M84 Effects of salts on foaming properties of milk protein concentrate at neutral pH. J. Han* and B. Vardhanabhuti, *University of Missouri, Columbia.*

Milk protein concentrate (MPC) is one of the major ingredients in foods due to its nutritional and functional properties including solubility, water binding, foaming, and emulsification. The foaming ability of milk proteins could potentially allow them to replace egg white protein; however, there is a lack of research on foaming properties of MPC. The goal of this study was to investigate the effects of salts on foaming properties of MPC. Calcium chloride, sodium citrate, and sodium chloride were mixed with MPC solutions and pH was adjusted such that the final solutions contained 5% w/w protein, 0–20 m*M* $CaCl_2$ and 0–40 m*M* citrate, or 0–100 m*M* NaCl at pH 7.0. Foam was generated by whipping MPC solutions in a KitchenAid mixer. Foaming properties were determined by measuring overrun and drainage ½ life. Physical properties of pre-foam solutions, including solubility, turbidity, and particle size were measured. Interfacial shear rheology was determined using a controlled- strain and rate rheometer with a bicone geometry. Addition of 20 m*M* $CaCl_2$ caused a reduction in overrun ($P < 0.05$) but no significant effect on foam stability. Either with or without of $CaCl_2$, increasing citrate concentration significantly increased overrun ($P < 0.05$) and drainage ½ life of MPC foam. However, samples having higher citrate concentration (40 m*M*) only showed improvement in overrun but a decrease in drainage ½ life. Sodium chloride significantly improved overrun ($P < 0.05$) but had no effect on drainage ½ life. Enhanced overrun corresponded to a reduction in particle size and turbidity and an increase in solubility of pre-foam solutions. Interestingly, interfacial rheology revealed that pre-foam solutions with $CaCl_2$ exhibited higher interfacial viscosity and interfacial elastic modulus, while the presence of citrate reduced interfacial viscosity and interfacial elastic modulus. These results indicated that appropriate concentrations and combination of salts are needed to optimize foaming properties of MPC.

Key words: milk protein concentrate, foaming properties, salts

M85 Microencapsulation of probiotic cultures using polymerized whey proteins as wall material. Z. Zheng[1], Y. Jiang[1], X. Chen[2], J. Wang[2], J. Cheng[1], H. Zhang[2], and M. Guo*[1], *[1]University of Vermont, Burlington, [2]Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China.*

Commonly used probiotics are sensitive to the environment and processing conditions. The objective of this study was to develop a microencapsulation technique using polymerized whey proteins (PWP) as a wall material for protecting probiotic cultures. PWP was prepared by heating whey protein isolate solution (12%, w/v, pH 8.0) at 85°C for 40 min. The solution was then adjusted to pH 7.0 after cooling. The ratio of the PWP solution to the probiotic culture *Lactobacillus acidophilus* NCFM (8.31×10^8 cfu/mL) was 7:3. The mix was extruded using a 50-mL syringe through a needle (0.6 mm) to the cross linker $CaCl_2$ solution (16.7%) containing Tween-20 (0.4%, v/v) at 40°C while stirring. After being kept in the $CaCl_2$ solution for 20 min and rinsed twice with 0.9% NaCl solution, the beads were freeze-dried after mixing with protecting agents including non-fat dry milk (20%), peptone (1%), and ascorbic acid (0.4%). Microencapsulation using sodium alginate (SA) was prepared as a control. The microencapsulated cultures by PWP and/or SA, and free culture were subject to artificial digestions (gastric juice for 3 h and intestinal juice for 6 h) to determine the survival rate. The diameters of PWP based and SA based beads were 2.70 ± 0.22 mm and 1.28 ± 0.09 mm, respectively. The entrapment yield for the PWP method (89.3 ± 4.8%) was significantly higher ($P < 0.01$) than the control method (73.18 ± 1.4%). Viable counts of the culture after digestion processes were $3.63 \pm 1.48 \times 10^4$, $1.83 \pm 1.10 \times 10^4$ and $1.35 \pm 0.72 \times 10^2$ cfu/mL for the PWP beads, SA ones and free culture, respectively. Results showed that the PWP based microencapsulation seems more effective in protection of probiotics than SA method in the model system. Survivability of PWP encapsulated probiotic cultures in fermented milk products will be further investigated.

