Graduate Student Competition: ADSA Dairy Foods Division Oral Competition

14 Tracking heat-resistant, spore-forming bacteria in the milk chain: A farm-to-table approach. M. Estrada*, J. Stratton, and A. Bianchini, *University of Nebraska-Lincoln, Lincoln.*

Thermoduric spore-forming bacteria *Bacillus* and *Paenibacillus* spp. can survive processing and refrigerated conditions causing spoilage of fluid milk, limiting the shelf life of the product beyond 14 d. The objective of this study was to evaluate the fluid milk chain for *Bacillus* and related genera to identify their potential sources. Raw milk, pasteurized milk and environmental samples representing the farm-to-table continuum were collected in the spring of 2012 from a dairy farm and a medium-size processing plant in the Midwest, supplied exclusively by that farm. Environmental samples from the dairy farm included feed, drinking water, bedding material, manure, milking parlor wash water, teat cloths and sponges (milking clusters and teats). Environmental samples from the processing plant included rinse water (tanks and mixer), and swabs (filler's surface and nozzles). Environmental and raw milk samples were heat-treated at 80°C for 12 min to eliminate vegetative cells. Pasteurized milk samples were evaluated for quality and, along with heat treated samples, stored at 6°C for 21 d. Samples were enumerated for microbial load and the plates used for bacterial isolation throughout storage. Colonies with the distinct morphology of *Bacillus* spp. were isolated and characterized using a subtyping method based on the DNA sequence of their rpoB gene. A total of 34 different rpoB allelic types representing *Bacillus* (99% of isolates) and *Paenibacillus* spp. (1%) were identified among 72 bacterial isolates. The *Bacillus* spp. clade was represented mainly by *B. licheniformis* (40% of isolates), *B. pumilus* (33%) and *B. subtilis* (14%). Two allelic types were identified in both raw and pasteurized milk, indicating the raw milk supply as a possible source of these bacteria. Six allelic types were found in milk and environmental samples (farm and processing plant), suggesting that these bacteria can enter the fluid milk chain from different sources. This data will help in the development of intervention strategies for controlling spoilage by spore-formers and extending the shelf life of pasteurized milk.

Key Words: HTST milk, spore-formers, subtyping

15 Pulse electric fields treatment maintains the antiproliferative activity of the milk fat globule membrane on colon carcinoma cells. S. Xu*[1], M. Walkling-Ribeiro[2,1], M. W. Griffiths[2,1], and M. Corredig[1], *[1]University of Guelph, Guelph, ON, Canada, [2]Canadian Research Institute for Food Safety (CRIFS), Guelph, ON, Canada.*

The milk fat globule membrane (MFGM) components have antiproliferative activities against colon cancer cells. The present work investigated the effect of processing of the cream on the antiproliferative activity of MFGM. Unheated cream and commercially pasteurized cream were collected from a local dairy immediately after separation. Pulsed electric fields (PEF) with 37 kV/cm field strengths for 1705 μs was applied to unheated cream, at 50°C and 65°C. The electrode gap was 0.21 cm and the flow rate of cream was 25 mL/min. PEF control treatments were carried out at 50°C and 65°C for 3min, the passage time in PEF chamber to mimic the effect of heating. For comparison, the unheated cream was also heat treated at 5 different temperature/time combinations: 50°C/10 min, 60°C/2 min, 60°C/10 min, 70°C/30 s and 70°C/2 min. After processing, all cream was processed to butter, and MFGM isolates were obtained from buttermilk using centrifugation at 70,000 × *g* for 45min at 15°C. The antiproliferative activity of extracts was tested on human adenocarcinoma HT-29 cells, incubated for 24 h then stimulated with 0.4, 0.2, or 0.1 mg/mL various MFGM isolates for another 24 h. Cell proliferation was tested using a colorimetric method (BrdU). All samples heated below 70°C showed significant cell reduction ($P < 0.05$, using ANOVA) from control at the highest 2 concentrations, with 70°C/30 s sample only at the highest concentration. The 70°C/2 min sample even helped cell growth, comparable to the commercially pasteurized sample. There was a reduction up to 52% in cell proliferation in PEF samples, with no significant difference compared with PEF controls, suggesting electrical field itself did not affect the anticarcinogenic activity of MFGM. Commercially pasteurized samples and heat treated samples showed a decrease in bioactivity. PEF did not change the phospholipids composition compared with PEF controls, while heating showed protein-protein interactions and a decrease in phospholipids as measured by ^{31}P NMR. PEF may be a promising process to maintain valuable bioactivity in the MFGM.

