Dairy Foods: Chemistry and Processing II

W1 Microfiltration and ultrafiltration process to produce micellar casein concentrate and milk protein concentrates with 80% protein content. P. Salunke, C. Marella*, and L. E. Metzger, *Dairy Science Department, Midwest Dairy Foods Research Center, South Dakota State University, Brookings.*

Milk protein concentrates (MPC) are used in applications that require dairy ingredients with higher level of protein relative to total solids (TS). MPC is essentially skim milk (SM) powder with reduced level of lactose. MPC has same ratio of casein to serum protein (SP) as found in SM. In some applications, protein ingredients with a reduced level of SP content may be preferred. The objective of the present research was to compare microfiltration (MF) and ultrafiltration (UF) processes for production of MCC and MPC with 80% protein relative to TS. 227 L of pasteurized SM was subjected to MF using 0.5-μm spiral-wound polyvinylede fluoride membrane. During the process, diafiltration (DF) water was added at 6 intervals, totaling to 100% of feed volume. In another process, 227 L of pasteurized SM from the same lot was subjected to UF using 10 kDa polyethersulfone membranes. During the process, DF water was added at 4 different intervals, totaling to a final addition of 40%. Both the processes used a volume reduction of 5. There were significant ($P <$ 0.05) differences in all of the compositional parameters except fat and casein for the MF retentate (MFR) and UF retentate (UFR). UFR had a higher total nitrogen (TN), TS, lactose, ash and calcium content as compared with MFR. This affected the TN/TS ratio found in both the retentates, MFR had a ratio of 81.7 and UFR had a ratio of 77.18. The differences in membrane pore sizes, operating pressures and level of DF used all contributed to the differences in final TN/TS ratio obtained. Capillary gel electrophoresis analysis of individual protein fractions present in the UFR and MFR showed that UFR has β-lactaglobulin to α-lactalbumin (α-LA) ratio of 2.57, which is close to the ratio found in SM. The MFR has a ratio of 3.57 indicating preferential transmission for α-LA by the MF membrane. The results from this study show that MF and UF processes could be used for production of MCC and MPC with similar TN/TS ratio with careful selection of operating parameters.

Key Words: MPC or MCC, micro- and ultrafiltration, capillary gel electrophoresis

W2 Understanding shear-induced aggregation in partially crystalline oil-in-water emulsions. G. Fuller*[1,2], T. Considine[1], M. Golding[2], L. Matia-Merino[2], and A. MacGibbon[1], *[1]Fonterra Co-operative Group Limited, Palmerston North, New Zealand, [2]Massey University, Palmerston North, New Zealand.*

Fat globules in food products such as cream are prone to irreversible aggregation due to the presence of both solid and liquid fat in the dispersed phase and weak interfacial films. This can lead to undesirable changes in both texture and functionality. Despite the need to understand and control this process, many questions still remain because the factors affecting aggregation are numerous and often interdependent. To study the aggregation process, 35% fat emulsions with different solid fat content (SFC) stabilized by 2% sodium caseinate were studied under shear (cone-and-plate geometry) at 5°C over 6 d. SFC was varied by combining hydrogenated palm kernel oil and canola oil. To study the effect of different interfacial film compositions, Tween 20 (0.5. 1.5 and 2.5% by wt) was added after homogenization. The results showed that emulsions containing 0.5% Tween 20 had distinctly different aggregation behavior compared with those with ≥ 1.5% Tween 20 regardless of solid fat content and the applied shear rate. At 0.5% Tween 20, aggregation time increased with increasing SFC whereas at ≥ 1.5% Tween 20, aggregation time decreased with increasing SFC. This behavior was likely due to a transition from a mixed protein-surfactant interface at 0.5% Tween 20 to a surfactant dominated interface at ≥ 1.5% Tween 20. By revealing how small changes in surfactant concentration significantly influence shear-induced aggregation behavior in oil-in-water emulsions with different SFC, these results will aid in the development of food products with improved shelf-life and stability.

Key Words: emulsion, shear-induced aggregation, partial coalescence

W3 Effect of Maillard-induced glycosylation on the molecular configuration of whey protein and its solubility and thermal stability for beverage applications. Q. Wang* and B. Ismail, *University of Minnesota, St Paul.*

Whey proteins are reasonably soluble in acidic beverages; however, thermal processing and prolonged storage can result in protein aggregation and subsequent deterioration of quality. Consequently, whey protein acidic beverages available on the market have a short shelf life and contain at most 4% protein, which is below the minimum percentage (4.2%) required by the FDA to claim a "high protein beverage." The objective was to determine the solubility, thermal stability, nutritional quality, and structural changes of partially glycosylated whey protein (PGWP). Maillard-induced glycosylation conditions were optimized to promote glycosylation of whey protein, while minimizing browning and maintaining nutritional quality. Solubility and thermal stability of PGWP and WPI were compared over a wide range of pH, protein concentrations, and heating temperatures and times. Differential scanning calorimetry and SDS-PAGE were employed to monitor onset of denaturation and polymerization, respectively. Samples were analyzed by surface-enhanced Raman spectroscopy and by matrix-assisted laser desorption ionization-time of flight mass spectrometry to determine secondary and tertiary structural changes and glycosylation sites, respectively. Compared with WPI, PGWP maintained higher solubility and thermal stability at protein concentrations greater than 4.2%, over a wide range of pH, including the pH around the isoelectric point (pI) of whey protein. The enhanced solubility and thermal stability was attributed to structural rigidity, unique glycosylation sites and resistance to denaturation. The nutritional quality was maintained and advanced stages of Maillard reaction were not detected. Our findings demonstrated the possibility of using PGWP in the production of high protein acidic beverages (>4.2% protein) and provided information that is essential to understand the structure/function relationship upon Maillard-induced glycosylation of whey proteins.

