M101 Inhibition of lipid oxidation in fish oil-in-water emulsions by the combination of bovine and caprine caseins with phospholipids. Adela Mora-Gutierrez*, Rahmat Attaie, Sela Woldesenbet, and Jeneanne Kirven, *Prairie View A&M University, Prairie View, TX.*

The nutritional benefits of polyunsaturated fatty acids from fish oil in terms of their role in growth and development and in health and disease are limited by their oxidative degradation. The objective of this study was to investigate the effect of bovine and caprine caseins in combination with phospholipids on the oxidative stability of 5% fish oil-in-water emulsions at pH 7.0. Menhaden oil was emulsified with phospholipids such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in a high-pressure valve homogenizer. The effect of bovine and caprine caseins in combination with phospholipids on oxidation rate was monitored by measurement of thiobarbituric acid reactive substances (TBARS) and lipid peroxides. Oxidation of 0.5% phospholipid-stabilized fish oil-in-water emulsions were performed at 25°C for 48 h, in the dark, in the presence of 0–0.3% bovine and caprine caseins and 0.1 m*M* Fe^{2+}. It was found that the emulsion stabilized with phosphatidylserine showed the best oxidative stability in terms of TBARS concentration and peroxide value. Formation of TBARS was inhibited 32% by addition of 0.3% caprine casein ($P < 0.05$). Inhibition of lipid oxidation by addition of 0.3% bovine casein was less effective, decreasing the concentration of TBARS by 24% ($P < 0.05$). The combination of casein and phosphatidylserine resulted in favorable structure and thickness of the interfacial layer, thus preventing lipid oxidation in the emulsion. These results indicated that the combination of casein and phosphatidylserine can be utilized to enhance the oxidative stability of emulsified fish oil.

Key Words: casein, phospholipid, fish oil

M102 Obtaining of casein fractions by preparative ion-exchange chromatography on weak anion-exchangers. Andrij Iukalo[1], Orysya Tsisaryk*[2], and Volodymyr Yukalo[1], *[1]Ternopil Ivan Puluj National Technical University, Ternopil, Ukraine, [2]Lviv National University of Veterinary Medicine and Biotechnology, Lviv, Ukraine.*

The aim of study was to obtain the main casein fractions (α_{S1}-CN, α_{S2}-CN, β-CN, and κ-CN) in preparative amounts using weak anion-exchangers. Total casein was obtained using isoelectric precipitation from fresh skim milk for fractionation. Fractional composition of total casein and the homogeneity of its fractions at different stages of the isolation were analyzed by electrophoresis on the vertical plates of polyacrylamide gel. At this stage, an alkaline buffer system containing 25 m*M* tris, 27 m*M* dietylbarbiturate, 3 m*M* ethylenediaminetetraacetate, and 4.5 *M* urea was used. Electrophoregrams were fixed and stained by conventional methods. The concentration of protein was determined by spectrophotometer (SF-46, λ-280 nm). Previously installed absorbtion coefficients for different fractions of casein (D 1% in 1 cm) were used: 10.0 – for α_{S1}-CN; 10,1 – for α_{S2}-CN; 4,6 – for β-CN; 9,6 – for κ-CN, and 8,2 – for total casein. DEAE-cellulose (DEAE-52 "Serva") and toyopearl DEAE 650M (Toyo soda) were used as anion-exchangers. Batch separation of total casein was performed in 0.02 *M* acetate buffer (pH = 6.5) in the presence of 3.3 *M* urea and 0.01 *M* 2-mercaptoethanol. $CaCl_2$ was used as eluent. This salt is more effective than NaCl due to the interaction with phosphoserine groups of caseins. For fractionation we used the following concentrations of $CaCl_2$: 0.0 *M* (I fraction); 0.005 *M* (II); 0.01 *M* (III); 0.02 *M* (IV); 0.035 *M* (V); 0.05 *M* (VI).The experiment was replicated 3 times. As a result, electrophoretic analysis shows that κ-casein is found in fraction I; II contains β-casein; III and IV – α_{S2}-casein; V – α_{S1}-casein with minor impurities of α_{S2}-casein, and VI contains only α_{S1}-casein. Obtained fractions were dialyzed, lyophilized and weighed. The total yield of caseins was 71% using DEAE-cellulose and 77% in the case of anion-exchangers toyopearl DEAE 650M ($P < 0.05$). Weak anion-exchangers are more effective than strong ones in caseins preparative fractionation. It allowed us to obtain a separate group of α_{S2}-caseins, which includes α_{S2}-, α_{S3}-, α_{S4}-, and α_{S6}-caseins. These fractions are characterized by the same sequence of amino acid residues and are precursors of many original bioactive peptides.