Key words: microencapsulation, polymerized whey protein, probiotic

M86 Proteolysis in UHT milk produced with CO_2 added raw milk. P. C. B. Vianna[1], E. H. M. Walter[2], M. E. F. Dias*[3], J. A. Faria[3], F. M. Netto[3], and M. L. Gigante[3], *[1]Universidade Norte do Paraná, Londrina, SP, Brazil, [2]Universidade Federal do Pampa, Bagé, SP, Brazil, [3]Universidade Estadual de Campinas, Campinas, SP, Brazil.*

The goal of this work was to evaluate the effect of CO_2 addition to raw milk on proteolysis of UHT milk during storage. Control milk (without CO_2 addition) and treated milk (added with CO_2 until pH 6.2) were stored in bulk tanks at 4°C during 6 d. After storage, milks were processed using indirect heating (140°C/5s). Samples were aseptically packed in low density polyethylene pouches and stored in the dark at room temperature. Raw milk was evaluated at reception and after 6 d-storage to standard plate and psychrotrophic bacteria count. UHT milk samples were analyzed for proteolysis twice a month until 120 d-storage. Increased in non casein nitrogen as a percentage of total nitrogen was used as index of proteolysis. Analysis of peptides of non casein nitrogen filtrates by RP-HPLC was performed after 1 and 120 d of storage. Split-plot design was used and the complete experiment was replicated 3 times. The results were evaluated by ANOVA and Tukey's test ($P \leq 0.05$). Raw milk presented standard plate and psychrotrophic bacteria counts of 2.8×10^3cfu/mL and 5.2×10^2 cfu/mL, respectively. After 6 d-storage, these counts increased 3 and 4 log cycles for milk without CO_2 addition, respectively, while in the CO_2 added milk the counts remained constant, resulting in a better microbiological quality of raw milk to UHT processing. The proteolysis increased significantly during 120 d of storage to both treatments, but the increase was 1.4 times faster for UHT control milk (UHT_C) than UHT produced from raw milk with CO_2 addition (UHT_{CO2}). One day after processing, the chromatograms showed similar peaks for both milks, corresponding to hydrolysis by plasmin and heat resistant psychrotrophic proteases. However, after 120 d the amount of peptides in UHT_{CO2} milk was almost constant, while peptides produced by plasmin and proteases of psychrotrophic increased in UHT_C, indicating higher proteolysis in this sample. The better microbiological quality of CO_2 added raw milk resulted in less proteolysis and possibly less susceptibility of age gelation in UHT milk, the main problem that limits its shelf life.

Key words: UHT milk proteolysis, carbon dioxide, psychrotrophic

M87 The effect of commercial sterilization regimes on micellar casein concentrates (MCC). C. M. Beliciu, A. Sauer*, and C. I. Moraru, *Cornell University, Ithaca, NY.*

The increasing interest of using micellar casein concentrates (MCC) obtained by membrane separation in the manufacture of shelf-stable,

high protein beverages creates a need to understand the effect of processing conditions, particularly sterilization, on the stability of this ingredient. In this work, MCCs of 5% - 10% casein concentration were subjected to both continuous-flow UHT treatment and in-container retorting, at the same cumulative value of the lethality factor (F_0 = 9.9). The effects of sterilization on the stability and physical properties of MCCs were investigated by determining their rheological properties, zeta potential, particle size and mineral distribution. The study was performed in triplicate. Significant differences among samples were determined at $P \leq 0.05$. Sterilization led to significant changes in zeta potential, from −37mV to −24mV for the controls (non-heat-treated MCCs), to −30 mV to −17mV in retorted MCCs and −34 mV to −20mV in the UHT treated MCCs. This was attributed to the re-distribution of minerals, specifically a loss in solubility of calcium phosphate at the micelle level; soluble Ca content decreased by an average of 30% as a result of the UHT treatment and by 21% as a result of retorting, as compared to untreated samples. Average particle diameter increased as a result of heat treatment: 240nm in retorted samples vs. 180nm in the untreated MCCs (control). UHT treatment led to formation of aggregates visible with the naked eye. Retorting of MCCs led to a 50% decrease in apparent viscosity as compared to the untreated samples, while the UHT treated MCCs had higher apparent viscosity than the controls. For the UHT treated samples, dynamic rheological testing revealed a solid-like behavior at a casein concentration >5%, which was indicative of structure formation. An evaluation of sterilization behavior of MCCs obtained by reconstitution of spray dried powders showed that drying enhanced the instabilities that occurred during sterilization. The results of this study are particularly relevant for using MCCs obtained by membrane separation for the manufacture of shelf stable milk protein beverages.

Key words: micellar casein, zeta potential, retorting

M88 The crystallization of large lactose crystals in skim milk concentrate. B. Toledo* and F. X. Milani, *University of Wisconsin-Madison, Madison.*

