Graduate Student Competition: ADSA Dairy Foods Graduate Student Oral Competition

70 Structural changes and texture development in milk protein concentrates induced by high hydrostatic pressure. Lee Cadesky*[1], Markus W. Ribeiro[1], Mukund V. Karwe[2], and Carmen I. Moraru[1], [1]*Cornell University, Ithaca, NY,* [2]*Rutgers University, New Brunswick, NJ.*

Milk protein concentrate (MPC) and micellar casein concentrate (MCC), obtained by membrane filtration, are increasingly available. This research focuses on utilizing high hydrostatic pressure (HHP) to induce structural modifications of milk proteins in MPC and MCC and enable the creation of new textures that may find application in milk protein based products. MPC and MCC powders were reconstituted by dispersing the powders in water to 2.5 and 10% casein (w/v), and allowed to fully hydrate. The concentrates were packaged in flexible pouches and subjected to 15 min HHP treatments at 150, 250, 350, and 450 MPa, at temperatures below 25°C, in a 10 L HHP unit. Pressure induced structural changes to casein micelles in HHP treated samples were assessed by (a) evaluating the renneting behavior of the protein concentrates using dynamic rheology; (b) mineral and proteomic profiling of ultracentrifugation supernatants. The study was replicated, and significant differences among treatments were assessed by statistical analyses. In all samples, HHP treatment increased calcium levels in the serum as compared with untreated controls ($P < 0.05$). HHP treatments at 250 and 350 MPa resulted in levels of serum calcium over 3.5 times higher than in untreated samples. HHP above 250 MPa induced significant ($P < 0.05$) concentration and pressure dependent changes in MCC and MPC. In both 10% MCC and 10% MPC, HHP above 350 MPa led to the formation of weak gel structures. HHP treatment also increased G' values of rennet gels and reduced coagulation times, which indicates a destabilization of casein micelles following pressure treatment. In 10% MCC, maximum gel strength was observed after 150 MPa treatment, with G' of 140 Pa as compared with 45 Pa in untreated samples. For 10% MPC, HHP treatment enabled rennet coagulation, which did not occur in untreated samples. MPC renneted gels were weak, with 450 MPa treatment resulting in the strongest gels (G' of 36 Pa). These observations suggest that HHP treatment of milk protein concentrates can be used for developing new types of dairy-based, protein rich foods with unique structure and texture.

Key Words: high-pressure processing, proteins, rheology

71 Solubilization of rehydrated frozen highly concentrated micellar casein concentrate for use in liquid food applications. Ying Lu*[1], Donald McMahon[1], Lloyd Metzger[2], Anil Kommineni[2], and Almut Vollmer[1], [1]*Western Dairy Center, Utah State University, Logan, UT,* [2]*Midwest Dairy Foods Research Center, South Dakota State University, Brookings, SD.*

Highly concentrated micellar casein concentrate (HC-MCC), a potential ingredient for protein fortification, containing ~20% casein with most whey proteins removed by microfiltration, diafiltration, and vacuum evaporation of skim milk. Our objective was to determine the conditions needed to obtain complete solubility of thawed frozen HC-MCC in water, and to understand its gelation upon cooling. Dispersibility (ability to pass through a 250-µm mesh sieve), suspendability (percentage of protein not sedimented at 80 × *g* within 5 min), and solubility (percentage of protein not sedimented at 20,000 × *g* within 5 min) were measured at various mixing conditions. Gelation upon cooling from 50°C to 5°C was monitored based on storage (G′) and loss (G″) modulus and gelled HC-MCC examined using transmission electron microscopy. Thawed HC-MCC was added to water (3% of protein) using high shear (7,500 rpm) for 1 min or low shear (800 rpm) for 30 min at 4, 12, 20, or 50°C and at pH 6.4 to 7.2. The HC-MCC completely dispersed at 50°C, or at ≤ 20°C followed by overnight hydration at 4°C. Suspendability at 50°C was ~90%, while mixing at ≤ 20°C followed by overnight hydration yielded only ~57%. Solubility followed a similar trend with ~83% at 50°C and only ~29% at ≤ 20°C. Mixing HC-MCC in 60 m*M* trisodium citrate increased dispersibility to 99%, and both suspendability and solubility to 81% at 20°C. Cold gelling temperature, defined as temperature at which G′ = G″, was positively correlated ($R^2 = 0.97$) with protein level in HC-MCC. Gelation occurred at 38, 28 and 7°C with 23, 20, and 17% of protein, respectively. Gelation was reversible upon heating. In micrographs of gelled HC-MCC, casein micelles were observed to be within the normal range and close packed together with only ~20 to 50 nm space between them. We propose that cold gelation of HC-MCC occurs when the kinetic energy of the casein micelles is sufficiently reduced to inhibit their mobility in relation to adjacent casein micelles. Understanding solubilization of rehydrated frozen HC-MCC and its rheological property can help designing process systems for using it as a potential ingredient in liquid food.