Key Words: casein, fraction, ion-exchange chromatography

M103 Size distribution of casein micelles in raw skim milk from individual cows as studied using cryo-TEM. Maneesha S. Mohan* and Federico M. Harte, *Food Science Department, Pennsylvania State University, University Park, PA.*

Casein micelles have a complex structure comprising of 4 caseins: α_{S1}, α_{S2}, β, and κ caseins, assembling as many as 20,000 casein proteins. Polydispersity is the degree of disparity or variation in size of particles. Traditionally, casein micelles have been considered highly polydisperse with relative polydispersity between 0.27 to 0.5 as reported by different studies using dynamic light scattering techniques. A recent study has defined casein micelles to be more monodisperse. Hence, we decided to get a visual confirmation of the polydispersity of casein micelles by studying the casein micelle size distribution in milk from 4 cows by cryo-transmission electron microscopy (cryo-TEM). The raw milk from 4 cows of first parity, mid lactation and low somatic cell count, was skimmed by centrifugation (6414 × *g* for 20 min). The diluted skim milk (10 µL in 500 µL of protein-free serum obtained by ultrafiltration of skim milk using a 3-kDa filter) was subjected to cryo-TEM at 200kV. The diameter of casein micelles was measured manually using ImageJ software. The relative polydispersity (relative standard deviation of size distribution) of casein micelles was a Gaussian distribution (x-axis = number of micelles as a fraction of the total micelles measured, y-axis = diameter of casein micelles) spanning between 10 to 341 nm (1545 micelles), 24 to 693 nm (1073 66 micelles), 11 to 514 nm (2621 micelles) and 11 to 532 nm (1891 micelles) for milk from 4 cows, respectively. The positively skewed distribution curve had a tapering tail of large casein micelles of diameter greater than 350 nm. The maximum number of casein micelles existed in the size range of 90 to 110 nm for milk from all the 4 cows. The relative polydispersity ranged between 0.39 and 0.47 for casein micelles with mean diameters (D_{10}) between 112 to 134 nm for the milk from the 4 cows. The results indicate high level of polydispersity in the casein micelles in milk of every cow. Hence it was possible to effectively utilize cryo-TEM to elucidate the size distribution of casein micelles and visually reconfirm the polydispersity of casein micelles in bovine milk from individual cows.

Key Words: polydispersity, electron microscopy, Gaussian

M104 Effect of casein micelle dissociation and casein modification on plasmin-induced hydrolysis. Hemang Bhatt[1,2], Aurelie Cucheval[1], Christina Coker[1], Hasmukh Patel*[3], Alistair Carr[2], and Rod Bennett[2], *[1]Fonterra Research and Development Centre, Palmerston North, New Zealand, [2]Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand, [3]Dairy Science Department, South Dakota State University, Brookings, SD.*