The presence of lactose crystals greater than 10 μm in concentrated skim milk is normally considered a quality defect. These large crystals can be avoided by manipulating process parameters such as cooling rate, seed concentration and agitation speed. The objective of the present study is to investigate the formation, characterization and performance of large α- lactose monohydrate crystals in concentrated skim milk at 40% total solids. A 2-level, fractional factorial design with 4 factors was used for the study. The factors tested were holding temperature (5 and 10°C), lactose seed concentration (0.005% and 0.010%), lactose seed size (200 and 40 mesh), and agitation speed (50 and 100 RPM). Lactose crystallization in concentrated skim milk was done by reconstituting skim milk powder to 40% total solids and allowed to hydrate for 24 h at 5°C. After reconstitution, the concentrate was pasteurized, cooled, and seeded with α- lactose monohydrate crystals. Lactose crystallized during 24 and 48 h in a 150 mL stirred reactor. Crystallization started at the selected temperatures and continued with a cooling rate of 0.02 to 0.04°C per hour. Photomicrographs were taken and analyzed with ImageJ 1.44i software. The formed crystals had mean sizes of 71 ± 35 μm and 93 ± 45 μm at 24 and 48 h, respectively. Analysis of the present model concluded that a 24 h crystallization period is significantly influence by the lactose seed size and holding temperature. As the lactose seed size increased, the mean value of crystals increased by 12 μm. When the holding temperature increased, the mean value of the crystals decreased 5 μm. Analysis for 48 h crystallization did not reveal significant influence from any of the tested factors. This is associated with shattering of large crystals, which diminished the influence of initial experimental design factors. The results from the present study are intended to be used as preliminary information in the design of skim milk processes and products that utilize large lactose crystals as a value added feature.

Key words: crystallization, lactose, skim milk

M89 Investigation of twin-screw extrusion puffing of non-fat dry milk powder and starch to produce puffs and crisps for snack and ingredient uses. A. J. Tremaine* and T. C. Schoenfuss, *University of Minnesota, Department of Food Science and Nutrition, St. Paul.*

The use of twin-screw extrusion to produce puffs and crisps for cereals and snacks is widely used in the food industry. Soy protein is the leading protein used in extrusion puffing, but caseinates and whey protein concentrates and isolates have also been researched extensively. Less research has focused on non-fat dry milk (NDM). NDM has the advantage of an abundant and inexpensive supply, has the full amount of calcium found in milk, and has a cleaner flavor than whey protein concentrates. One disadvantage of NDM is the difficulty in creating protein-protein interactions in the extruder to obtain a stable puff. The objective of this study was to evaluate the effect of acid addition and varying moisture levels on the attributes of expanded puffs containing high levels of NDM. Varying concentrations of low-heat NDM were combined with modified cornstarch and processed on a Buhler 44mm twin-screw extruder. Extruded product was collected in ropes, cut into 2 inch lengths, and dried on a fluidized bed dryer. The experimental parameters included three NDM concentrations (45, 65, 85%), three lactic acid levels (0, 33, 50% of the moisture) and two moisture levels (6.5, 7.3 kg/h). Process (die temperature, die pressure, motor torque and specific mechanical energy) and product responses (color, solubility, expansion ratio and bulk density) were statistically analyzed to assess the effects of NDM, acid and moisture levels, and response surface plots were generated. The results obtained indicate that NDM concentration, moisture and acid level all affected process and product responses. These results will be useful in product development in the food industry when incorporating NDM into extruded products.

Key words: extrusion, non-fat dry milk, response surface plots

M90 Browning and pH of UHT whole milk as influenced by time and temperature of storage. M. E. F. Dias*[1], P. C. B. Vianna[2], and M. L. Gigante[1], *[1]Universidade Estadual de Campinas, Campinas, SP/Brazil, [2]Universidade Norte do Paraná, Londrina, PR/Brazil.*

The CO_2 addition has been used in maintaining the quality of refrigerated storage of raw milk. The objective of this work was to evaluate the effect of time and temperature of storage on pH and browning of UHT whole milk produced from refrigerated raw milk with or without CO_2 added. Raw milk (250 L) with and without CO_2 addition was stored in bulk tanks at 4°C during 6 d before UHT treatment. The milk was sterilized by direct steam injection (143°C/4 s, homogenization pressure 220 bar), cooled to 25°C and packaged in Tetra Brik (125 mL). The samples were stored at 25, 35 and 45°C for 180 d. During storage, samples were taken at random after 1, 30, 60, 90, 120, 150 and 180 d and evaluated for pH and browning. The color was assessed with a colorimeter Hunterlab ColorQuest II, with D65 illuminant according to conditions provided by the manufacturer. The increase in Hunter b* value (measures blue [-] to yellow [+]) was used for determination of browning. The pH was measured in milk samples at 20°C using a combined pH glass electrode fitted to a pH meter (Digimed Model DM 22).

The split-split-plot design with 3 replications was used. The results were evaluated by ANOVA (ANOVA) and Tukey′s test ($P \leq 0.05$). The CO_2 addition to raw milk significantly affected the pH of UHT milk and it was 6.66 and 6.70 for samples with or without CO_2 added, respectively. The rate of browning for UHT samples obtained from raw milk without CO_2 was higher than the rate obtained for samples from raw milk added of CO_2. During storage the pH of all samples decreased, while browning, as indicated by b* values, increased. The lower pH and increase of browning was greater at 45°C, followed by samples at 35°C and 25°C. After 6 mo storage period, UHT milk stored at 45°C showed pH value of 6.24 while this value was 6.74 at 25°C. At the same time, b* values were 18.93 and 7.03 for UHT milk stored at 45°C and 25°C, respectively. Changes in pH and browning are related to Maillard reactions.

