) is not known. The objective was to study the possibility of an acquired resistance of these 2 bacterial strains to higher doses of the 2 antimicrobial compounds after prior exposure to lower doses of these antimicrobials. Treatments consisted of the transfer of previously grown cultures at the lower concentrations i.e., 100 and 1000 ppm of both antimicrobials, after 24, 48 and 72 h, into 2 higher concentrations 10,000 and 100,000 ppm, of both antimicrobials and growth was measured after 0, 24, 48 and 72 h of incubation. Data were analyzed using Proc Mixed model of Statistical Analysis System with a repeated measures design. Experiments were replicated 3 times. There was a significant interaction effect between the antimicrobial × microorganism × treatment × transfer hour × growth hour, on the growth of the bacterial strains ($P = 0.0015$). There were significantly higher counts ($P < 0.005$) of *Streptococcus thermophilus* compared with the control. For *Lactobacillus bulgaricus* the growth was similar to the control which suggests there are no significant differences between the growth at higher concentrations and the control. *Streptococcus thermophilus* exhibited resistance to the higher doses of antimicrobial compounds.

Key Words: resistance, yogurt culture, antimicrobial

W82 Isolation of an oligotrophic *Lactobacillus* species that may be associated with late gas production and splits in cheese. C. J. Oberg*[1,2], M. Culumber[1], T. Oberg[2], J. R. Broadbent[2], and D. J. McMahon[2], [1]*Department of Microbiology, Weber State University, Ogden, UT,* [2]*Western Dairy Center, Utah State University, Logan.*

A wide variety of facultative and obligate heterofermentative bacteria, including several lactobacilli, have been associated with late gas production in aged cheese. Such cheese can suffer from splits and slit defects during cheese storage, especially when storage temperature is increased to accelerate flavor development. The objective of this study was to identify bacteria in aged Cheddar cheese that causes late gas production. We isolated a novel heterofermentative *Lactobacillus* species (WDC04) following incubation on MRS agar at 6°C for 35 d. BLAST analysis against the 16S rRNA gene database of GenBank revealed WDC04 had 97% sequence identity with *Lactobacillus suebicus* strain CECT5917 (AJ575744), *Lactobacillus vaccinostercus* (AB218793), and an uncultured compost bacterial sequence (FN667177). Cellular morphology and colony morphology were consistent with related species. API CH50 fermentation panels showed a preference for utilization of ribose and galactose over other carbohydrate sources, and WDC04 is difficult to grow expect on MRS broth supplemented with galactose and ribose. As a nonstarter lactic acid bacteria (NSLAB), WDC04 ecologically falls into the category of being an oligotroph that undergoes slow growth in conditions of low nutrient availability. In contrast, lactococcal starter bacteria have copiotroph attributes and exhibit high growth rates when resources are abundant such as occurs in milk. In the harsh environment of ripening cheese (no residual lactose, low pH, low temperature and high salt concentration), viability of starter bacteria usually declines. At the same time, oligotrophic NSLABs utilize amino acids and bacterial debris to supply their energy needs, and will slowly increase in numbers until they are the predominant organism(s) of aged cheese microflora. Gas production by WDC04 was observed at 5 d in MRS broth incubated at 25°C with significant gas production by 9 d. Gas production was also observed after incubation for 28 d in MRS at 8°C. It was concluded that WDC04 is a potential cause of gas production during storage of cheddar cheese.

Key Words: cheese, nonstarter, heterofermentative

W83 Influence of various health beneficial spices on the acid tolerance of *Streptococcus thermophilus* ST-M5. M. Sanchez-Vega*[1,2] and K. Aryana[2,1], [1]*Louisiana State University,* [2]*Louisiana State University Agricultural Center.*