Key Words: whey protein, glycosylation, solubility

W4 Development of a multiclass method for determination of 38 veterinary drugs in milk by ultra-high-performance liquid chromatography-tandem mass spectrometry. R. W. Han[1,3], N. Zheng[1,2], J. Q. Wang*[1,2], Z. N. Yu[3], X. M. Xu[1,2], Y. P. Zhen[1,2], X. Y. Qu[1,2], and L. C. Huang[1,2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Ministry of Agriculture–Milk and Dairy Product Inspection Center (Beijing), Beijing, China, [3]College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, Shandong, China.*

A simple, selective and rapid multi-residue method was developed to determine 38 veterinary drugs simultaneously in milk by ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The selected veterinary drugs include 14 β-lactams, 8 quinolones, 8 sulfonamides, 5 tetracyclines, and 3 macrolides. The analytes were extracted by water-acetonitrile (1:3), and then purified using Oasis HLB cartridge. The elution with water-acetonitrile (8:1) was injected into the UPLC-MS/MS system on a Waters UPLC C_{18} column in gradient mode. Multiple reaction monitoring (MRM) experiments in the positive ionization mode were performed to achieve data acquisition under MS/MS. Results showed good accuracy and repeatability. Average recoveries for different veterinary drugs in milk were 67.9–117.5% for β-lactams, 79.3–117.7% for quinolones, 71.3–106.1% for sulfonamides, 76–115.5% for tetracyclines and 78.2~106.1% for macrolides. The coefficients of variation (C.V.) of the recoveries were less than 15% for intraday and interday precisions. The limits of quantification (LOQs) for β-lactams, quinolones, sulfonamides, tetracyclines and macrolides were 0.3–10 ng/mL, 0.03–0.6 ng/mL, 0.03–0.3 ng/mL, 0.6 ng/mL and 0.03–0.6 ng/mL, respectively. Finally, the method was applied to 25 raw milk samples and traces of some veterinary drugs under allowable levels were detected, such as flumequine, sulfapyridine, sulfamethoxazole and lincomycin.

Key Words: veterinary drugs, milk, UPLC-MS/MS

W5 A UPLC-MS/MS method to simultaneous determine aflatoxin M1, ochratoxin A, zearalenone and α-zearalenol in milk. L. C. Huang[1,3], N. Zheng[1,2], J. Q. Wang*[1,2], J. B. Cheng[1,3], R. W. Han[1,2], X. M. Xu[1,2], and S. L. Li[1,2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Ministry of Agriculture–Milk and Dairy Product Inspection Center (Beijing), Beijing, China, [3]College of Animal Science and Technology, Anhui Agricultural University, Hefei, China.*

Our objective was to develop an ultra-high-performance liquid chromatography triple-quadrupole spectrometry (UPLC-MS/MS, TQ-S, waters, USA) method to simultaneously determine aflatoxin M1, ochratoxin A, zearalenone and α-zearalenol in milk. The analytes were extracted by acetonitrile, and purified by solid phase extraction (SPE) using Oasis HLB cartridge, and then injected into the UPLC-MS/MS system. The mycotoxins were separated by UPLC BEH C_{18} column in gradient mode and determined in multiple reaction monitoring mode under positive- and negative-mode electrospray ionization. The optimized conditions of purification were SPE pH 5.0, eluting solution of 100% methanol, SPE flow rate of 1.5 mL/min and washing water of 2 mL. The matrix effects of 3 milk matrices, including raw milk, liquid milk and milk powder, were evaluated by the signal suppression-enhancement and compensated by external matrix-matched calibration. Correlation coefficients (r^2) of external matrix-matched calibration curves were higher than 0.996 in their respective linear ranges (0.01–1.00 μg/kg). The LOD and LOQ ranges of 4 mycotoxins selected were 0.001–0.005 μg/kg and 0.003–0.015 μg/kg, respectively. The method validation in 3 matrices at low (0.025 μg/kg) and high (0.5 μg/kg) spiked levels obtained reasonable recoveries (87.0–109%) and repeatability (CV < 10%). Intra- and inter-day tests at levels of 0.025 μg/kg also got satisfactory recoveries (23.1–25.4 μg/kg) and RSDs (7.4–9.9%). Finally, the method was successfully applied to milk samples, and traces of 4 mycotoxins were detected. The results demonstrated that the proposed method was sensitive, reliable and robust. Therefore, the developed method was suitable for the simultaneous determination of 4 mycotoxins in milk and could be performed for their routine analysis in mycotoxin study and survey.