Plasmin-induced hydrolysis of casein in milk can lead to many defects including proteolysis, age gelation, and bitterness. We hypothesized that the susceptibility of casein micelles to plasmin can be affected by its micellar structure and modification of the lysine residues on caseins. Different levels of dissociation of the casein micelle structure were achieved by calcium chelation using EDTA and by succinylation by attaching a succinate group at the ε-amino group of lysine residues, leading to the formation of succinyl-lysine. The target sites for succinylation were identified using liquid chromatography-tandem mass spectrometry. Changes in the particle size and levels of casein micelle dissociation in skim milk were determined using a Zetasizer and sodium dodecyl sulfate PAGE, respectively. The subsequent plasmin hydrolysis was monitored by quantifying the hydrolyzed product using reverse phase high performance liquid chromatography. The EDTA-induced calcium chelation and dissociation of the casein micelle increased plasmin-induced hydrolysis. In contrast, succinylation had an inhibitory effect on plasmin-induced hydrolysis. Calcium chelation and succinylation of the skim milk resulted in dissociation of caseins from the casein micelle, a decrease in turbidity, extensive unfolding, and expansion of the polypeptide chain; all of these changes collectively reduced steric hindrance and made the protein more readily hydrolyzed by plasmin. However, the formation of succinyl−lysine rendered β-casein unrecognizable to the substrate-binding pocket of plasmin, resulting in a nonlinear decrease in the hydrolysis because of the competitive effect of micelle dissociation. These results clearly indicate the importance of the casein micelle structure and its susceptibility to plasmin action. They will be useful in understanding and controlling the plasmin-induced hydrolysis of milk proteins in food systems.

Key Words: plasmin, succinylation, liquid chromatography-tandem mass spectrometry

M105 The role of lactose and whey proteins in plasmin resistance of heat-treated milk. Hemang Bhatt[1,2], Aurelie Cucheval[1], Christina Coker[1], Hasmukh Patel*[3], Alistair Carr[2], and Rod Bennett[2], *[1]Fonterra Research and Development Centre, Palmerston North, New Zealand, [2]Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand, [3]Dairy Science Department, South Dakota State University, Brookings, SD.*

Sterilization and UHT treatment are commonly used to increase the shelf life of milk and dairy beverages. However, the high heat resistance of the plasmin system enables it to partially survive UHT treatment, which could lead to defects in the texture (gelation, sedimentation) and the taste (bitterness) of these products. The present study investigated the effect of high heat treatment on plasmin-induced hydrolysis using a sequential approach. A high-heat-treated (120°C/15 min) skim milk had been found to have higher resistance to plasmin-induced hydrolysis, compared with a control skim milk. The effect of heat treatment on the resistance of skim milk was studied with a sequential approach. Different milk systems were prepared by removing whey protein and then lactose from skim milk and reconstituting each milk separately, to observe the effect of whey protein and lactose both individually and in combination. The milk systems were heated at 120°C for different times to achieve different levels of heat treatment. All treated milk systems were then hydrolyzed by adding plasmin and the reaction was monitored using reverse-phase HPLC. The extents of whey protein denaturation, dissociation of casein from the micelles and association of whey protein with the micelles were monitored using sodium dodecyl sulfate-PAGE. Particle size and levels of lactosylation were also measured. Plasmin-induced hydrolysis was negatively affected by an increase in the heat treatment. Both whey protein association with the casein micelles and lactosylation decreased the availability of protein to plasmin. These effects were compared and mechanisms for them have been proposed. Whey-protein-free milk was the most resistant to plasmin, followed by skim milk and lactose-free milk, suggesting that lactosylation plays a more major role than whey protein association with the casein micelles in making protein resistant to plasmin-induced hydrolysis.

Key Words: plasmin, lactosylation, sterilization

M106 Oxidative stability of an Iranian ghee (butter fat) versus soybean oil during storage at different temperatures. Mahshid Azizi*[1], Maryam Enteshari[2], and Mohammadreza Dolatkhahnejad[3], *[1]Islamic Azad University of Birjand, Chemical Engineering and Food Industries Department, Tehran, Iran, [2]Department of Food Science and Technology, Shahid Beheshti University of Medical Sciences, Tehran,Iran, [3]Islamic Azad University of Ayatollah Amoli, Food Industries and Scientific Engineering Department, Tehran, Iran.*