Key Words: milk fat globule membrane (MFGM), anticarcinogenic activity, pulsed electric field (PEF)

16 Effect of the heating of whey proteins in the presence of milk fat globule membrane extract or phospholipids from buttermilk. M. Saffon*[1], R. Jiménez-Flores[2], M. Britten[3], and Y. Pouliot[1], *[1]STELA Dairy Research Center, Institute of Nutrition and Functional Food (INAF), Laval University, Quebec City, QC, Canada, [2]Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo, [3]Food Research and Development Center (FRDC), Agriculture and Agri-Food Canada, St-Hyacinthe, QC, Canada.*

Previous work suggests that phospholipids from buttermilk were involved during the heat-induced formation of protein aggregates from whey and buttermilk proteins. It was hypothesized that "free" phospholipids from buttermilk initiates the formation of whey proteins aggregates. Whey protein isolate (WPI) was dispersed in water to a concentration of 5% protein (w/v), and the pH was adjusted to both 4.6 and at 6.8. Solutions were heated to 65°C or 80°C for 15 min under constant stirring. Milk fat globule membrane (MFGM) extract isolated from whey buttermilk or commercial phospholipids (PL) powders were dispersed in the WPI solutions at 1.0% (w/v) before heating. The aggregate composition was characterized with Ellman's reagent (free thiols), SDS-PAGE electrophoresis, thin layer chromatography (TLC), and 3-dimensional confocal laser-scanning microscopy (CLSM). All experiments were performed in triplicate and the concentration of free thiol groups were tested according to a factorial design. Addition of phospholipids or MFGM extract did not significantly affect the liberation of free thiol groups of whey proteins but delayed the loss of the native form of major whey proteins by 5 min at pH 6.8. TLC profiles showed no trace of phospholipids after 20 min of heating WPI-PL mixtures at 80°C at pH 4.6. CLSM images confirmed that only a few interactions occurred between whey proteins and MFGM proteins/phospholipids in the whey solution, while the interactions were frequent in the presence of MFGM extract or phospholipids. Overall, our results show evidence that phospholipids from buttermilk are involved in the formation of protein aggregates through the MFGM fragments at 65°C, whereas they can directly interact with the proteins at 80°C. Results allow the consideration of

new potential applications for the use of dairy products that are rich in phospholipids such as regular or whey buttermilks.

Key Words: buttermilk, phospholipid, aggregation

17 Effect of ultra-high pressure homogenization on physicochemical properties of pasteurized skim milk. M. S. Mohan*, R. Ye, and F. Harte, *Department of Food Science and Technology, University of Tennessee, Knoxville.*

Recent developments in material science and engineering enabled us to study the effect of ultra-high pressure homogenization (HPH; up to 500 MPa) on the physicochemical properties of milk. We report the effect of HPH of pasteurized skim milk (0 to 500 MPa followed by immediate chilling) on pH, apparent casein micelle size (dynamic light scattering; pH 2 to 10), turbidity (absorbance at 550 nm; pH 2 to 10), heat stability (120°C; pH 6.3 to 6.7), viscosity (flow curve), and viscoelasticity after rennet addition (G′; 90 min, 1Hz, 0.1% strain). All HPH milks were stable for more than 10 d without visible coagulation. The pH of all except 500 MPa milk were slightly higher (up to 0.13 pH units) than 0 MPa milk (pH 6.81). Maximum casein micelle size was observed after HPH of milk at 500 MPa (ca. 500 nm) compared with 0 MPa milk (ca. 250 nm) at pH 5. However, 500 MPa milk remained stable above this pH and coagulated together with the other milks below pH 5. From pH 6 to 8 the casein micelle size of 500 MPa milk decreased until it was same as 0 MPa milk at pH 9 and 10. Although the absorbance of 0 MPa was higher than 500 MPa milk at pH 8 to 10, indicating differences in particle size and absorbance observations, the trend was similar for both measurements at pH 6 and 7. At normal milk pH (6.6 and 6.7) all the HPH milks remained stable for 24 min at 120°C. With increase in pressure the casein micelles were first partially broken down (at 100 MPa), re-aggregated (until 400 MPa) and then re-dispersed (at 500 MPa), confirmed by microscopic image and particle size analysis. The rennet coagulation of 500 MPa milk did not occur even after 90 min. Overall, 500 MPa pressure did not significantly affect the pH, heat and shelf stability of milk, but altered the viscosity and renneting properties. Further understanding these changes will enable the novel use of HPH milk proteins as ingredient in products including cheeses.