Key Words: mycotoxin, milk, UPLC-MS/MS

W6 Comparison of amino acid composition of milk from different species. J. X. Zhang[2,3], J. Q. Wang*[1,2], D. P. Bu[2], J. H. Yang[2], L. Ma[2], and J. T. Chen[2], *[1]Agronomy College of Heilongjiang August First Land Reclamation University, Heilongjiang, China, [2]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [3]Xinjiang Agricultural University, Urumqi, China.*

The objective of this study was to investigate variation of milk amino acids composition of different dairy animals. Milk samples from cows, goats, yaks and buffalos (n = 20 for each species) were obtained from different farms, and transported to the laboratory as packed for analysis. Amino acids were extracted by hydrolyzed with 7.8 mol/L HCl for 24 h, and concentrations of amino acids were determined by Hitachi L-8900 high speed amino acid analyzer. Data was processed by Excel and analyzed by SAS 9.0 and PCA with The Unscrambler 9.8 procedure. Results showed that concentrations of all tested amino acids were different among the cow, yak, buffalo and goat ($P < 0.01$). Concentration of total AA was found to be yak > buffalo > goat > cow (11024.49, 8271.16, 6325.35, 5688.38). According to Ser (118.60, 171.55, 234.44), Thr (119.39, 181.45, 230.47), Val (203.83, 287.27, 376.91) and Tyr (107.62, 132.26, 172.70) profiles base of the PCA scores and loading plots, cow, buffalo, and yak milk were grouped together, whereas goat milk (the 4 amino acids were 170.45, 176.08, 254.19, 128.09) was in a second group. Buffalo and yak milk showed higher Pro (483.01, 613.23) and Ile (263.96, 321.50) concentrations compared with the cow (Pro 132.50, Ile 230.16) samples, whereas yak (518.21) milk was characterized by a higher concentration of Lys compared with the milk from buffalo (368.53). It was concluded that the 4 specials showed distinct amino acids profiles.

Key Words: milk, species, amino acid

W7 Determination of milk composition using near-infrared transflectance spectrum. L. Ma[1,2], J. Q. Wang*[1], D. P. Bu[1], J. H. Yang[1], J. X. Zhang[1], and J. T. Chen[1], *[1]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China.*

Near-infrared transmission/reflection spectrum has been used in food and agricultural products test extensively. The objective of this study was to investigate main components in milk using near-infrared transflectance spectrum. Milk samples (n = 150) were collected from one dairy farm, and protein (3.70 ± 0.24%), fat (4.56 ± 0.69%), lactose (5.02 ± 0.14%), total solids (14.09 ± 0.79%) and non-fat solids (9.74 ± 0.27%) in milk were measured by MilkoScan FT 120. Milk transflective spectra ranged from 400 to 2500 nm were collected using NIRS DS2500, with sample 2 mm thick. Data was exported to the Unscrambler 9.8 (CAMO, Oslo, Norway) for multivariable analysis. The same samples were employed for calibration and full cross validation. Regression model of spectroscopy was constructed via partial least squares. The loading plots showed that middle and long wavelength (1300–2500 nm) spectroscopy contributed much to milk components model. Satisfied results of calibration and prediction were obtained in this study, for high determination coefficients (R^2) of calibration and validation for protein, fat, lactose, total solids and non-fat solids above 0.90. Root mean square error (RMSE) of validation suggested better prediction accuracy for milk protein, lactose, and nonfat solids, which were less

than that of fat and total solids. Excellent ratio of prediction to deviation (RPD) value was observed for protein, while RPD for fat, lactose, total solids and non-fat solids were acceptable. It was concluded that near infrared transflectance spectrum would be capable of in milk composition determination.

Table 1. Parameters of milk composition model

			Calibration			Validation			
Milk composition	Pre-treatment	PC	Predicted Y vs. measured X	RMSE	R^2	Predicted Y vs. measured X	RMSE	R^2	RPD
Protein	No	9	Y =0.969x +0.114	0.036	0.98	Y=0.960x +0.148	0.045	0.97	4.97
Fat	No	6	Y =0.916x +0.383	0.177	0.93	Y=0.910x +0.410	0.197	0.92	3.06
Lactose	MSC	12	Y =0.928x +0.362	0.029	0.96	Y=0.911x +0.448	0.042	0.91	3.15
Total solids	SNV	5	Y =0.922x +1.093	0.219	0.92	Y=0.915x +1.188	0.234	0.91	3.41
Nonfat solids	SNV	11	Y =0.940x +0.569	0.067	0.94	Y=0.927x +0.695	0.077	0.92	3.54

Key Words: near-infrared transflectance spectrum, milk composition

W8 Evolution of milk calcium content during the year. C. Hurtaud*[1], M. Johan[1], S. Leurent[2], Y. Gallard[2], and L. Delaby[1], *[1]INRA-Agrocampus Ouest UMR 1348 PEGASE, Saint-Gilles, France, [2]INRA Domaine du Pin-au-Haras, Exmes, France.*