The oxidative stability of an Iranian ghee (butter fat) and soybean oil, which were stored at 4 different storage temperatures (4, 25, 45, and 60°C) and during one-month storage was investigated. Oxidation changes were monitored every 2 weeks by analytical parameters including acid value (AV), peroxide value (PV), *p*-anisidin value (*p*-AV), thiobarbituric acid value (TBA) and oxidative stability index (OSI). In addition, iodine value (IV) and fatty acids profile were determined to elucidate intrinsic differences between ghee and soybean oil. The PV, *p*-AV and TBA were gradually increased during storage time, while the OSI decreased. The ghee in contrast to soybean oil, showed significantly ($P < 0.05$) lower amounts of PV, *p*-AV and TBA and higher OSI. However higher amounts of AV were obtained for ghee samples at all of mentioned storage temperatures which indicated higher free fatty acids (FFA %) as a result of production procedure for this kind of fat. Moreover, obtained data revealed that the effects of applied different temperatures on the mentioned oxidation parameters were significant during studied storage time ($P < 0.05$); additionally, at higher temperatures (45 and 60°C) these effects were more intensive particularly for the soybean oil samples. Results confirmed that ghee displayed better oxidative stability behavior as evidenced by lower PV, *p*-AV and TBA with higher values of OSI at accelerated storage conditions.

Key Words: oxidative stability, Iranian ghee, soybean oil

M107 Effect of hydrodynamic cavitation on acid gelation properties of skim milk. Harsh Dahiya*[1], Hasmukh A. Patel[1], and Thom Huppertz[1,2], *[1]South Dakota State University, Brookings, SD, [2]NIZO Food Research, Ede, the Netherlands.*

Acid gelation is an important property of milk with relevance to yogurt manufacturing. Hydrodynamic cavitation (HC) is an emerging technology involving a process of vaporization, bubble generation followed by bubble collapse in a flowing liquid brought about by a decrease in pressure followed by a subsequent increase in pressure. In this study, we compared the effects of HC with conventional heat treatments used in dairy industry on acid gelation property of skim milk. Pasteurized skim milk (3.5% protein and 9% total solids) was preheated to 50°C and

then subjected to 2 sets of HC treatments, namely, HC at 20, 40, and 60 Hz at sufficiently high flow rate (950 L/h) to avoid any temperature increase during HC (T1) and HC at 60 Hz at low flow rates (200 L/h) to allow scale-free heating of skim milk increasing its temperature to desired temperature up to 90°C (T2) using APV Cavitator (supplied by SPX, Denmark) fitted with 4-row rotor in 6mm housing. A portion of skim milk was also heated at 90°C for 10 min. Acid milk gels were prepared from untreated (control), skim milk samples obtained from T1, T2 as well as the skim milk samples conventionally heated to 90°C for 10 min using glucono-δ-lactone to obtain final pH4.6 ± 0.05 after 4h incubation at 30°C. Rheological characteristics of acid gels were studied using small amplitude oscillatory rheology (SAOR) at 1% strain and 0.1 Hz frequency. The soluble (serum) phases obtained by centrifugation of control, cavitated and heated milk samples at 25000g/1h were characterized using sodium dodecyl sulfate PAGE (SDS-PAGE). It was observed that the storage modulus (G′) of acid gels prepared with skim milk samples subjected to T1 was significantly lower ($P < 0.05$) than those prepared with samples subjected to T2. Acid gels prepared from the samples subjected to T2 with temperatures reaching 90°C had highest final G′, which was attributed to significant denaturation of whey proteins in these samples. It was also observed that this value of G′ was similar to the one obtained from the conventional heating of milk, suggesting that the cavitation treatment of milk do not adversely affect the acid gelation property of the milk.