There is a great deal of public interest in the use of herbal remedies. Garlic is said to be antibacterial, antiviral, and antifungal and also prevent cardiovascular diseases and some types of cancer. Ginger is effective against nausea and cardiovascular diseases and is also an analgesic and has antibacterial properties. Onion is effective against the common cold and reduces the risk of developing diabetes; it also has antiinflammatory, anticholesterol, anticancer and antioxidant properties. Many studies have been conducted using spice extracts, but the effect of pure spice juice has not been studied. According to some studies, spices might have the capacity of enhancing the growth of certain probiotic strains while acting as a bactericide for harmful bacteria. The main objective of this study was to elucidate the influence of garlic, ginger, and onion on the acid tolerance of *Streptococcus thermophilus* ST-M5. Freshly thawed culture was inoculated in acidified MRS broth at pH 2 and 1% v/v of freshly extracted spice juice was added. Control was without spice juice. Growth was determined hourly during 2 h of incubation at 37°C. The data were analyzed using Proc Mixed model with a Tukey adjustment of Statistical Analysis System. Experiments were replicated 3 times. All 3 spices showed a significant ($P < 0.05$) increase in counts at 0 h of incubation when compared with control. After 1 h of incubation, all 3 spices had significantly ($P < 0.05$) higher counts than control. After 2 h of incubation, ginger showed no significant ($P > 0.05$) difference compared with control (5.5 log cfu/mL), while there were slight yet significantly lower counts for garlic (4.3 log cfu/mL) and onion (5.1 log cfu/mL). Among the spices, ginger had the best overall effect. These 3 spices can be used with *Streptococcus thermophilus* enabling health benefits from both sources.

Key Words: spice, culture, acid tolerance

W84 Bile tolerance of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 subjected to mild sonication intensities at different temperatures. M. Moncada*[1,2] and K. Aryana[2,1], [1]*Louisiana State University,* [2]*Louisiana State University Agricultural Center.*

Low sonication intensity is a non-destructive technique that consists on the application of low energy, high frequency (1–10 MHz) and power intensities below 1 W/cm2. It has been reported that the survival of lactic acid bacteria is increased with the application of "mild" sonication

condition. One of the requirements for a bacterium to be called probiotic is the ability to survive the gastrointestinal stress factors such as acid and bile conditions. *Lactobacillus bulgaricus* is widely used in the fermentation of dairy products. The objective of this study was to determine the effect of various mild sonication intensities at different temperatures on bile tolerance of *Lactobacillus bulgaricus* LB-12. Freshly thawed *Lactobacillus bulgaricus* LB-12 culture was suspended in 0.1% peptone water and sonicated using a 13 mm diameter probe set at a maximum acoustic power output of 750 W, frequency 24 kHz. Before sonication, the inoculated samples were set at 3 temperatures (4, 22, and 40°C). Four sonication treatments with intensities of 8.07, 14.68, 19.83 and 23.55 W/cm^2 were performed in a random manner at the 3 different temperatures mentioned above. Control samples did not receive any sonication treatment. Bile tolerance of samples was determined every 2 h for 12 h of incubation. Data were analyzed using ANOVA of Statistical Analysis System. Three replications were conducted. At 4°C, bile tolerance of cultures subjected to control and 14.68 W/cm^2 was significantly ($P <$ 0.05) higher compared with the rest of mild sonicated intensities. Bile tolerance at 22°C was significantly ($P < 0.05$) higher than at 4 and 40°C during the 12 h of incubation. Log reduction at 22 and 40°C showed that samples treated with mild sonication intensities had higher bacterial growth than the control. It is concluded that certain mild sonication conditions improved bile tolerance of *Lactobacillus bulgaricus* LB-12.

Key Words: sonication, culture

W85 A new approach to make milk calibration standards for electronic somatic cell counters. J. Podoll, D. M. Barbano,* and K. L. Wojciechowski, *Cornell University, Department of Food Science, Northeast Dairy Foods Research Center, Ithaca, NY.*