Key words: browning, UHT milk, CO_2

M91 Evaluation of vacuum packaging on physical properties and solubility of dry dairy ingredients. H. Eshpari* and P. Tong, *California Polytechnic State University, San Luis Obispo.*

Dry dairy ingredients can have a longer shelf life if packaged and stored properly. Vacuum packaging can be an attractive method for keeping quality and provides added value because of the inherent compactness of the products. Vacuum packaged dry dairy ingredients may also have added ease of handling for end users. However little is known about the impact of vacuum packaging on the properties of dry dairy ingredients. The objective of this study was to determine the effects of vacuum packaging on particle size, (particle, bulk, and tapped) densities, flowability, compressibility, color, and solubility of 6 types of dry dairy ingredients. Commercial samples of nonfat dry milk powder, whole milk powder, buttermilk powder, milk protein isolate whey protein concentrate 80, and sweet whey powder were repackaged in duplicate using multi-wall foil side gusseted bags under varying degrees of vacuum (1, 0.7, 0.4 bar) and a control with no vacuum, and then stored for 3, 6, and 12 mo at 25°C and 60% relative humidity. Each powder was sampled and analyzed in duplicate for all the quality attributes mentioned above upon receiving and after 3, 6, and 12 mo storage. At $\alpha = 0.01$: particle size, (particle bulk and tapped) densities, and flowability of the powders increased (P-values = 0.001), while the compressibility decreased ($P = 0.004$) due to the significant effect of storage time. Powders packaged under vacuum showed a higher mean of L- color value ($P = 0.003$), but significantly lower means of (a- and b-) color values, (p-values = 0.005, and 0.001 respectively) due to the significant effect of vacuum pressure. This change in color values was more dramatic in high fat containing powders such as whole milk powder. No significant change was observed in solubility of the powders. The results suggest that the proposed vacuum packaging method may be beneficial to maintain the quality of the powders studied.

Key words: vacuum packaging, dairy powders, solubility

M92 Hydrophobic aroma encapsulation in whey protein nanoparticles. H. J. Giroux and M. Britten*, *Food Research and Development Centre, Agriculture and Agri-Food Canada, St-Hyacinthe, (QC), Canada.*

The development of foods with health benefits prompts the manufacturers to review products formulation and processing conditions. The modification of food components or processing can impact directly on flavor release and perception. Moreover, aroma compounds are volatile, sensitive and might be degraded during manufacturing, storage or digestion. Encapsulation technologies have been proposed to entrap, protect and deliver sensitive or bioactive components and improve the sensory properties of functional foods. Because the interest in reducing capsule size to further reduce the impact of encapsulated ingredients on food texture, protein nanoparticles present a good potential to serve as carriers and delivery systems. The objective of this study was to encapsulate hydrophobic aroma in whey protein nanoparticles. Aroma-loaded nanoparticles ($d < 300$ nm) were prepared by cross-linking denatured whey protein through pH-cycling. The effect of nanoparticulation conditions (aggregation pH, calcium addition) and aroma concentration on the physicochemical characteristics of nanoparticles and the dynamic release profile of aroma was studied. The release behavior of aroma encapsulated in nanoparticles was compared with those of aroma added to native or denatured whey protein. Better retention of aroma was observed for nanoparticles produced at pH 5.0 and 5.5 without calcium addition. These nanoparticles are characterized by a less compact and more porous internal structure allowing a higher loading of aroma. Increasing aroma concentration increased the diameter and the voluminosity of the aroma-loaded nanoparticles. The percentage of aroma retention showed an increase from 7 to 24% over the tested concentration range while the value averaged 2% for native or denatured whey protein. Encapsulation of ethyl hexanoate in whey protein nanoparticles reduced the mass transfer of aroma at the surface of the matrix and improved its retention.