Key Words: casein micelle, microfiltration, solubility

72 Development of a method for characterizing high-protein dairy powders with an ultrasonic flaw detector. Mary Hauser* and Jayendra Amamcharla, *Kansas State University, Manhattan, KS.*

When choosing a high-protein dairy powder such as milk protein concentrate (MPC), dissolution behavior is an important property to consider. Current methods for testing powder dissolution are time consuming, difficult to reproduce, and subjective. Ultrasound spectroscopy is a rapid and precise method, but expensive equipment and skilled technicians are needed. An economical alternative is to use an ultrasonic flaw detector (UFD). The objective of study was to develop a method to characterize the dissolution behavior of MPC with an UFD. The experimental setup included an UFD (Epoch LTC) in pulse-echo mode and a 1MHz immersion transducer that was kept a constant distance from the reflector plate. Powder concentration, stirring speed, UFD settings, and path length were optimized. To validate the method, 2 batches of MPC80 from a commercial manufacturer were procured and stored at 25°C and 40°C for 4 weeks. MPC was slowly added to water at a 5% concentration. During the dissolution, A-Scan data from UFD was acquired to calculate velocity and attenuation. Focus beam reflectance measurement (FBRM) and solubility index were used as reference methods. Velocity had a trend of fluctuating and then stabilizing with fluctuation time increasing with storage time. Due to the variation in fluctuation, the standard deviation from 900 to 1800s was set as a parameter. Day 0s standard deviation was 0.01 m/s and increased to 0.1 m/s after 4 wk at 40°C. During dissolution, attenuation increased and then gradually decreased. From the attenuation, 3 parameters were extracted. The area under the curve from 0 to 1800s showed the most changes. From Day 0 to wk 4 at 40°C, the area decreased from 100 to 110 Np·s/m to below 30 Np·s/m. After comparing all the data, it was observed that fresh powders dispersed quickly, had a low standard deviation and a larger area. As the MPC aged at 40°C, the

particle dispersion rate decreased and an increase in standard deviation and reduction in area were observed. Overall, the UFD can be a low cost method to characterize the dissolution behavior of dairy powders.

Key Words: ultrasonic flaw detector, milk protein concentrate, solubility

73 The effect of spray-drying parameters on the flavor of skim milk powder. C. W. Park*, M. A. Stout, and M. A. Drake, *Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC.*