Our objective was to develop a procedure to make milk somatic cell count (SCC) reference materials for calibration of electronic somatic cell counters (ESCC). The key innovation was the use gravity separation to produce a naturally occurring high and low SCC milk. One batch of whole raw milk was separated into 2 portions. One portion was gravity separated at 4°C for 24 h and the second portion was centrifugally separated at 4°C to produce raw skim milk that was also gravity separated. The somatic cells concentrated at the top of both gravity separation tanks. Milk was collected from the top and the bottom of the tanks to produce a high and low SCC milk from each tank. These 4 bronopol preserved milks were analyzed using direct microscopic somatic cell count by 2 laboratories. A set 12 milk dilutions of the high SCC whole and skim milks were made (mass/mass) combinations of the high and low SCC skim and whole milks, respectively. The experiment was replicated 3 times. The range of SCC within a set was from approximately 5,000 to 950,000 somatic cells per mL. Two laboratories analyzed 3 sets of these milks per week by ESCC over a period of about 21 d of 4oC storage. ANOVA was used to determine the effect of laboratory, set type (skim or whole milk), and sample age on the observed electronic milk SCC. No effect of laboratory or set type ($P > 0.05$) on observed milk ESCC was detected. There was a mean decrease ($P < 0.05$) in ESCC with time of storage (about 7,000 cells per mL at 350,000 cells per mL during 2 weeks of refrigerated storage). Gravity separation can be used to produce a set of milk SCC reference standards with a wide range somatic cell count with 12 incremental SCC distributed evenly from low to high across the range.

Key Words: somatic cells, gravity separation, reference standards

W86 Freezing and thawing milk calibration standards for electronic somatic cell counters. L. V. Marzo[1] and D. M. Barbano*[2], *[1]University of Sao Paulo, Pirassununga, Brazil, [2]Cornell University, Department of Food Science, Northeast Dairy Foods Research Center, Ithaca, NY.*

Our objective was to determine the effect of freezing and thawing on electronic milk somatic cell count (ESCC) standards. Whole raw milk (350 kg) was pasteurized and split into 2 portions. One portion was gravity separated at 4°C for 22 h and the second portion was centrifugally separated at 4°C to produce skim milk that was also gravity separated. After 22 h the somatic cells were concentrated at the top of both gravity separation tanks. Stock solutions (3: high SCC skim and whole milk stock and a low SCC skim stock) were prepared and preserved (bronopol). Standards were formulated by diluting high SCC (whole or skim milk) stock with low SCC skim stock to make 1600 g of each of a series of 12 whole and 12 skim standards. The range of SCC was from approximately 2,000 to 1,150,000 SCC/mL and from 2,000 to 800,000 SCC/mL for the skim and whole milk sets, respectively. Half of the sets were kept refrigerated at a 4°C and the other half were frozen at −80°C for 24 h, and then moved to a −20°C freezer. On each day of analysis, 1 set of skim and 1 set whole refrigerated milks were tested and 1 set of skim and 1 set whole frozen milks were tested on 2 different cell counters in different labs. Refrigerated and frozen samples were put in the water bath at the same time and were analyzed when their temperature reached 40 to 42°C. Analysis was repeated 4 times during a 2 week storage and replicated 3 times. Effects of freezing, laboratory, set type (skim or whole milk), and sample age on the ESCC were determined. For the skim SCC standards there was no effect of freezing on mean ESCC during a 2 week period. For the whole milk SCC standards there was an effect of freezing with the frozen and thawed set having a mean SCC of about 6,000 SCC/mL lower than the unfrozen samples over a 2 weeks period. Skim milk SCC standards had a larger range of SCC than whole milk. Frozen and thawed skim standards may have better homogeneity with longer frozen storage time and avoid problems of oiling off during thawing and heating of milks.

Key Words: somatic cells, freezing, gravity separation

W87 Protease activity of *Streptococcus thermophilus* ST-M5 subjected to mild sonication intensities at different temperatures. M. Moncada*[1,2] and K. Aryana[2,1], *[1]Louisiana State University, [2]Louisiana State University Agricultural Center.*

Streptococcus thermophilus is a bacterium used widely for the production of many fermented dairy products. Protease activity degrades the milk proteins to peptides and influence the quality characteristics namely texture and flavor of several aged dairy products. "Mild" sonication intensity is a technique that uses sound waves to cause cavitation in liquid solutions and may improve the permeability of the cell membrane. The objective of this study was to evaluate the influence of "mild" sonication intensities on protease activity of *Streptococcus thermophilus* at different temperatures. Freshly thawed *Streptococcus thermophilus* ST-M5 culture was suspended in 0.1% peptone water and sonicated using a 13 mm diameter probe set at a maximum acoustic power output of 750 W, frequency 24 kHz. Before sonication, the inoculated samples were set a 3 different temperatures (4, 22 and 40°C). Four sonication treatments with intensities of 8.07, 14.68, 19.83 and 23.55 W/cm^2 were performed in a random manner at the 3 different temperatures mentioned above. Control samples did not receive any sonication treatment. Protease activity was determined at 0, 12 and 24 h of incubation spectrophotometrically at 340 nm. The experimental design was completely randomized design. Data were analyzed using ANOVA of Statistical Analysis System.