Calcium content is regarded as being relatively stable in cows' milk during lactation. However, results from commercial herds, have suggested that changes in milk calcium (Ca) content can occur. The objective of this experiment was to compare the characteristics of milk (especially milk Ca content) based on low input grass based systems compared with corn silage based systems, across 2 breeds of dairy cows (Holstein, HO vs. Normande, NO).The experiment took place on the INRA experimental farm of Le Pin-au-Haras. Sixty dairy cows were observed from calving to drying off. Two feeding systems were compared. The Intensive system (IS) was designed to maximize individual performance, with a high energy diet (in winter, corn silage with 30% concentrate; in spring, summer and autumn periods, pasture with 4 kg/d of concentrate supplemented with corn silage from July). The Grass system (GS) was designed to decrease inputs (in winter, conserved grass with no concentrate; in spring, summer and autumn, pasture with no concentrate). The experimental design was a continuous design. No significant interaction was detected between feeding system and breed for milk yield and composition. During the whole year, GS treatment reduced milk yield (−5 kg/d), with no significant effect on protein and total Ca contents. The NO cows produced less milk, but with higher protein and total Ca contents. During winter, GS treatment reduced milk yield, protein and total Ca contents. Milk Ca content decreased from January to June and rapidly increased after July irrespective of breed. During May and June, HO milk had a Ca concentration below the French limit of 1.2 g/L of the European health allegation of milk for consumption. There was a significant effect of stage of lactation and month on Ca content. Month included numerous significant factors such as maximum daily temperature, day length and radiance duration. This study clearly showed that both cow genotype and lactation stage affects milk Ca content. However, the lactation stage did not explain all the seasonal variations of milk calcium content observed in this trial.

Key Words: milk, dairy cow, calcium

W9 Quantitative analysis of supercritical carbon dioxide (sc-CO_2) treated β-lactoglobulin tryptic peptides. C. Kembel* and R. Jimenez-Flores, *California Polytechnic State University, San Luis Obispo.*

Whey protein is an abundant source of biologically active peptides that have a diverse set of functional properties. One limitation in the production of novel peptides is the native folding of the proteins secondary and tertiary structure. β-Lactoglobulin is a barreled protein with a hydrophobic core capable of binding other proteins. Due to its abundance in whey, it represents an important source of bioactive peptides. We present a method with the potential for the production of novel bioactive peptides from whey. Supercritical CO_2 is known to change protein conformation due to its hydrophobic properties, high diffusion coefficient, low viscosity, and low surface tension. Because β-Lactoglobulin contains a large hydrophobic core where binding sites are present, sc-CO_2 treatment is likely to expose these hydrophobic sequences. These sequences are normally confined to the interior of the protein away from enzymatic attack under normal conditions. Therefore upon exposure to sc-CO_2, these sequences were shown to be more susceptible to trypsin hydrolysis. This method increases the potential for the production of novel peptides. Minor changes in the temperature of the sc-CO_2 treatment induced subsequent conformational changes resulting in different peptides. Variable conditions (pH, temperature, and pressure) sc-CO_2 was shown to yield unique fingerprints by SDS-PAGE. Capillary electrophoresis was then used to discern the peptide profiles of the sc-CO_2 treated peptides as well as native peptides. Distinct differences in peptide profiles were noted. Subsequent analyses will include HPLC-MS to determine peptide sequences and quantity differences in peptide treatments.

Key Words: supercritical CO_2, whey protein, bioactive peptide

W10 AFM imaging and analysis of phospholipid monolayers. J. Cuthbert*[1], S. Gallier[2], D. Gragson[1], and R. Jimenez-Flores[1], *[1]California Polytechnic State University, San Luis Obispo, [2]Massey University, Palmerston North, New Zealand.*

Membrane structure analysis in biological systems is fundamental for understanding its relationship with function. Membrane analyses still relies on observations of the physical phenomena of its components in model systems. Dairy foods are complex systems that incorporate parts of colloidal properties with emulsions. The emulsion in a dairy system is unique due to the presence of the milk fat globule membrane (MFGM). This membrane is composed of phospholipids and proteins, and it has an important biological function in the initial stages of mammals' lives, and in humans it has an important nutritional role throughout their life. In our work to elucidate the structure/function relationship of the MFGM, we are exploring some structural aspects of its phospholipids. Therefore, in this work we present how the properties of the phospholipid/protein monolayers found in milk fat globules were investigated using atomic force microscopy (AFM) in a model system with phospholipids extracted from raw milk and either β-casein or β-lactoglobulin. Slides of the phospholipid and phospholipid-casein monolayer films were prepared as models from a Langmuir trough. The aim was to determine if the monolayers would form liquid-ordered domains or alternatives. Our observations indicate that this technique distinguishes liquid ordered domains in AC contact mode. In this mode, the cantilever oscillates above the surface. It is gentle enough to image phospholipid monolayers without damage. When the tip approaches the surface, the sample's intermolecular and electrostatic forces change the cantilever's amplitude and phase angle. As the cantilever is affected by surface forces, a piezoelectric scanner adjusts its height. The monolayer

domains were best observed in the height and phase traces where the ridges were salient. Because the monolayer domains were more elastic than the surrounding surface, they can be observed in the phase trace. A typical monolayer region was approximately 2 nm high and showed a change of 4° in the phase trace. We conclude that the AFM is a good technique to measure changes in monolayers that could happen during digestion of lipids.