Key Words: high pressure homogenization, pasteurized skim milk, casein micelle

18 Performance of modified milk protein concentrates in model high-protein nutrition bars. J. Banach*, S. Clark, and B. Lamsal, *Iowa State University, Ames.*

The nutritional value and flavor of milk protein concentrates (MPC) are desirable for high-protein nutrition (HPN) bar applications. However, rapid hardening and crumbly texture upon incorporation limit its use. Extrusion and toasting were used to modify MPC at 80% protein (MPC80) and performance was evaluated in model HPN bars. MPC80 was extruded in a twin-screw co-rotating extruder at 2 ramped temperature profiles with die temperatures of 65 or 120°C. Extrudates were dried and finely ground (<250 mm). Toasting of MPC80 was done in a convection oven at 75 or 110°C for 4 h. Model bars contained 30% protein, 22% glycerol, 19% palm kernel stearin, 12% maltitol syrup, 10% high-fructose corn syrup, and water. Dough was sealed into cylindrical molds and in water activity sample cups, and stored at 22, 32, or 42°C. Bar texture (hardness, fracturability, and shear), water activity, and color change were measured over 42 d. Disulfide bond formation in bars was studied with sodium dodecyl sulfate PAGE (SDS-PAGE). Sample means were compared with Tukey's adjusted *P*-value ($P < 0.05$). Toasted MPC80 in bars had texture similar to unmodified MPC80 (control). Bars prepared with MPC80 extruded at 65°C were significantly softer than bars made with control MPC80. Significant difference in hardness and fracturability between bars formulated with MPC80 extruded at 120°C and those prepared with control MPC80 was intermittent. Water activity of the bars increased slightly during storage, but remained less than 0.65, which assured shelf stability. Total color change was limited at 22°C storage, but increased significantly at 32°C and 42°C. Non-reduced SDS-PAGE showed that the whey protein in bars prepared with extruded MPC80 was not soluble on Day 0; its solubility under reduced conditions indicated that disulfide bonds were formed before bar manufacture. Internal protein aggregation did not occur because free sulfhydryls were previously reacted, and thus low temperature (65°C) extrusion of MPC80 may improve performance in HPN bars.

Key Words: milk protein concentrate, high-protein nutrition bar, texture

19 Concentration of milk by ultrafiltration modifies the acid-induced gelation properties of casein micelles. Y. Li* and M. Corredig, *University of Guelph, Guelph, ON, Canada.*

