323 From giant unilamellar vesicles (GUVs) to lipid organization of bovine milk fat globule membrane (MFGM). H. Zheng*[1,2], R. Jiménez-Flores[2], and D. Everett[1], *[1]Riddet Institute and Department of Food Science, University of Otago, Dunedin, New Zealand, [2]Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

The milk fat globule membrane (MFGM) is believed to play an important role in many physiological processes. The bio-functionalities of the MFGM are regulated, not only by functional molecules (e.g., phospholipids and membrane proteins), but also by their structural organization. However, to date, the structure and dynamics of MFGM are not fully understood. Giant unilamellar vesicles (GUVs) were introduced and developed in biological and physiological studies to investigate the morphology of lipid organization. Lipids were selected [phosphatidylethanolamine (PE, 18:1), phosphatidylcholine (PC, 16:0), sphingomyelin (SM, 16:0 and 23:0) and cholesterol] to construct GUV systems under an alternating current (AC) electrical field in a specially designed chamber. The unitary, binary, ternary and quaternary systems from model combinations of PE, PC, SM and cholesterol were applied to form GUVs, and a comparative study was conducted to find out differences in structural morphology and conditions of GUV formation using microscopic techniques. Images obtained from confocal laser scanning microscopy showed both head group-labeled (lissamine rhodamine B, Rd) and fatty acid tail-labeled (NBD) dyes stained well on GUVs and emitted decent fluorescent signals from laser excitation. The PE-labeled dye was also well-stained in unitary PC-GUV systems. The diameter of the generated GUVs (formed in a sucrose buffer medium) were from 20 μm to more than 100 μm. The current fundamental study provides a reproducible procedure for constructing GUVs from bovine milk phospholipids.

Key Words: giant unilamellar vesicles (GUV), milk fat globule membrane (MFGM), phospholipid

324 Centrifugal washing processes reveal lipid organization of bovine milk fat globule membrane (MFGM). H. Zheng*[1,2], R. Jiménez-Flores[2], and D. Everett[1], *[1]Riddet Institute and Department of Food Science, University of Otago, Dunedin, New Zealand, [2]Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

The milk fat globule membrane (MFGM) is constructed from a backbone of phospholipids and proteins that encapsulate native bovine milk fat globules. To date, the organization of the phospholipid structure is not fully understood. In this study, an innovative approach was carried out to reveal the lipid organization in the MFGM by applying washing procedures with different degrees of stringency to MFG surfaces in simulated milk ultra-filtrate buffer. Three washing methods, M1 (mild: 3000 × *g*, 5 min, 3 washes), M2 (reference: 3750 × *g*, 15 min, one wash) and M3 (intensive: 15000 × *g*, 20 min, 3 washes) were selected to isolate and wash MFGs. The results showed that M3 removed more phospholipids (PLs, 1.38 mg/m^2) from raw MFGs than M1 (1.21 mg/m^2, as evaluated by HPLC) but induced an enrichment of cholesterol (0.50 mg/ m^2) on the surface of MFGs ($P < 0.05$), suggesting that the MFGM structure may be significantly damaged by the M3 process. Moreover, once the native MFGM is damaged, cholesterol may be enriched in the MFG fraction during centrifugal washing rather than lost with the MFGM fragments, which suggests that most of the cholesterol is located in the outer leaflet of the native MFGM. HPLC-ELSD results showed that phosphatidylinositol (PI), phosphatidylserine (PS) and sphingomyelin (SM) were more strongly retained on MFG surface than the more loosely bound phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Relative quantities of PL loss from MFGs during washing were estimated, and the micro-structural morphology was assessed by confocal laser scanning microscopy (CLSM) based on changes in intensities of fluorescent stains. CLSM images confirmed damage to the outer leaflet phospholipid double layer after M3 washing. The current results provide fundamental information about how centrifugal washing can alter the native MFGM, thus further developing the published schematic models of the structure of the MFGM.