Key words: encapsulation, whey protein, aroma

M93 Formation of β-lactoglobulin/alginate nanoemulsion containing coenzyme Q10. H. N. Choi*, M. R. Lee, and W. J. Lee, *Division of Applied and Life Science (Institute of Agriculture & Life Science), Jinju-si, South Korea.*

The bioavailability of poorly water soluble nutrients, such as coenzyme Q10 (Co Q10), can be enhanced by the use of nanoemulsion system. It is hypothesized that exposed hydrophobic residues of β-lactoglobulin (β-lg) from heat treatment and additional protection from the complex formation of β-lg/alginate may affect physicochemical properties of nanoemulsion as a Co Q10 delivery system. The objectives of this study were to produce oil-in-water β-lactoglobulin/alginate nanoemulsion loaded with Co Q10 (β-lg/AL NE) and to investigate how processing variables, such as heating temperature and alginate concentration, affect the physicochemical properties and encapsulation efficiency of β-lg/AL NE. β-lg/AL NE was prepared at different heating temperatures from 60 to 70°C. Alginate concentration was varied from 0 to 0.05%. Morphologies of β-lg/AL NE were observed using a transmission electron microscopy. Size and zeta-potential values of β-lg/AL NE were measured by electrophoretic light scattering spectrophotometer. High performance liquid chromatography was used to assay encapsulation efficiency of Co Q10. The spherical shapes of β-lg/AL NE with the size of 150 to 250 nm were successfully formed. There was an increase in size from 160 to 240 nm and encapsulation efficiency from 70 to 80% with increasing heating temperature from 60 to 70°C. A significant ($P < 0.05$) increase in zeta-potential value from −5 to −13 mV was observed with increasing heating temperature from 60 to 70°C. Increasing alginate concentration from 0 to 0.05% resulted in a significant ($P < 0.01$) increase in encapsulation efficiency from 70 to 80%. In Split plot design, both heating temperature and alginate concentration had a significant ($P < 0.05$) effect on encapsulation efficiency of Co Q10. In conclusion, heating temperatures and alginate concentrations, which were β-lg/AL NE manufacturing variables,

were the key-processing factors to affect size, zeta-potential value, and encapsulation efficiency of β-lg/AL NE.

Key words: β-lactoglobulin, nanoemulsion, coenzyme Q10

M94 Homogenization and lipase addition influence methyl ketone generation. M. Cao*, E. L. Anderson, and S. A. Rankin, *University of Wisconsin-Madison, Madison.*

Specific methyl ketones contribute to the characteristic flavor of blue cheese. Various metabolic and enzymatic pathways contribute to the generation of these methyl ketones including the β-oxidation of fatty acids by the mold *Penicillium roqueforti*. Little recent research has explored means by which methyl ketone production can be altered or controlled. Earlier work and current industry practices have implicated milk or cream homogenization and exogenous lipase addition as means to alter methyl ketone production. Thus, the objective of this work was to determine the effects of milk homogenization and lipase addition on methyl ketone production in milks inoculated with *P. roqueforti*. Milks were homogenized pressures (e.g., 3.5 MP/3.5 MP, 10.5 MP/3.5MP). Homogenized milks were randomly treated with one of 3 lipases (porcine pancreatic, *P. roqueforti* and calf), inoculated with *P. roqueforti* spores and allowed to incubate (24 h, 20°C). Changes in milkfat size and milkfat globule surface areas were determined by laser scattering. Fatty acid and methyl ketone concentrations were determined using GC/MS. In general, average milkfat globule size decreased from 3.5 to 0.37 μm and surface areas increased from 1.8 to 25 m2/g. Concentrations of FFAs varied dramatically as a function of lipase treatment. Even-chain fatty acids from C4 to C10 increased as a function of homogenization treatment with approximately 10- and 2-fold increases in fatty acid concentrations for the calf and *P. roqueforti* lipase treatments, respectively. FFA levels from the porcine lipase treatment were not consistently affected by homogenization, yet had high levels of FFA. There was an interaction between pressure and lipase treatment that affected the concentrations of specific methyl ketones. In general, the *P. roqueforti* lipase treatment resulted in the highest levels of methyl ketone generation. Ketone concentration did not correlate well to particle size, milkfat globule surface area or initial level of free fatty acid. This study demonstrates that lipase treatment and homogenization alter accumulation of methyl ketones as affected by the metabolic activity of *P. roqueforti*.

Key words: blue cheese, methyl ketone, lipase

M95 Use of fluorescence spectroscopy for monitoring vitamin D fortification of skim milk. J. K. Amamcharla* and L. E. Metzger, *Midwest Dairy Foods Research Center, Dairy Science Department, South Dakota State University, Brookings.*