Membrane filtration is a widespread unit operation in dairy technology, and little is understood on how concentration by ultrafiltration (UF) and diafiltration (DF) may change the processing functionality of the casein micelles. This study investigated the acid (gluconoδ-lactone, 1.3% wt/wt for control; 1.8% wt/wt for 2×; 3.0% wt/wt for 4×, at 40°C) induced gelation behavior of milk concentrated by UF and DF with and without heat treatment at 80 ± 1°C for 15 min. Measurements of soluble and insoluble calcium by ion chromatography suggested that there was a lower amount of colloidal calcium phosphate in the casein micelles concentrated (4 ×) by UF compared with those in single strength milk. In addition, the amount of colloidal calcium in the heated concentrated milk was significantly lower than for unheated concentrated milk. Size exclusion chromatography on the soluble fraction demonstrated that DF caused compositional changes in the serum fraction compared with UF. The processing history (DF, UF and heating) strongly affected the gelation behavior of the concentrated milk: The gelation pH, measured by rheology and diffusing wave spectroscopy, significantly increased with the extent of concentration ($P < 0.05$, as measured by ANOVA), due to a reduction in the interparticle distance and because of the changes occurring to the soluble fraction. Concentrated samples formed significantly ($P < 0.05$) stiffer gels than control milk because of an increased amount of linkages in the network. DF milk showed a significantly higher gelation pH compared with the UF milk at the same volume fraction. In addition, compared with UF milk, heated DF milk had even higher pH of gelation, but no significant difference in the gel stiffness (pH = 4.6) between the 2 treatments. This work clearly demonstrated for the first time that UF and DF changes the composition of the soluble fraction, and affects the acid induced behavior of concentrated milk.

Key Words: concentrated milk, unheat and heat treatment, gelation behavior

20 The effects of sodium reduction, with and without KCl, on blue cheese. A. Pataky*[1], S. Rankin[2], Z. Vickers[1], and T. Schoenfuss[1], *[1]University of Minnesota, Saint Paul, [2]University of Wisconsin, Madison.*

Blue cheese contains approximately 400 mg sodium per 28-g serving, twice the amount found in Cheddar. Salt is essential to blue cheese ripening and microbial safety. Because blue cheese is often surface-salted, the effects of sodium reduction may be more noticeable in the center of the cheese wheel. The purpose of this study was to evaluate the effects of sodium reduction (25%) on blue cheese composition, flavor, sensory, and proteolytic properties with and without the use of potassium chloride (KCl) at 2 locations in the cheese wheel. Three-kg wheels of pasteurized milk blue cheese were produced from 2,100 kg of milk (in duplicate), and 3 salting treatments were applied to randomly selected wheels. Salt was applied by % weight of the wheel in the following treatments: Control (C; 3.5 wt% NaCl), reduced sodium (R; 2.63 wt% NaCl), and reduced sodium with KCl (RK; 2.63 wt% NaCl, 1.17 wt% KCl). Wheels were evaluated monthly during 5 mo of aging, sampling both inner and outer portions of the cheese wheel. Sodium and potassium concentrations, fat, moisture, pH, a_w, volatile free fatty acids, and extent of proteolysis (as measured by free amino acids) were measured. Sensory attributes (aroma, taste, aftertaste) and volatile flavor chemicals were measured at mo 3 and 5. Salt reductions of 24% and 21% in RK and R, respectively, were achieved. The water activity of C and RK treatments was 0.935 at 5 mo of age. R was higher (0.945). A greater extent of proteolysis was observed in R compared with C. A descriptive sensory panel found higher overall flavor intensity and "waxy" aroma in the inner portion of the cheese compared with outer portions ($P = 0.013$), and in RK when compared with C ($P = 0.036$). Concentrations of medium chain fatty acids were higher in RK than C. Flavor volatiles associated with blue cheese were found in higher concentrations in RK treatment, specifically 2-octanone, 2-nonanone, butanoic acid, and 2-hexanone. A consumer panel (n = 95) ranked overall liking for all treatments similarly, and higher texture liking for RK. Reduction in sodium with and without KCl produced consumer-acceptable blue cheese despite sensory and compositional differences.

Key Words: sodium reduction, blue cheese, KCl

21 Improving the quality of low sodium Cheddar cheese. M. Ozturk*[1], S. Govindasamy-Lucey[2], J. J. Jaeggi[2], M. E. Johnson[2], and J. A. Lucey[1,2], *[1]University of Wisconsin, Madison, [2]Wisconsin Center for Dairy Research, Madison.*