Key Words: atomic force microscopy, milk fat globule membrane, monolayer

W11 Correlation between solubility and solubility index of high protein milk protein concentrates. H. Patel*[1], P. Salunke[1], and J. Amamcharla[2], *[1]Dairy Science Department, South Dakota State University, Brookings, [2]Animal Sciences and Industry, Kansas State University, Manhattan.*

Solubility of high protein ingredients such as milk protein concentrate (MPC) is an important primary property. Measuring the solubility of MPC is a lengthy and tedious process. Developing a simple, quick, and reliable procedure can save time and efforts. Solubility Index (SI) is a well-established method for the determination of solubility of milk powders. The SI is a good indicator of insoluble constituents of milk powders. However, no reports are available in the literature indicating the use of SI for predicting solubility of MPC. The objective of present study was to find out whether correlations exist between SI and percent solubility of MPC80 under a wide range of reconstitution conditions. Five representative samples of MPC80 with a similar protein (81–82%), lactose and mineral contents were obtained from different countries. Solutions (5% w/w) were prepared by reconstituting MPC at 4 different temperatures (20, 40, 50 and 60°C). During reconstitution, aliquots from each of this solution were withdrawn at different time intervals (2, 10, 20, 30, 45 and 60 min). A total of 120 samples were analyzed in this way. The SI of these samples was determined using a centrifugation method at 700g for 10 min). Percent solubility was determined by the standard gravimetric method. The data were analyzed using PROC REG available in SAS and the correlation coefficient (r^2) between percent solubility and SI was obtained. The results of the present study indicated that there was a strong correlation between the solubility and SI measured at different time and temperature of reconstitution. As the temperature and time of reconstitution increased, there was a significant ($P < 0.05$) increase in the solubility. Consequently, there was a significant ($P < 0.05$) decrease in SI. The r^2 value of 0.94 for different reconstitution temperatures and that of 0.81 for different reconstitution time indicated that SI can be used as a quick and routine method in place of the traditional solubility method for characterizing the solubility of MPCs as it is simple, fast, reliable, and easy to perform.

Key Words: milk protein concentrate (MPC), solubility, solubility index (SI)

W12 Phospholipids from milk help cancer prevention in skin cell culture. L.-A. Nguyen*[1], L. H. Laiho[1], and R. Jiménez-Flores[2], *[1]California Polytechnic State University, Biomedical Engineering Department, San Luis Obispo, [2]Dairy Products Technology Center, San Luis Obispo.*

Milk phospholipids (MPL) have a unique composition due to their biological role of engulfing the milk fat globules as milk is synthesized. This unique combination of MPL has been studied and found to have diverse biological activities or functions. In this study we focused on measuring the protective effect of milk phospholipids on skin cell culture after exposure to UV light. This model system has been used as means to identify skin cancer preventing agents. Our study focused on evaluating the expression of a UV-induced DNA damage marker, cyclin-dependent kinase inhibitor, p21WAF1/CIP1. Our previous work had shown some preliminary histology and MTT tissue viability results, which suggested that MPL act upon skin cells in a protective manner against UV (UV) radiation. Western Blots were used to quantify p21 expression in human keratinocytes in 4 categories of samples: No-UV, UV, UV+MPL, MPL and in HeLa (p21 positive control). In the No-UV samples, cells were not irradiated by UV light. Treatment consisted on exposure to a UV dosage of 10 mJ/cm^2. After UV treatment, the same amount of protein from each sample (determined by BCA assay) was loaded into a 4–12% Bis-Tris SDS-PAGE gel, run under denaturing, non-reducing conditions then blotted and treated with antibodies for the quantitative detection of p21 proteins. Finally, intensities of p21 protein bands were analyzed. Under non-reducing conditions, 3 p21 proteins covalently bonded with each other showed up as 63 KDa molecules on the PVDF membrane. The UV and HeLa samples showed a 2.28 fold, and 1.23 fold increase in p21 expression, respectively, compared with the No-UV samples control. The MPL samples showed a 0.948 fold decrease in p21 compared with the No-UV samples, and the UV+MPL samples showed only a 1.13 fold increase in p21. Less p21 expression in the UV+MPL samples compared with the UV samples suggested that less DNA damage occurred in the samples that were treated with MPL. Conclusion: Milk Phospholipids reduced UV-induced DNA damage in human keratinocytes through incorporation in cell media and could potentially be incorporated as a chemopreventive agent.

Key Words: milk phospholipid, cancer, UV

W13 Reduction of aflatoxin M_1 content during manufacture and storage of Egyptian Domiati cheese. M. Motawee*, *National Organization for Drug Control and Research, Cairo, Egypt.*

Elevated levels of aflatoxin M_1 (AFM_1) in milk and milk products is considered to pose certain hygienic risks for human health. The maximum level of AFM_1 allowable in Egyptian milk is 50 ng/L and while a previous study found the majority of milk was below this level, some milk contained up to 250 ng/L. Domiati cheese is the most popular soft white pickled cheese in Egypt and accounts for 75% of the cheese produced and consumed in Egypt. During manufacture of Domiati cheese from 5% to 14% of salt is added to the milk depending on the season and cheese ripening temperature. The aim was to determine what proportion of initial AFM_1 in milk is retained after manufacture into Domiati cheese and remains during 90 d storage. Milk was inoculated with 1µg/kg AFM_1 then pasteurized at 63°C for 30 min and made into Domiati cheese using salt additions of 6%, 8% and 10% (wt/wt). Cheese making was performed on 3 separate occasions. The AFM_1 levels in milk, cheese and whey were determined using an ELISA test kit. Pasteurization of milk caused ≤ 10% loss of AFM_1. About 60%, 58%, and 56% of total AFM_1 remained in cheese curd made using 6%, 8% and 10% salt respectively, with the residual being lost in the whey. After 2 wk storage at 20°C, all of the cheeses had a 17% reduction in AFM_1 compared with their levels after manufacture. With continued storage through 90 d the losses of AFM_1 were significantly different ($P < 0.05$) with reduction in AFM_1 or 20.5%, 21.4%, 22.0% for cheeses made using 6%, 8% and 10% salt respectively. Thus, including pasteurization of milk, conversion of milk into Domiati cheese and its subsequent storage period for 3 mo produced an overall 32% reduction of AFM_1. In conclusion, as well as avoiding contamination of milk with AFM_1 there is a lower health risk to the population from the presence of AFM_1 in