Unit operations during production influence the sensory properties of skim milk powder (SMP). Off-flavors associated with SMP decrease consumer acceptance of ingredient applications. Previous work has shown that spray drying parameters such as inlet temperature and feed solids concentration affect physical and sensory properties of whole milk powder and whey protein concentrate. The objective of this study was to determine the effect of inlet temperature and feed solids concentration on the flavor of SMP. Pasteurized skim milk was evaporated to 50% total solids by a falling film evaporator. The condensed milk was then spray dried at either 160, 210, or 260°C inlet temperature and either 30, 40, or 50% total solids in a randomized order. Warm deionized water was added to achieve 30 and 40% solids condensed milk. The entire experiment was replicated 3 times. Flavor of the SMP was evaluated by sensory and instrumental analyses, which included descriptive analysis and solvent extraction with solvent-assisted flavor evaporation followed by gas chromatography mass spectrometry. Surface free fat and particle size were also analyzed. Both main effects (30, 40, 50% solids and 160, 210, and 260°C inlet temperature) and interactions between solids concentration and inlet temperature were investigated. Interaction effects were not significant ($P > 0.05$). Decreasing inlet temperature decreased overall aroma, sweet aromatic and cooked flavors, and 2-acetyl-1-pyrroline and furaneol concentrations and increased cardboard flavor and hexanal, heptanal, nonanal, furfuryl alcohol, and 2,4-decadienal concentrations ($P < 0.05$). Increasing solids concentration increased sweet aromatic flavor and 2-acetyl-1-pyrroline concentration, while 30% solids concentration increased cardboard flavor and hexanal, nonanal, DMTS, and furfuryl alcohol concentrations compared with 40 or 50% solids ($P < 0.05$). Particle size increased and surface free fat decreased with increasing inlet temperature and solids concentration ($P < 0.05$). These results demonstrate that increasing inlet temperatures and solids concentration during spray drying decrease off-flavor intensities in SMP.

Key Words: skim milk powder, spray drying, flavor

74 Using membrane filtration to fractionate acid whey into value-added ingredients. Bang Chen*[1], Karen E. Smith[2], John A. Lucey[2,1], Rebecca Kalscheuer[2], and Michael Molitor[2], *[1]University of Wisconsin-Madison, Madison, WI, [2]The Wisconsin Center for Dairy Research, Madison, WI.*

There has been a huge expansion in acid whey production due to the rapid growth in Greek yogurt manufacture, therefore it is critical to find an economically feasible way to process acid whey. The objective of this study was to determine the suitability of novel experimental nanofiltration (NF) membranes to reduce the calcium content of acid whey, so that value added streams (e.g., lactose) could be produced from this whey. Approximately 1,000 L of acid whey was obtained from a local Greek yogurt manufacturer for each trial. A 10,000 Da UF membrane was used to produce a permeate stream (i.e., remove any residual proteins). Acid whey was then processed by one of 4 different NF membranes. Three novel NF membranes were evaluated for divalent ion permeation and compared with a control NF membrane. Permeates were concentrated to 1× and 2×, and were sampled at processing pressures of 1380, 2760 and 4140 kPa, and at operating temperatures of 4, 21, 43 and 54°C. Flux also was recorded. Total solids, lactose, galactose, lactic acid and calcium contents of the NF permeates/ retentates were determined. The calcium/lactose ratio in the permeate was determined. We wanted a high ratio of calcium to lactose in the permeate so that we could successful reduce the ash content of acid whey while retaining lactose. Higher temperatures and higher pressures yielded higher rates of component permeation for each type of membrane. The novel NF membranes had higher permeation of all components, compared with the control NF membrane. Lactose and calcium permeated the novel NF membranes to differing degrees, in contrast to the control NF membrane. Permeates were further concentrated (6×) by 1 of 2 novel membranes, or a control membrane. The composition of final concentrates and samples taken during the process of concentration were analyzed. The results indicated that the novel membranes had good retention of lactose while calcium permeation increased as the permeate concentration increased. Lactose hydrolysis tests were performed on NF concentrates of acid whey by utilizing a commercially available lactase enzyme with an acidic pH optimum. The end product of this hydrolysis was a dairy syrup with enhanced sweetness.