Three replications were conducted. Differences of least squares means were used to determine significant differences at $P < 0.05$ for main effect (mild sonication intensity) and interaction effect (mild sonication intensity × time × temperature). Absorbance units increased over time from 0 to 24 h. At 4°C, protease activity of cultures subjected to 8.07 W/cm^2 was significantly ($P < 0.05$) higher than the control at 12 and 24 h. At 22°C the protease activity at all sonication intensities and all time points were lower than the control except for 19.83 W/cm^2 at 24 h. At 40°C, 23.55 W/cm^2 showed significant ($P < 0.05$) increase in protease activity compared with the control at 0, 12 and 24 h. The optical density (OD) values at 0, 12, and 24 h after using 23.55 W/cm^2 were 0.11, 0.16 and 0.17 absorbance units while OD values for control were 0.09, 0.12 and 0.15 absorbance units at 0, 12, and 24 h, respectively, at 40°C. *Streptococcus thermophilus* treated with some mild sonication intensities improved its protease activity.

Key Words: protease, culture, sonication

W88 Prediction of fatty acid chain length and unsaturation of milk fat by mid-infrared milk analysis. K. L. Wojciechowski[1], D. M. Barbano*[1], and E. de Jong[2], *[1]Cornell University, Department of Food Science, Northeast Dairy Foods Research Center, Ithaca, NY, [2]Delta Instruments, Drachten, the Netherlands.*

Our objective was to predict mean fatty acid chain length (mCL, carbon number) and mean fatty acid unsaturation (mUnsat, double bonds per fatty acid) of milk fat from mid-FTIR spectra. Calibration models for both parameters were calculated, using partial least squares, based on spectra for 268 samples collected over a period of 1.5 year. Of which 219 samples (largely herd milks) were selected to cover a wide variation in regional and dietary dependent milk fat composition. The set was complemented with 49 modified milks spanning an orthogonal set in fat, protein and lactose. Milks were analyzed for fat by ether extraction and fatty acid composition by GLC. Standard errors for calibration, determined using full cross validation (SECV) on the calibration set were 0.11 carbons for mCL and 0.012 double bonds for mUnsat. Validation was on the basis of an independent set of 47 milks, for which mean and range in mCL were 14.47, 14.00 to 14.85 carbons and mean and range in mUnsat were 0.319, 0.236 to 0.394 double bonds, respectively. The mean difference, standard deviation of the differences, relative coefficient of variation for prediction of mCL compared with GLC reference values was 0.056 carbons, 0.063 carbons, and 0.4%, respectively, and 0.003 double bonds, 0.011 double bonds, and 3.3%, respectively. Future work will determine if estimates of fatty acid composition by mid-FTIR can be used in real time to improve the accuracy of prediction of total fat content of milk.

Key Words: mid-infrared, fatty acids, chain length

W89 A ruggedness study: Casein content of milk by Kjeldahl analysis for milk concentrates and non-bovine milks. K. L. Wojciechowski and D. M. Barbano,* *Cornell University, Department of Food Science, Northeast Dairy Foods Research Center, Ithaca, NY.*