milk when the milk is pasteurized and converted into Domiati cheese that is then stored for the customary 3 mo.

Key Words: aflatoxin, Domiati cheese, storage

W14 Limited glycerolysis and transesterification reactions to change the fatty acid composition and crystallization properties of butterfat. D. Sanchez-Macias[2,1], A. Laubscher*[1], and R. Jimenez-Flores[1], *[1]California Polytechnic State University, San Luis Obispo, [2]Agroindustrial Engineering Department, Universidad Nacional del Chimborazo. Riobamba, Ecuador.*

Saturated fatty acids in butterfat have been regarded as a nutritional disadvantage in some dairy products. Several different strategies have been tried and tested for the modification of the fatty acid composition of bovine milk. The nature of the rumen forces the cow to produce saturated fatty acids, and bypass techniques have limited results. Enzymatic modification of milk fat has also limits in efficiency since enzymes work only in an aqueous media and therefore only in the interface of emulsions. Fractionation of butterfat based on differential crystallization had in the past some limited success, but no clear difference was obtained by removing crystals formed at relative high temperatures, where saturated fatty acids were assumed to be present in higher concentration. We approached this strategy, by attempting a chemical rearrangement of fatty acids in the triglycerides of milk fat by a limited glycerolysis and transesterification, in which glycerol was used as an intermediary for rearrangement of fatty acids, and limited amounts of oleic acid were added. Glycerolysis and transesterification took place in the same vessels with minimal amount of alcoholic KOH as catalyzer and a temperature of 200°C for 2 h. Resulting combinations of triglycerides from this reaction showed significantly different fatty acid distribution; Control butterfat heated without any catalyzer served as control and reference. The major differences were observed when comparing the crystals separately from the remaining oil fraction. Even in the group without oleic acid, the crystals showed fatty acids increase in 16:0 and 18:0 of 17 and 45% respectively; changes of fatty acid composition on the other tested fractions was even more pronounced. The crystal fractions were drastically different in morphology and color in the treated samples compared with controls. We conclude that there is some technological potential in partial chemical glycerolysis and transesterification to modify the composition of butterfat fractions

Key Words: fatty acid, butterfat, glycerolysis transesterification

W15 The effects of microfluidization on the particle size distribution of liposomal aggregates between whey buttermilk and commercial sweet buttermilk. T. Nguyen* and R. Jimenez-Flores, *California Polytechnic State University, San Luis Obispo.*

Milk-derived ingredients from the production of cheese and butter can be used as vehicles for nutrients. One of the advantages of ingredients from milk that contain milk fat globule components such as phospholipids is that they form emulsions with fat, or liposomes when treated with high shear. Our objective in this work was to measure the effect of shear on regular buttermilk and whey buttermilk. The effects of microfluidization at 2000 psi on the particle size distribution of liposomal aggregates between whey buttermilk and commercial sweet buttermilk at pH 4.6 and 6.8 were compared with whey protein isolate. At pH 4.6, the average aggregate size increased in sweet buttermilk after every one of 3 passes total through the microfluidizer. There was a slight decrease in the average aggregate size of whey buttermilk after the first pass and an increase in the average size for the second and third passes. Similarly, a slight decrease was seen in the average particle size of whey protein isolate after the first 2 passes and an increase in the average size after the third pass. The aggregate size distribution of whey buttermilk resembled that of whey protein isolate at pH 6.8. There was an alternate decrease and increase in the average aggregate size after each pass. In contrast, a slight decrease in average particle size was seen in sweet buttermilk after the first 2 passes and an increase in size after the final pass at pH 6.8. At pH 4.6, whey protein isolate and both buttermilks had a greater number of small particles after microfluidization. In contrast, at pH 6.8, each dairy product had a greater number of larger particles. This could be due to hydrophobic interactions between the aggregates. This study suggests that microfluidization at various pH affects the size distribution of whey buttermilk aggregates or liposome particles so that size can be manipulated and therefore can be utilized as a novel ingredient and in the processing of dairy foods to deliver nutrition.