Key Words: acid whey, membrane processing, nanofiltration

75 Hydrodynamic cavitation as a tool to improve texture, mouthfeel, and creaminess in formulated and high-protein, low-fat Greek yogurts. Gopinathan H. Meletharayil* and Hasmukh A. Patel, *South Dakota State University, Brookings, SD.*

Greek yogurt (GY) manufacturing involves a straining step that generates acid whey, which has led to processing and environmental concerns. Non-strained yogurt (GSY) can be the solution to this problem. However, GSY is unacceptable to consumers because of its tart astringent taste, gritty and chalky texture and lack of creamy mouthfeel, taste, and flavor. We explored the use of hydrodynamic cavitation in conjunction with CO_2 treated functional milk proteins to develop a low fat GSY having rheological and organoleptic profiles similar to GY. CO_2 treated milk retentate was added to skim milk and NFDM base to yield 9% (w/w) protein and 17% (w/w) total solids. The mixture was heated to 90°C for 10 min followed by cooling to 42°C. The milk was fermented with yogurt culture to pH 4.6, followed by rapid cooling to less than 10°C. The yogurt was then subjected to hydrodynamic cavitation using an APV cavitator (SPX Flow Technology, Denmark). Rheology, large deformation studies, % lactic acid (LA) organic acid profiles using HPLC, graininess using microscopy and organoleptic profile (mouthfeel and creaminess) using sensory panel were investigated. Experimental data were tested for ANOVA and statistical significance ($P < 0.05$) was determined, using statistical software SAS. Hydrodynamic cavitation reduced the consistency coefficient of GSY to values similar to those obtained in commercial GY. Cavitation of GSY reduced the number of grains from 2389 to 35 grains/g compared with 293 grains/g in commercial GY. There was no significant difference in the LA content between the cavitated GSY and commercial GY. Organic acid profiles of the strained and non-strained cavitated yogurts were superimposable. GSY subjected to hydrodynamic cavitation had better mouthfeel and creaminess compared with commercial GY. Based on these results, it can be concluded that the combination of CO_2 treated proteins and hydrodynamic cavitation can be used as a promising solution to manufacture GSY with rheological and organoleptic profiles better or similar to GY. This processing innovation will help to reduce processing times,

capital investments and more importantly the vexing problem of acid whey disposal.

Key Words: hydrodynamic cavitation, Greek yogurt, texture

76 Engineering of infant formula emulsions to enhance protein thermal stability through Maillard conjugation. Kamil P. Drapala*, Daniel M. Mulvihill, and James A. O'Mahony, *School of Food and Nutritional Sciences, University College Cork, Cork, Ireland.*

Comfort-type infant formula (IF) emulsions are frequently manufactured using whey protein hydrolysate (WPH) ingredients; however, stability of these emulsions to heating is often poor. The objective of this study was to improve heat stability of such emulsions by conjugation of WPH with maltodextrin (MD) through wet heating. Model IF emulsions (1.55% protein, 3.50% oil, 7.00% carbohydrate) were prepared using whey protein isolate (WPI), WPH, heated WPH or WPH-MD conjugate. The conjugate was prepared by heating a WPH/MD solution (5.00% protein and 5.00% maltodextrin, pH 8.2) at 90°C for 8 h; heated WPH was prepared in a similar manner but in the absence of MD. Emulsions were heated at 75°C or 95°C for 15 min using a rheometer, with viscosity data recorded throughout the heat treatments, or at 100°C for 15 min using an oil bath. Emulsions were recovered after all heat treatments and changes in viscosity, fat globule size distribution (FGSD) and microstructure, determined using confocal laser scanning microscopy (CLSM), were used to monitor the effects of heating on the structure/stability of the emulsions. Emulsions with similar, monomodal size distributions (mean oil droplet diameter $\leq$ 1.0 μm) were formed with all protein ingredients (no significant differences, $P < 0.05$). Heat stability of emulsions increased in the order WPH < WPI $\ll$ heated WPH $\lll$ WPH-MD conjugate. A sharp increase in viscosity during heating at 75°C (WPH) or 95°C (WPI) and significantly higher viscosity ($P < 0.05$) post-heat treatment (as compared with initial viscosity) indicated structural rearrangement/coagulation in the WPH and WPI stabilized emulsions; no changes in viscosity on heating were observed for emulsions stabilized with heated WPH or WPH-MD conjugate. After heat treatment at 100°C, flocculation and coalescence of oil droplets in emulsions stabilized by heated WPH were mediated by protein aggregation (as evidenced by CLSM analysis) while no changes in FGSD or microstructure were observed in emulsions stabilized by WPH-MD conjugate. Modification of WPH through conjugation with MD yielded a protein ingredient with superior thermal stability in oil-in-water IF emulsions.