It is well recognized that a sufficient level of vitamin (vit.) D is necessary in the human diet. Most animals and plants have the ability to synthesize vit. D when exposed to sunlight. However, various factors can reduce the cutaneous synthesis of vit. D and products can be fortified with vit. D. In this regard, retail milks in the United States are fortified with 400 IU/quart of vit. D. In a recent release of the Dietary Guidelines for Americans 2010, USDA and Department of Health and Human Services recommended 600 IU/day of vit. D for children and most adults. As per the regulations of the FDA, milk fortified with vit. D is required to be at least equal to the declared value on the label with no regulations on the upper limit. In a recent study, 51% of 120 retail milk samples contained either below 400 IU/quart or above 501 IU/quart of vit. D. The lack of control of vit. D fortification of milk may be due to the fact that the current methods for vit. D analysis are laborious and precision of the method is dependent on the experience of the analyst. The objective of the present work was to develop a rapid method for measurement of vit. D in skim milk using front face fluorescence spectroscopy. For this purpose, comingled raw whole milk was fat separated, pasteurized and stored under refrigerated conditions. Sixty-six skim milk samples with various concentrations of vit. D (0, 200, 400, 600, 800, and 1000 IU/quart) were prepared using water dispersible vit. D and A mixture. Fluorescence spectra of vit. D (excitation: 360nm; emission: 400 to 600nm) were collected on each sample at 37°C using a front-face accessory. The spectra were normalized, mean-centered, and partial least square regression (PLSR) and neural network (NN) models were developed. Coefficient of determination (r^2), root mean square error of cross validation (RMSECV, IU/quart), and relative standard error of prediction (RSEP, %) were 0.98, 41.8, and 0.9, respectively, for PLSR and 0.97, 54.0, and 1.2, respectively, for NN model. The results indicate that the proposed method can be used as an effective tool to monitor vit. D fortification of skim milk.

Key words: vitamin D, skim milk fortification, fluorescence spectroscopy

M96 Milk composition evaluation as screening criteria to investigate fraudulent addition of cheese whey to milk. M. M. Falcão, F. A. P. Paula, M. O. Leite*, C. F. A. M. Penna, L. M. Fonseca, M. M. O. P. Cerqueira, and M. R. Souza, *Universidade Federal de Minas Gerais.*

Fraudulent addition of cheese whey to milk is a detected practice in several countries around the world. Low cheese whey costs and high costs for fraud detection, together with other aspects are factors that result in this problem for the dairy sector. The method worldwide accepted for detection of cheese whey addition to the milk is the caseinomacropeptide (CMP) index determination by HPLC. However, it is a high cost method for routine screening. To evaluate the milk composition as a principal components analysis for screening of fraudulent addition of cheese whey to milk, 30 individual raw milk samples were pooled to 6 samples containing milk from 5 animals. Each of the 6 samples was split and cheese whey was added to a final concentration of 0%, 5%, 10%, 20%, and 40% (vol/vol) of whey in the milk. Milk composition was evaluated by infrared (Bentley CombiSystem 2300), and obtained data evaluated by ANOVA with average composition by Student Newman Keuls (SNK) test. Average composition for the normal samples was 4.04 g/100g, 4.11g/100g, 4.38g/100g, and 13.56g/100g for, respectively, fat, protein, lactose and total solids. For the samples added with 40% (vol./vol.) cheese whey average composition was 2.48 g/100g, 2.36 g/100g, 4.73 g/100g, and 10.74 g/100g for, respectively, fat, protein, lactose and total solids. The results were statistically different among different treatments, and in practice this method can be used as screening tool with a logistic regression treatment of data in the results database.

Key words: cheese whey, milk quality, milk fraud

M97 Measuring milk treatments and storage temperature effects on fat globules aggregation. N. Fucà[1], G. Impoco[1], M. Caccamo*[1], and G. Licitra[1,2], *[1]CoRFiLaC, Regione Siciliana, Ragusa, Italy, [2]DISPA, Catania University, Catania, Italy.*

Milk treatments and storage temperatures cause changes to the microstructure of the lipidic phase. This study aims to demonstrate that confocal microscopy (CLSM) and quantitative analysis of resulting

images can give useful clues to estimate fat globules' aggregation. Two experiments were carried out. First, 5 types of milk subject to different treatments (homogenization-UHT, homogenization-high pasteurization, homogenization-pasteurization, pasteurization, microfiltration) and one sample of raw milk (used as control) were compared, to evaluate the effects of treatments on the lipidic phase. Then, the same milk types were analyzed at 2 different storage temperatures (4 and 16°C). Milk samples were stained with Nile Red to visualize lipids. Images were acquired using 60x objective lens and 1.5 zoom factor of a confocal laser scanning microscope. Image analysis was used to quantify fat globules aggregation in CLSM micrographs. Aggregation favors non-homogeneity both of distribution and volume of fat clusters. These 2 effects can be measured more reliably than aggregation itself. Eleven measures were computed on each image to capture variation in size and distribution of fat globules. After pairwise correlation assessment, 6 out of these 11 measures were chosen for their statistical independence and significance, and for their realistic description of the geometry of the lipidic phase. As expected, treatments did affect distribution and size of clusters. High significant difference ($P < 0.0001$) was found among the measurements related to the 5 types of milk. Statistical analysis revealed that storage temperature significantly affected distribution and size ($P < 0.0001$) as well, promoting aggregation. Quantitative analysis of CLSM micrographs turned out to be capable of capturing fundamental effects of fat globules' aggregation due to milk treatments and storage temperature.