Key Words: phospholipid, microfluidization, nutrition delivery

W16 Fast and easy screening of whey protein types using a novel portable infrared spectroscopy. T. Wang* and L. Rodriguez-Saona, *The Ohio State University, Columbus.*

Whey proteins are attractive ingredients to the food industry because of their high nutritional value and wide functionality. Whey protein powders are available from different suppliers using various processing methods and conditions, resulting in variability in their macromolecular structure, components levels and thus having an effect on their functionality. There are 3 major types of whey protein including whey protein isolates (WPI), whey protein concentrates (WPC) and whey protein hydrolysates (WPH) providing diverse functionality to food applications. Our objective was to develop a simple and rapid method to differentiate whey protein types by combining a portable infrared spectrometer and pattern recognition analysis. Whey protein powders including WPI (n = 23), WPC (n = 8) and WPH (n = 14) from different suppliers were evaluated. A portable infrared spectroscopy (Cary 630, Agilent Technologies) was used for spectra collection by pressing the whey protein onto an ATR-IR diamond crystal with a pressure clamp. Spectra were analyzed by soft independent modeling of class analogy (SIMCA) for powder classification. SIMCA model showed a strong ability to differentiate whey protein types by forming tight clusters far from each other (interclass distances > 3). Major band responsible for separation was associated with carboxylic acid side chain in amino acids present in whey proteins. Portable IR units enable to quickly assess the quality of the incoming raw material allowing for timely corrective measures during manufacture. Portable systems are simple to use and require minimal or no sample preparation, thus reducing assay time and helping to streamline the analytical procedure so that it is more applicable for field-based screening and higher sample throughput.

Key Words: whey protein, infrared spectroscopy, pattern recognition analysis

W17 Effect of transglutaminase treatment on the functionality of MPC and MCC: Functional properties. P. Salunke*, C. Marella, and L. E. Metzger, *Dairy Science Department, Midwest Dairy Foods Research Center, South Dakota State University, Brookings.*

Milk protein concentrate (MPC) and micellar casein concentrate (MCC) are products manufactured using ultra- and micro-filtration

respectively. Functional properties such as alcohol stability (AS), heat stability (HS), solubility and solubility index (SI) are affected by presence or absence of constituents in MPC and MCC particularly, casein, whey proteins, lactose, and minerals. However, MPC and MCC alone are unable to deliver critical functional properties required in certain products such as high heat stable products, emulsions, and processed cheese products. The use of cross-linking enzymes such as transglutaminase (TGase) has the potential to modify the physical properties of MPC or MCC and may improve their functional properties. The objective of this study was to determine the effect of TGase treatment of MPC and MCC retentates on the functionality of MPC and MCC. Three lots of MCC and MPC retentate were produced using 3 different lots of pasteurized skim milk. Each replicate of retentate was divided into 3 equal portions. One portion of the retentate was treated with TGase at 0.3 units/g of protein, one portion was treated with TGase at 3.0 units/g of protein and one had no TGase addition. All the retentates were incubated for 25 min at 50°C, heat treated at 72°C for 10 min, cooled to 4°C and then spray dried. Various functional properties including AS, HS, solubility and SI at room temperature (RT) and hot water were tested using 5% protein solution of MCC and MPC. MPC samples had significantly ($P \leq 0.05$) higher AS, HS, solubility and significantly ($P \leq 0.05$) lower SI as compared with respective MCC treatments. TGase treatment significantly ($P \leq 0.05$) increased the AS, HS, SI, and significantly ($P \leq 0.05$) decreased the solubility. The study demonstrates that TGase treatment was found to significantly ($P \leq 0.05$) affect and modifythe functionality of MCC and MPC. These TGase treated ingredients can be used in products where whey protein have detrimental effect including high heat stable products, emulsions or processed cheese products.

Key Words: transglutaminase, MPC or MCC, functional properties

W18 Effect of transglutaminase treatment on the functionality of MPC and MCC: Yogurt formulation. P. Salunke*, C. Marella, and L. E. Metzger, *Dairy Science Department, Midwest Dairy Foods Research Center, South Dakota State University, Brookings.*

Milk protein concentrate (MPC) can be used in yogurt formulations to increase the protein content of yogurt. In contrast micellar casein concentrate (MCC) produced using microfiltration has a reduced level of whey protein and has not been utilized in yogurt. The use of transglutaminase (TGase) has the potential to modify the physical properties of MPC or MCC and may improve its functionality in yogurt. The objective of this study was to determine the effect of TGase treatment of MPC and MCC retentates on the functionality of MPC and MCC when they are used in yogurt formulation. Three lots of MCC and MPC retentate were produced using 3 different lots of pasteurized skim milk. Each replicate of retentate was divided into 3 equal portions. One portion of the retentate was treated with TGase at 0.3 units/g of protein, one portion was treated with TGase at 3.0 units/g of protein and one had no TGase addition. All the retentates were incubated for 25 min at 50°C, heat treated at 72°C for 10 min, cooled to 4°C and then spray dried. Each MCC and MPC was then used in a yogurt formulation that was standardized to 8.5% MSNF and 5.75% protein using deproteinized whey powder and distilled water. In each formulation, the MPC or MCC utilized contributed 5% protein. All the ingredients were mixed and were subjected to heat treatment in the rapid visco analyzer (RVA) at constant speed of 150 rpm using a profile where it was heated from 45°C to 93°C over a period of 10 min, held for 6 min at 93°C, and cooled to 45°C in 10 min. After cooling to 45°C, the sample was acidified using glucono-δ-Lactone and mixed thoroughly at 500 rpm for 1 min, incubated at 45°C in water bath for 2.5 h and subsequently stored at 4°C overnight. The yogurt manufactured using MPC had significantly ($P \leq 0.05$) higher RVA-viscosity, water holding capacity (WHC) and syneresis as compared with the MCC samples. In the MCC yogurt samples there was significant ($P \leq 0.05$) decrease in RVA-viscosity, WHC and syneresis as enzyme level increased. TGase treatment was found to significantly ($P \leq 0.05$) affect the functionality of MCC and MPC in yogurt formulations.