Key Words: protein-carbohydrate conjugation, emulsion thermal stability, infant formula

77 Improvement of the physicochemical and functional properties of whey protein hydrolysates by conjugation. Eve M. Mulcahy*, Daniel M. Mulvihill, and James A. O'Mahony, *School of Food and Nutritional Sciences, University College Cork, Cork, Ireland.*

Proteins can be modified by enzymatic hydrolysis to alter their functionality. The objective of this study was to determine the effect of conjugation on selected physicochemical functionalities (i.e., solubility and solution clarity/heat stability) of either intact whey protein isolate (WPI) or hydrolysed whey protein isolate (WPH). Conjugation of WPI or WPH (degree of hydrolysis of 8%) with maltodextrin (dextrose equivalent value of 6; MD6) was achieved by heating solutions of 5% WPI or WPH and 5% MD6, initial pH of 8.2, at 90°C for up to 24 h. Samples were taken after 3, 5, 8 and 24 h of heating; the greatest reduction ($P < 0.05$) in available amino groups (21.0% reduction in AAG, measured by the o-phthaldialdehyde assay) occurred during the first 8 h of heating for WPH-MD6, with a considerably smaller decrease in AAG between 8 and 24 h of heating, which was consistent with the limited (0.8%) reduction of AAG in WPI-MD6 after 8 h. The number of amino groups available to react with the carbonyl groups of MD6 were 55% higher in WPH than in WPI which contributed to a greater extent of conjugation with the former. Unheated WPI had a protein solubility of 87.3% at pH 4.5; WPI-MD6 conjugate solutions (8 h of heating) had a protein solubility of 40.0% at pH 4.5 while WPH-MD6 conjugate solutions (8 h of heating) had enhanced protein solubility of 78.6% at pH 4.5. Unheated WPI increased in turbidity after thermal treatment for 3 min at 85°C, with 40 m*M* NaCl (48.5% decrease in transmission compared with control) while WPI-MD6 and WPH-MD6 conjugates retained, to a greater extent, solution clarity after heating (3.5 and 0.2% decrease in transmission compared with respective controls). Conjugation of WPH with MD6 resulted in higher levels of conjugation than for WPI and resulted in a conjugate with enhanced functional properties including improved solubility and lower levels of turbidity development in a heated high ionic strength environment compared with unconjugated WPI and conjugated WPI-MD6.

Key Words: conjugation, whey protein hydrolysate, physicochemical functionality

78 Novel application of a fungal catalase preparation to control spore-forming bacteria in the dairy industry. Nuria Garcia-Fernandez*[1,2] and Ashraf Hassan[1], [1]*Dairy Science Department, South Dakota State University, Brookings, SD,* [2]*Midwest Dairy Foods Research Center, South Dakota State University, Brookings, SD.*