Key words: milk, microstructure, quantitative analysis

M98 Effects of residual lactose and galactose on cheese moisture determination. H. Lee*, F. X. Milani, and S. A. Rankin, *University of Wisconsin-Madison, Madison.*

Accurate measurement of moisture in cheese is important to maximize yield and ensure economic parity. Official methods determine moisture based on the mass lost due to the thermal volatilization of available water. Because cheese is a chemically complex and variable medium, there is a potential that additional volatile compounds may be created during analytical moisture determination. Maillard browning and other various thermally catalyzed reactions may alter the final mass after drying. Based on their reactivity, the residual sugars lactose and galactose are 2 components in cheese that may be natively present at varying levels and that may participate in reactions capable of artificially elevating moisture determination. The objective of this study was to examine the effects of residual lactose and galactose on cheese moisture determination. In this study, 4 medium cheddar and 4 mozzarella cheese samples were analyzed for moisture content in triplicate using a microwave system (CEM Corporations, Matthews, NC). Cheese samples were manufactured with 5% added α-lactose monohydrate or galactose as the reducing sugar treatments. For reasons of control and comparison, an untreated sample and treatments containing 5% added sucrose or natrium carboxylmethyl cellulose (non-reducing carbohydrates) were included. There was an effect ($\alpha < 0.05$) for the main treatments of cheese type and sugar type. In general, the lactose and galactose samples had higher moisture levels than the non-reducing treatments. Compared with the untreated controls, the reducing treatments overestimated the moisture content by approximately 1.4% for the cheddar samples and 2.4% for the mozzarella samples. The samples with reducing sugars also displayed substantial dark brown color after the drying process as compared with minimal color change for other samples. There was no difference between the lactose and galactose treatments. This study demonstrates that the presence of residual reducing sugars may result an overestimation of cheese moisture concentration and that these effects may be different for cheeses of varying composition.

Key words: cheese, moisture, sugar

M99 Quantification of textural properties of composite milk gels using laser-scanning fluorescence confocal microscopy and image texture analysis. R. Hennessy*[1], L. Laiho[1], A. Laubscher[2], and R. Jimenez-Flores[2], *[1]Cal Poly Biomedical Engineering, San Luis Obispo, [2]Cal Poly, DPTC, San Luis Obispo.*

Current techniques of food texture analysis require destruction of the sample, ignore the spatial relationship between principal constituents, or require subjective data that depends on the skill of human subjects. A 2-dimensional, non-destructive, objective measurement technique is needed to quantify the spatial relationship between the principal constituents of dairy products. Our purpose was to investigate whether textural properties can be measured using laser-scanning fluorescence confocal microscopy (LSFCM) by quantifying the spatial relationship between the principal constituents of dairy products. In this study, 2 different types of composite milk gels were created, and stabilized by either freeze drying or baking. The milk gels were stained with the fluorescent markers; Nile red, for lipids, and fast green FCF, for protein. LSFCM was used to image the stained composite milk gels. For each sample, a stack of 30 images, each 5μm apart, were captured to create a 3-dimension set of data. Maximum intensity projection (MIP) was then performed on the stack of images to create a single image where the entire field of view contains pixels that are in focus. Using the MIP image, the following parameters were calculated: 1) fat/protein ratio (FP), 2) fat and protein overlap (OL), and 3) the image texture (T). All three parameters were calculated using an algorithm written in MATLAB. FP was calculated by counting the number of pixels labeled as fat and divides that number by the number of pixels labeled as protein, OL was calculated by counting the number of pixels labeled as both fat and protein and dividing that number by the total number of pixels in the image, and T was calculated using the gray level co-occurrence matrix of the image. OL was found to be the best parameter for distinguishing between baked and freeze dried gels, with $OL = 0.67 \pm 0.12$ for baked gels, and $OL = 0.23 \pm 0.09$ for freeze dried gels. A high OL was found to indicate a chewy texture, while a low OL was found to indicate a more brittle texture that commonly occurs from freeze drying.

Key words: texture, confocal microscopy, composite gels

M100 Evaluation of two kits based on microbial inhibition for detection of antimicrobial residues in milk. A. D. Lage, L. P. Freire, N. M. A. Silva, M. M. P. Araújo, R. D. P. Santos, G. M. Resende, A. F. Cunha, M. R. Souza, C. F. A. M. Penna, L. M. Fonseca, M. O. Leite, and M. M. O. P. Cerqueira*, *Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.*

Two kits based on microbial inhibition (Charm Cow Side II Test and Charm Blue Yellow II Test) were evaluated for detection of antimicrobial residues in milk in different concentrations. The ability of both kits to detect the tetracycline group in a lower concentration of that considered by the Brazilian law was also tested. Milk samples were inoculated with standard solutions of 23 different antimicrobial agents and metabolites of ceftiofur in 2 different concentrations: the lower limit of detection stated by the manufacturer (level 1) and the maximum residue limit (MRL) (level 2) established by the Brazilian legislation. The results were submitted to the McNemar test at 95% of confidence.