Key Words: milk fat globule membrane, phospholipid, confocal laser scanning microscopy

325 Production of dairy-based functional peptides and their fractionation by membrane adsorption chromatography. E. Leeb*, S. Cheison, and U. Kulozik, *Technische Universität München, Freising, Germany.*

The production of functional peptides out of milk proteins offers 2 advantages. First, milk proteins are known to be potential precursors for several functional peptides. Second, milk proteins are a natural source offering good consumer acceptance. However, a target production of peptides requires the application of specific enzymes. Therefore, a comprehensive study using the 2 specific enzymes trypsin and Lys-C was carried out to investigate the production of functional peptides. β-Lactoglobulin was used as model substrate and the release of the ACE-inhibitory peptides f(9–14) and f(142–148) was examined. Peptide composition of the hydrolysate was analyzed by mass spectrometry (MS) over the hydrolysis process and the reproducible release of peptides was determined. For both enzymes the heterogeneity of the hydrolysates was decreasing with increasing hydrolysis duration. Using trypsin a reproducible production of the target peptides was only feasible by total-hydrolysis. In contrast, Lys-C enables the release of the peptide f(9–14) at earlier stages of hydrolysis. However, a target use of the functional peptides is only possible after their separation from the hydrolysate. Therefore a food-grade process to fractionate the hydrolysates was developed using ion-exchange membrane adsorption chromatography (MAC). The application of a 2-step purification process, whereby a strong anion and cation MAC module were coupled, enables the fractionation of all peptides. Process conditions for maximum separation efficiency were determined, using 0.03 mol/L phosphate-buffer at pH 7 for the anion exchange process. This gave 7 fractions in the first step. In the second step the flow-through of the anion exchange process was further separated using a cation exchanger and 0.03 mol/L phosphate buffer (pH 3). With a step-wise NaCl-gradient for both fractionation processes the production of 12 fractions in total was achieved. MS analysis of the fractions showed a recovery of the peptide f(9–14) with 97% and of the peptide f(142–148) with 80% in enriched fractions.

Key Words: enzymatic hydrolysis, ACE-inhibitory peptide, membrane adsorption chromatography

326 Correction of mid-IR fat test for sample to sample variation in fatty acid chain length and unsaturation. 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 use predicted 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 to improve the accuracy of fat estimation using the classical carbon hydrogen stretch (Fat B) and carbonyl stretch (Fat A). Calibration models for both mCL and mUnsat 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. Twelve sets of USDA Federal milk market individual farm control milks (10 milks per set), collected over a period of 5 mo, were analyzed for fat by ether extraction and fatty acid composition by both mid-IR transmittance and GLC. The fatty mCL and mUnsat measured by mid-IR were used to apply a linear correction factors to the Fat B and Fat A estimates of fat concentration for each sample. The mean (MD) and standard deviation of the difference (SDD) and Euclidean distance (ED) were calculated for each set of 10 samples for 6 different linear methods of fat estimation by IR in comparison to ether extraction results. The mean ED for Fat A, Fat B, 70% Fat B + 30% Fat A, Fat A corrected for mCL, Fat A corrected for mCL and mUnsat, and Fat B corrected for mUnsat were 0.072, 0.034, 0.040, 0.018, 0.016, and 0.028, respectively. The corrections for mCL and mUnsat improved ($P < 0.05$) the accuracy of Fat A predictions of fat content more than the same corrections applied to Fat B, with a MD between ether extraction and IR for the 120 bulk tank milks of −0.005% fat and a SDD of 0.0123 for the Fat A corrected for mCL and mUnsat.. This work has demonstrated the results from PLS based model predictions of mCL and mUnsat can be used in real time to improve the accuracy of fat testing by mid-IR transmittance.