Key Words: acid gel, cavitation, rheology

M108 Molar mass of a crude extract of *Streptococcus thermophilus* St-143 exopolysaccharide during fermentation of milk.
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Some lactic acid bacteria produce high molecular weight polysaccharides (EPS) during milk fermentation. It is unclear if there is any change in the properties of EPS during the fermentation. The molar mass of EPS was investigated during fermentation of milk with *Streptococcus thermophilus* (St-143). We used crude extracts, instead of highly purified samples, to observe more native EPS systems. Reconstituted nonfat milk samples were fermented by *S. thermophilus* at 40°C until pH 4.5. Crude extracts of EPS were obtained at 4 different fermentation times starting from 150 min (pH ~5.3) to 330 min (pH~4.5). Whey from fermented milk was ultrafiltered using 100 kDa membrane to remove soluble milk sugars and proteins. The UF retentate was centrifuged and supernatant was further concentrated using single pass pressure UF cell with 100 kDa membrane. The concentrated EPS solution was analyzed for protein and sugar contents. Molar mass of EPS was estimated using size exclusion chromatography-multi angle laser light scattering (SEC-MALLS). Polysaccharide content of the EPS concentrates varied from ~0.3 to 0.4 mg/mL. Protein impurities in the EPS concentrate were high when sampled at pH 5.3, and significantly decreased ($P < 0.05$) until pH 4.5. Extracts separated using SEC gave an initial peak that appeared to be mostly high M_w protein aggregates and a second peak that corresponded to EPS. The molar mass of EPS ranged from 1.57×10^6 to 2.86×10^6 g/mol. EPS sampled at pH 5.3 had significantly higher ($P < 0.05$) molar mass than samples obtained at pH 4.5. EPS samples also appeared to be monodisperse (i.e., $M_w/M_n = 1$). The radius of gyration of EPS was ~54 nm and was not significantly different between the different pH samples. It appears that the properties of EPS may vary during milk fermentation.

Key Words: exopolysaccharide, molar mass, ultrafiltration

M109 Formation of hydroxymethylfurfural and other caramelization products during extrusion of lactose blends.
Catrin E. Tyl, Elizabeth M. Reid, and Tonya C. Schoenfuss*, *University of Minnesota, St. Paul, MN.*

As part of our ongoing efforts to finding value-added uses for lactose, we carried out reactive extrusion on blends of lactose, glucose, and citric acid to polymerize lactose to indigestible carbohydrates. This treatment can also induce the formation of color and hydroxymethylfurfural (HMF). The objective of this study was to investigate the effect of formula and extrusion conditions on color and HMF formation. Data analysis was carried out with ANOVA followed by Tukey's HSD test. Three citric acid (CA) concentrations (2, 4, and 6% w/w) were tested. Glucose comprised 20% w/w and lactose made up the remainder. All blends were extruded in duplicate at 2 feed rates: 15 and 30 kg/h. Color was measured as Hunter *L*, *a*, and *b* values and absorption at 420 nm, indicative for yellow-brown pigments. Absorption was higher for samples of all CA levels extruded at 15 kg/h and these extrudates also had lower *L* and higher *b* values (i.e., samples were darker and more yellow). Thus, the extent of caramelization increased when mixtures spent more time in the extruder at the low feed rate. Higher CA levels lead to more pigment formation at 15 kg/h, but not at 30 kg/h. Along with other caramelization products, HMF is formed in sugars heated with acids. HMF has shown toxicity in in vitro and animal studies. Thus, reducing its formation through optimum processing conditions and/or sample treatments is needed. HMF content was measured by RP-HPLC/ UV. Increasing CA in the raw mix produced extrudates with lower amounts of HMF. The lower feed rate resulted in significantly higher levels of HMF. We evaluated sample treatments to remove HMF using Amberlite (an ion exchanger) and activated charcoal. Filtration over Amberlite lead to 27–57% less HMF. Preliminary results indicate that charcoal treatment may further reduce HMF (>95%). However, because it also lead to losses in oligo- and polysaccharide yields, further optimization of this method is necessary.

Table 1 (Abstr. 109). Effect of formula (citric acid at 2, 4, and 6% wt/wt) and feed rate (15 and 30 kg/h) on color and hydroxymethylfurfural (HMF) formation

	15 kg/h			30 kg/h		
Item	2	4	6	2	4	6
A_{420}	0.554	0.654	0.934	0.194	0.195	0.200
L	83.84	83.59	80.99	87.41	88.50	89.17
b	27.23	30.01	35.03	14.83	15.21	15.79
HMF (mg/g)	1.20	0.91	0.73	0.71	0.55	0.52
HMF after Amberlite (mg/g)	0.51	0.46	0.53	0.42	0.38	0.31

Key Words: hydroxymethylfurfural, lactose, extrusion