Key Words: transglutaminase, MPC or MCC, yogurt functionality

W19 Adiponectin concentrations in cow milk during induced negative energy balance. S. P. Singh*[1], S. Häussler[1], J. J. Gross[2], R. M. Bruckmaier[2], and H. Sauerwein[1], *[1]Institute of Animal Science, Physiology and Hygiene Group, University of Bonn, [2]Veterinary Physiology, Vetsuisse Faculty University of Bern.*

Induced negative energy balance (NEB) in dairy cows decreased milk protein concentration. Fat mobilization during NEB may affect the concentrations of the adipokine adiponectin (Aq) in blood but also in milk. We therefore aimed to investigate the effect of a deliberately induced NEB on Aq concentrations in these body fluids. Multiparous Holstein cows (n = 21) were allocated to either a control (C, n = 10) or restriction (R, n = 11) group after 12 wk of lactation. Feeding in the preceding period and in the C group was according to the recommendations of Society of Nutrition Physiology. In R, an energy deficit was induced of at least 30% of the calculated requirements. Skim milk samples from 2 consecutive milkings each in wk 2, 12 and 17 of lactation (wk 17 = 2nd wk of R or C feeding) and plasma samples from these times were used. Milk protein content was assessed by infrared analyzer and Aq was measured by ELISA (Mielenz et al., 2013, Domest. Anim. Endocrinol.). The intra- and interassay variations were 4.5% and 11.9%. The limit of detection was 0.03 ng/mL. Assay accuracy was determined by linearity of serial samples dilutions. Milk Aq (μg/mL and ng/mg milk protein) were calculated and data (means ± SEM) were analyzed by Mixed Model (SPSS). Milk Aq concentrations (μg/mL) were higher in wk 2 than in wk 12 of lactation (0.88 ± 0.05 vs. 0.47 ± 0.03; $P < 0.001$). Numerically less Aq was observed in milk of R compared with C cows (0.43 ± 0.03 vs. 0.56 ± 0.06; $P > 0.05$); plasma Aq was not different between R and C. Across all animals in wk 12 and 17, milk Aq was correlated with plasma Aq (r = 0.342; $P = 0.026$). Blood Aq during this period was about 70 fold higher than in milk (35.02 ± 1.15 vs. 0.48 ± 0.02). When expressed as per mg of milk protein, Aq concentrations in wk 2 were also higher (1.7 fold) than in wk 12 ($P < 0.001$), and R animals had slightly lower concentrations (−15%, $P > 0.05$) than C cows. Our results indicate a significant decline in milk Aq both per volume and per mg milk protein as lactation advances. The lack of differences between the R and C group indicates that an induced NEB at this stage of lactation does neither affect Aq secretion nor transfer from blood to milk.

Key Words: adiponectin, milk, feed restriction

W20 Effect of transglutaminase treatment on the functionality of MPC and MCC: Imitation mozzarella cheese manufactured in twin screw cooker. P. Salunke*, C. Marella, and L. E. Metzger, *Dairy Science Department, Midwest Dairy Research Center, South Dakota State University, Brookings.*

A critical parameter in dairy based imitation mozzarella cheese (IMC) is the amount of intact casein provided by dairy ingredients in the formulation. From a functionality perspective, rennet casein is the preferred ingredient to provide intact casein in a formulation. However, the use of transglutaminase (TGase) has the potential to modify the physical properties of MPC or MCC and may improve its functionality in IMCs. The objective of this study was to determine the effect of TGase treatment of MPC and MCC retentates on the functionality of MPC and MCC when they are used in IMCs. Three lots of MCC and MPC retentate were produced using 3 different lots of pasteurized skim milk. Each replicate of retentate was divided into 3 equal portions. One portion of the retentate was treated with TGase at 0.3 units/g of protein, one portion was treated with TGase at 3.0 units/g of protein and one had no TGase addition. All the retentates were incubated for 25 min at 50°C, heat treated at 72°C for 10 min, cooled to 10°C and then spray dried. Each MCC and MPC was then used in IMC formulation that was standardized to 21% fat, 1% salt, 48% moisture, and 20% protein. In each formulation, the MPC or MCC utilized contributed all the protein. A preblend of all the ingredients (except lactic acid) was prepared (4.0 kg) in the Blentech twin-screw cooker by mixing at 50 rpm for 20 min at 20°C. The temperature of the preblend was then increased to 74°C over 5 min and held for an additional 4 min maintaining the auger speed of 120 rpm. The IMC formulation using either MCC or MPC treated with the highest TGase level did not form an emulsion. The IMC made from MCC treatments had significantly ($P \leq 0.05$) higher TPA-hardness and stretchability on pizza as compared with their respective MPC treatments. The IMC made from TGase treated MCC and MPC had significantly ($P \leq 0.05$) lower melt area, and significantly ($P \leq 0.05$) higher transition temperature and stretchability as compared with their respective controls. The study demonstrates that TGase treatment modifies the functionality of MCC and MPC in IMC applications.

Key Words: transglutaminase, MPC or MCC, IMC functionality