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

327 The role of milk immunoglobulins in gravity separation of somatic cells in raw skim milk. S. R. Geer* and D. M. Barbano, *Cornell University, Ithaca, NY.*

Our objective was to determine if immunoglobulins (Ig) play a role in gravity separation (rising to the top) of somatic cells (SC) in skim milk. Understanding the mechanism of gravity separation might be used to develop a continuous flow technology to remove SC, bacteria, and spores from milk. Other researchers have shown that gravity separation of milk fat globules is enhanced by IgM. Our recent research found that bacteria and SC gravity separate in both raw whole and skim milk and that heating milk to > 74.5°C for 25s stopped gravity separation of milk fat, SC, and bacteria. Bovine colostrum is a good natural source of Ig. A series of 6 gravity separation columns were used: one contained raw skim and the remaining 5 contained pasteurized skim (>74.5°C for 25s) with increasing amounts of added colostrum to achieve about 0, 0.4, 0.8, 2.0, and 4.0 g/L added Ig. The milks were allowed to gravity separate at 4°C for 22 h, and 6 fractions were collected and analyzed for SC: the bottom 90%, and then each successive 2% layer by weight. The experiment was replicated 3 times using different milk and colostrum. Addition of colostrum restored gravity separation with the SC found in the top 2% of column as a function of added Ig from colostrum increasing ($P < 0.05$) in a dose response relationship (linear regression $R^2 = 0.879$). No difference in SC in the top 2% between the raw skim (82% of total SC) and the pasteurized skim with 4.0 g/L added Ig (77% of total SC) was detected ($P > 0.05$), indicating that 4.0 g/L added Ig restored gravity separation of SC. The percent of SC in the top 2% layer for the other 4 treatments of colostrum (0, 0.4, 0.8, and 2.0 g/L) were lower ($P < 0.05$) than the raw skim and pasteurized skim with 4.0 g/L of added Ig. The spore count of the upper 2% and lower 90% of raw skim milk for the 3rd replicate was determined. Gravity separation of spores was apparent in the raw skim milk with about 72% of total spores in the top 2%, but no spore separation was observed in the pasteurized skim. Igs appear to be at least one of several possible factors involved in the gravity separation of SC, spores and bacteria in milk.

Key Words: gravity separation, somatic cell, colostrum

328 Effect of seasonal variation on the heat stability of UHT and in-container sterilized milk. B. Chen*, A. Grandison, and M. Lewis, *University of Reading, Reading, UK.*

The objective of this study was to investigate the effect of seasonal variation on the heat stability of UHT and in-container sterilized milk produced from 25 batches of raw milk over one year. Heat stability was assessed by measuring the amount of sediment in the bovine milk. The milk produced in summer has more sediment than the other seasons after UHT treatment. In contrast, heat stability was not significantly different for the different seasons for in-container sterilization. Sediment formation in both heat treatments was always accompanied by an increase in casein micelle size. The range of for sediment for UHT and in-container sterilized milk was from 0.10 to 0.29% and from 0.02 to 0.56% respectively. Correspondingly, the casein micelle size for UHT and in-container sterilized milk varied from 224 to 337 nm and from 273 to 381 nm, respectively. In addition, the roles of different stabilizing salts (di-sodium hydrogen phosphate and tri-sodium citrate) on both heat treatments were evaluated. Without stabilizing salts bovine milk produced more sediment when subjected to in-container sterilization compared with UHT processing. Addition of up to 10 m*M* stabilizing salts resulted in a significant ($P < 0.05$) increase in sediment for in-container sterilization, but only a slight increase in UHT processing. Adding up to 2 m*M* calcium chloride increased sediment formation significantly ($P < 0.05$) more after UHT treatment than after in-container sterilization. These results for bovine milk are in agreement with trends found for heat stability of caprine milk, which have been published previously. It was concluded that the effect of seasonal variation on heat stability of UHT milk was significant, but not for in-container sterilized milk. There is no single mechanism or set of reactions that cause milk to produce sediment during heating and that the kinetics are different for UHT and in-container sterilization processes.