Low Na cheeses often exhibit acidic and bitter flavor and pasty texture. We proposed that starter-induced acidity could be prevented by decreasing microbial activity by the application of high hydrostatic pressure (HHP), and by increasing curd buffering with use of ultrafiltration (UF) retentates. Camel chymosin was used as a coagulant to reduce proteolysis and thus bitterness. Three types of low Na (0.8% NaCl) cheeses were manufactured: non-UF fortified, no HHP applied; UF fortified (17.2 ± 0.6% TS), no HHP applied; and UF fortified, HHP (500 MPa for 3 min applied at 1 d). Regular salt (2% NaCl) non-UF fortified, no HHP applied cheese was also manufactured. Average composition of all cheeses was 36.3 ± 1.4% moisture, 33.8 ± 0.8% fat, and 25.4 ± 0.8% protein. Analysis was performed at 4 d, 2 wk, 1, 3 and 6 mo after cheese manufacture. Cheese functionality during ripening was assessed using texture profile analysis (TPA) and dynamic low-amplitude oscillatory rheology. Quantitative descriptive analysis was conducted with 9 trained panelists to evaluate texture and flavor attributes using a 15 point scale. Pressure treated low Na cheese had ~1.5, and ~3 log lower starter culture numbers than all samples at 4 d and 2 wk, respectively. Cheese milk retentate fortification and HHP treatment resulted in low Na cheeses with significantly ($P < 0.05$) higher acid/base buffering capacity and pH values. For low Na cheeses, retentate fortification significantly ($P < 0.05$) increased cheese firmness measured at 4 d of ripening; however, by 1 mo all low Na cheeses exhibited similar hardness values, which were lower than regular salt cheese. Pressure treatment significantly ($P < 0.05$) increased maximum loss tangent (meltability) and decreased melt temperature. Sensory results indicated only very slight bitterness (<2 out of 15 point scale) development for all cheeses during 3 mo of ripening. Pressure treated and UF-fortified cheese was rated significantly ($P < 0.05$) lower in acidity during ripening. Pressures of 500 MPa and milk retentate fortification could be used to improve the quality of low Na cheese.

Key Words: high pressure processing, low sodium cheese, milk retentate

22 Influence of depletion flocculation and continuous phase viscosity on the stability of sodium-caseinate-stabilized emulsions. Y. C. Liang*[1,2], H. Patel[3], L. Matia-Merino[2], A. Q. Ye[4], G. Gillies[1], and M. Golding[2,4], *[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, [4]Riddet Institute, Massey University, Palmerston North, New Zealand.*

Creaming has a detrimental effect on the quality of a protein-rich oil-in-water emulsion because the emulsion tends to separate into a droplet-rich phase and a droplet-poor phase during aging, which reduces its visual appearance and mouthfeel. We explored the stability and rheological properties of sodium-caseinate-stabilized emulsion. The formation of the transient gel network was studied by microstructure, and by large and small deformation rheology. Sodium caseinate was reconstituted to a 3% (wt/wt) solution. Corn oil (60% wt/wt) was mixed with the protein solution. The mixture was homogenized to yield a stock emulsion, which was then mixed with stock caseinate (20% wt/wt), xanthan gum (2% wt/wt), and maltodextrin (40% wt/wt) solutions to produce final 30% oil-in-water emulsions containing 2 to 10% caseinate, 0.01 to 0.2% xanthan gum, and 5 to 20% maltodextrin, respectively. The pH was adjusted to 6.8 ± 0.04 for all model emulsions. All the samples were prepared separately in duplicates. The emulsions displayed 2 types of behavior. At 1.5 to 4% caseinate, the emulsion separated rapidly, whereas a droplet network with stronger attractions formed slowly at high concentrations (5 to 10%), arresting the phase separation transiently. Small deformation rheology showed that the development of the transient droplet network depended markedly on the unadsorbed caseinate concentration. Droplet rearrangements were possibly influenced by both the strength of the depletion force and the continuous phase viscosity at high caseinate concentrations. Interestingly, droplet network was weakened with the addition of maltodextrin, with a stabilizing mechanism that differed from the prediction that the low shear viscosity will prevent phase separation of the emulsion. The understanding of the development of caseinate-induced droplet network provides a mechanism capable of controlling the creaming behavior of emulsion. We find a good correlation between visual observation and small deformation rheology. More stable samples exhibit a longer droplet network formation time than the less-stable ones.

Key Words: caseinate, depletion flocculation, stability