The objective of our work was to develop a modification of Association of Official Analytical Chemists International (AOACI) method 998.05 (optimized for a raw bovine milk matrix) so that it could be applied to milk concentrates and milks of other species (goat, sheep, and water buffalo). A ruggedness study was carried out that demonstrated that as concentration of protein in milk increased (either in bovine milk concentrates or in milks of other species), the amount of buffer needed in the noncasein nitrogen sample preparation method to achieve a filtrate pH of 4.6 increased. In the first part of the study using a series of bovine milk ultrafiltration concentrates, it was demonstrated that the method gave more consistent predictions of casein as a percentage of true protein when the NCN filtrate pH was between 4.5 and 4.6, regardless of total protein concentration. The study was designed with a series of UF retentates ranged from 3 to 9% protein but all contained the same casein as percentage of true protein, regardless of TP concentration. Thus, when the reagent concentration was correct the casein as a percent of true protein at all protein concentrations. When the amount of buffer added to the sample was not sufficient (i.e, the filtrate pH was too high), the filtrates were not clear. A polynomial equation was developed for prediction of the amount of acetic acid - sodium acetate buffer required to achieve pH for milk protein concentrations from 3 to 9% protein. The modified method was tested on goat, sheep, and water buffalo milks. The results of this study will be used as the basis for proposed changes in the official (AOAC and International Dairy Federation) methods for measurement of the casein content of milk to expand the scope of the method so it can be used to achieve accurate results for milk concentrates and milks of other species.

Key Words: casein, milk concentrates, sheep and goat

W90 A review of the pH influenced casein-whey protein interactions in heated milk. H. Taterka,* B. Guamis, and M. Castillo, *Universitat Autònoma de Barcelona, Barcelona, Spain.*

During the heat treatment of milk, denatured whey proteins associate to the casein micelle, affecting the functional properties of milk in downstream applications such as milk and yogurt. Although the specific mechanism of attachment is not entirely understood, after partial denaturation, β-lactoglobulin (β-LG) attaches via available sulfide groups to κ-casein (κ-CN). Additionally, there is conflicting evidence, apart from the more commonly accepted mechanism in which the κ-CN/β-LG complex is formed on the surface of the micelle, that may indicate that β-LG does not attach to κ-CN on the surface of the casein micelle, but rather that κ-CN first interacts with β-LG preferentially in the serum matrix and later the κ-CN/ β-LG complex can re-associate with the casein micelle. Furthermore, it is believed that α-lactalbumin (α-LA) does not attach directly to the micelle, but instead forms an association with β-LG portion of the κ-CN/β-LGcomplex, and is therefore dependent on this association to attach to the casein micelle. After heat-treatment and attachment, the milk matrix contains 3 types of whey proteins: native whey proteins, protein aggregates, and those aggregates that have formed an association on the surface of the casein micelle. The percentages of these whey protein-types are highly dependent on the extent of heat treatment (temperature and time) as well as milk pH. Methods have been used to separate and quantify these types of whey proteins in heated milk including acid separation, rennet separation, and ultracentrifugation. To quantify the whey protein forms in milk, different analytical methods can be used such as HPLC, capillary electrophoresis and gel electrophoresis. This work reviews the mechanism of the attachment of partially denatured whey proteins to the surface of the casein micelle, the various types of whey proteins that can be found in the milk matrix after heat treatment, as well as the various methods of determination and quantification of whey proteins.

Key Words: whey protein, casein, denaturation

W91 Gel-based shotgun proteomics analysis of cow milk fat globules. T. J. Yuan, J. Q. Wang,* Y. X. Yang, D. P. Bu, J. H. Yang, P. Sun, and L. Y. Zhou, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

This study was designed to characterize protein expression profiling of cow milk fat globules (MFG). Four different lysis buffers were applied to improve protein extraction from the cow MFG, and extracts were subjected to SDS-PAGE separation followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for identification and characterization. The result indicated different lysis buffers led to different quality of protein extraction and resolution. All 224 MFG proteins (MFGP) were identified by 2 or more unique peptide sequences. The identified MFGP ranges from 8 kDa to 546 kDa in molecular weight mass, and 4.61 to 11.36 in isoelectric point. The Gene Ontology (GO) classification for localization and function of these proteins was presented. Among all identifications, 69 were not reported before in bovine MFG or MFGM, and the majority of these proteins are involved in membrane and vesicular trafficking, cell signaling, immune function, protein synthesis, binding and folding, fat transport/metabolism, enzymatic activity or poorly defined functions. Our work presents a detailed picture of the cow MFG proteome at the physiological conditions based on gel LC-MS/MS analysis, providing a useful source for future studies on lactation biology and on physiopathological evaluation of the mammary gland on the basis of their proteomic assortment.

Key Words: milk fat globule, shotgun proteomics, Gene Ontology classification