Key Words: seasonal variation, heat stability, UHT and in-container sterilization

329 Effect of chemical-physical properties of raw milk on the quality of dairy products in the UK. B. Chen*, A. Grandison, and M. Lewis, *University of Reading, Reading, UK.*

The objective of this study was to investigate the effect of raw milk composition on some selected properties of milk products. Raw bulk cow milk was collected and its composition and physical properties were measured every 2 wk. This milk was then converted to a range of products using standardized methodology and selected properties of these products were measured. Products include evaporated milk, soft cheese, skim milk powder and whipping cream. This project has been replicated with a minimum of 25 batches of raw milk. The range of values for fat and protein were 3.62 to 4.77% and 2.89 to 3.56%, respectively. Ranges for lactose (4.52 to 4.69%), total solids (12.31 to 13.37%), pH (6.73 to 6.87), buffering capacity (pH change from 0.78 to 0.88) and ethanol stability (84 to 100%) were narrower. Ca^{2+}concentration ranged

from 1.68 to 2.55 m*M*. Sediment in raw milk was very low, ranging from 0.03% to 0.13% (dry weight basis). Viscosity ranged from 1.52 to 2.36 cp and density from 1026 to 1031 kg/m^3. Casein micelle size ranged from 132 to 202 nm and freezing point depression from −0.530 to −0.514°C. The highest foaming times were 205 and 96 s for raw and skim milk, respectively, and the shortest were 24 and 19 s. The viscosity range for evaporated milk greatly varied from 9.5 to 243.7 cp and was also affected by the level of added stabilizer. For the whipping cream, the range of value for overrun and whipping time were 94 to 202% and 169 to 261 s which were more variable than foam stability (5.00 to 18.75 mL). The heat stability of skim milk powder showed considerable variability. The ranges of the moisture content and hardness in soft cheese were 46.17 to 64.33% and 0.63 to 4.00 N respectively. It was concluded that variations in raw milk composition influence the properties of manufactured milk products.

Key Words: raw milk quality, physico-chemical properties, best use for milk

330 In vitro and in vivo assessment of the antioxidant activity of whey protein hydrolysates prepared using commercial enzymes. B. Mann*, A. Kumari, K. Prajapati, R. Kumar, and R. Sharma, *National Dairy Research Institute, Karnal, Haryana, India.*

Whey proteins are potential source of antioxidant peptides. Hydrolysis by various food-grade enzymes leads to the production of such peptides. In this study, whey protein hydrolysates (WPHs) were prepared from whey protein concentrate (WPC-70) using commercial proteases (flavorzyme and alcalase) and hydrolysis conditions were optimized by applying response surface methodology. The antioxidant activity was evaluated using in vitro and in vivo assays along with molecular and techno-functional characteristics of these WPHs. These WPHs showed very high antioxidant activities; i.e., 1.41 ± 0.08 (alcalase WPH) and 1.25 ± 0.06 (flavorzyme WPH) μmol of trolox/ mg of protein as compared with intact whey proteins (0.19 ± 0.07μmol of trolox/ mg of protein). Further, these WPHs were evaluated for antioxidant activity by carrying out in vivo studies in mice as animal model. The result showed that the antioxidant enzymes viz. catalase, superoxide dismutase, glutathione peroxidase activities in blood as well as catalase and glutathione activities in liver homogenates increased significantly ($P < 0.05$) in the groups fed with WPHs + oxidized oil compared with oxidized oil fed group. The level of thiobarbituric acid reactive substances in blood and liver homogenates of the group fed with WPHs decreased significantly ($P < 0.05$) compared with the groups fed with oxidized oil. The peptides in WPHs have been identified using LC/MS and the results showed that these hydrolysates contained fragments of β-LG and α-LA. Sequence of these peptides were synthesized and assessed for the antioxidant activity. The peptide α-LA (99–108) showed the maximum antioxidant activity (1.720 ± 0.164 μmol of trolox/ mg of protein). The techno-functional properties of WPHs were slightly inferior to the unhydrolyzed whey protein except whipping ability which showed increase in case of WPHs. The incorporation of these hydrolysates showed increase in the antioxidant activity of flavored milks from 14.4 to 43.3% vis-à-vis control. These WPHs with antioxidant activity could lead to the development of novel foods relevant in health promotion and disease prevention.

Key Words: whey protein, whey protein hydrolysate, antioxidant activity