Both kits were effective in detecting most of antimicrobials tested in 2 concentrations. It is necessary to review information from the manufacturer of the kit Charm Cow Side II Test for the detection of residues of oxacillin, penicillin G, spiramycin, and sulfonamides and Charm Blue Yellow II Test for the detection of erythromycin, cloxacillin, sulfadiazine, tylosin, and penicillin G in milk. Both kits detected residues of tetracyclines given the MRLs required by the Brazilian law and can be safely used for monitoring these drugs in milk. Ceftiofur and their metabolites were detected by the 2 kits and can be safely monitored by the methods tested.

Key words: milk, antimicrobials, residues

M101 Validation of CombiScope FTIR for milk urea evaluation in raw milk. M. C. P. P. Oliveira*, N. M. A. Silva, L. P. F. Bastos, R. S. Conrrado, L. M. Fonseca, M. M. O. P. Cerqueira, R. Rodrigues, and M. O. Leite, *Veterinary School/Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*.

The measurement of milk urea nitrogen (MUN) concentration in raw milk is a useful tool for herd nutritional evaluation. The aim of this study was to validate the CombiScope FTIR (Advanced/Delta Instruments) for analysis of urea content in raw milk samples, based on non protein nitrogen calculated urea (NPN-CU). A total of 513 samples of bulk tank raw milk were screened in the Laboratory for Milk Quality Analysis (Veterinary School, Universidade Federal de Minas Gerais) for this study. Calculated urea results generated by Fourier Transform Infrared in the CombiScope FTIR were compared with urea concentration obtained from analysis of the same samples by enzymatic automated method (Chemspec 150 Analyzer; Bentley Instruments). The repeatability of CombiScope was tested using 20 pools of raw milk samples preserved with bronopol. Each pool was distributed in 10 vials and each vial was placed in a rack, to complete 10 racks containing 20 samples each. There was no significant difference ($P > 0.05$) between the results generated by both methods. The FTIR showed an average of 9.93 mg/dL of urea, while the average for the automated enzymatic method was 9.49 mg/dL of urea. The standard deviation and coefficient of variation were, respectively, 3.31 mg/dL and 33% for FTIR and 4.22 mg/dL and 44% for enzymatic methods. The result of repeatability of the FTIR analysis of urea showed an average of 10.22 mg/dL, standard deviation of 0.87 mg/dL, coefficient of variation of 8.42% and repeatability limit of 2.43 mg/dL. Therefore, FTIR is a reliable method to be used for urea analysis in raw milk, with the advantage of being a rapid, efficient, versatile and low cost method. Acknowledgments: FAPEMIG, CNPq, CAPES, Laboratório de Análise da Qualidade do Leite/EV-UFMG/FUNDEP.

Key words: milk, FTIR, urea

M102 Identification of starch in cheese using laser scanning confocal microscopy. W. R. McManus, E. N. Oberg, R. Wadhwani, K. M. Brown, and D. J. McMahon*, *Western Dairy Center, Utah State University, Logan.*

Polysaccharides such as starches and gums have been used to modify texture in dairy products. However, there has been little published on the microstructure of these additions to foods unless they are large microparticles because of the difficulty in identifying them in a complex matrix. Laser scanning confocal microscopy (LSCM), is able to optically dissect thin layers through a food sample and identify multiple components when they have an attached fluorophore. For example, in cheese, fat can be labeled with Nile red, a hydrophobic fluorophore, excited by light with wavelength of 488 nm, and protein can be labeled with several fluorophores, including Rhodamine B (RHODB) excited at 568. There has not been a method for readily attaching fluorophores to polysaccharides. To do so, we have developed a method for chemically modifying starch in a food gel (after fixing protein and fat), so that it can bind a fluorophore. Milk gels, Cheddar and Mozzarella cheese containing starches were fixed with osmium tetroxide, as vapor or as a 1.0% (aq) solution, then oxidized using 0.5%(aq) periodic acid, stained with 1% (aq) Acriflavin HCl (ACRFL), and 0.01% (aq) RHODB. The fluorophores were excited and their fluorescence collected separately (using filters of 512 to 532 nm and above 585 nm, respectively) to generate images in which there was strong imaging of the locations of both polysaccharide by ACRFL and protein by RHODB. Control images of samples containing no polysaccharides demonstrated there was no cross reactivity of ACRFL with the fixed protein. Fixation in LSCM is not commonly used, however, using periodic acid to produce reactive dialdehyde groups on starch that can bind ACRFL mandates fixation, so that the proteins are not degraded. This also physically traps the polysaccharides within the protein gel, assuring that in an LSCM image of a milk gel or cheese, the relationship of the polysaccharides to fats and proteins has not changed from their original position.

Key words: cheese, starch, microstructure