Spores can resist pasteurization, germinate, and grow in the dairy products during storage, causing spoilage. The aim of this work was to investigate the antimicrobial properties of several catalase preparations (Cat) against spore-forming bacteria isolated from dairy sources and their possible applications in milk and cleaning of separation membranes. The antimicrobial activity of a food-grade (FG) commercially available Cat produced by *Aspergillus niger*, a non-food-grade Cat (NFG), and a Cat from bovine liver (BL) against *Bacillus sporothermodurans* (Bs), *Geobacillus stearothermophilus* (Gs), *Bacillus mojavensis* (Bc), *Bacillus licheniformis* (K1), and *Bacillus* spp. (10/1) was assessed by the agar diffusion assay and broth microdilution. The effect of FG Cat on biofilms formed by 2 single strains of slime-producing *Bacillus* (Bc and K1) and a cocktail of 4 strains (Bc, Bs, Gs and 10/1) was studied alone (150 mg/mL) and after a pretreatment with 0.1% Tween 20-PBS buffer. Last, a cocktail of 10^3 spores/mL of 4 strains of *Bacillus* (Bc, Gs, Bs, and 10/1) inoculated in UHT milk was challenged with 0, 6.25 and 12.5 mg/mL of FG Cat for 30 min and the number of survivors was determined. The FG and NFG Cat inhibited the growth of vegetative cells of all tested species of *Bacillus* at 12.5 mg/mL with inhibition zones of up to 25 mm, while BL did not show any inhibition. The FG Cat reduced the number of viable cells in one-day-old Bc and K1 biofilms by 2 and 4.87 log cfu/cm^2 respectively ($P < 0.05$). While the application to a 3-d-old multispecies biofilm reduced the viable counts by only 0.73 log cfu/cm^2, a pretreatment with Tween 20 followed by FG, resulted in a 1.18 log cfu/cm^2 reduction ($P < 0.05$). The FG Cat at 12.5 mg/mL reduced the number of germinating spores in milk by 36.7%. The FG Cat lost antimicrobial activity after heating at 100°C for 10 min. This works describes for the first time the antimicrobial activity of Cat preparations against bacterial spores, which would create new opportunities for the dairy industry to control germination and outgrowth of spore-forming bacteria (patent pending).

Key Words: catalase, antimicrobial, *Bacillus*

79 Impact of buttermilk serum fractions on the rennet coagulation properties of bovine milk. M.-P. Gauvin*[1], M. Britten[1,2], and Y. Pouliot[1], *[1]STELA Dairy Research Center, Institute on Nutrition and Functional Foods (INAF), Université Laval, Québec, Québec, Canada, [2]Food Research and Development Center (FRDC), Agriculture and Agri-Food Canada, St-Hyacinthe, Québec, Canada.*

Buttermilk is the aqueous phase obtained after churning of cream into butter. Its composition is similar to that of skim milk except for higher proportion of milk fat globule membrane (MFGM) components. These components seem to interfere with rennet coagulation of milk. The purpose of the present study was to separate buttermilk serum into 3 fractions using centrifugation and determine the effect of each fraction on milk rennet-induced coagulation. Buttermilk and pasteurized skim milk (control) were centrifuged (31,000 *g*, 34°C, 1 h). Three distinct layers of the supernatants were collected: low-density opalescent (LDO), clear (CL) and high-density opalescent (HDO) layers. Protein and fat in these fractions were characterized by gel electrophoresis (SDS-PAGE) and estimation of phospholipid content by colorimetric phosphorus assay after fat extraction by the Mojonnier procedure. Casein micelles from skim milk were collected by centrifugation (31,000 *g*, 20°C, 1 h), redispersed in the different fractions and used to monitor rennet aggregation and coagulation kinetics using dynamic light scattering and rheology. MFGM protein and residual casein micelles were mainly concentrated in the HDO fraction. This fraction from buttermilk contained 3 times more MFGM protein than the same fraction from skim milk, but 35% less casein. Phospholipids were also concentrated in this fraction, 200 mg/100 g and 17 mg/100 g, representing 43 and 39% total fat of this fraction in buttermilk and skim milk, respectively. All buttermilk serum fractions, especially HDO, showed negative effect on rennet coagulation kinetics. Compared with the results obtained with their respective CL fraction (which is the LDO and HDO-depleted fraction), buttermilk HDO slowed down the aggregation rate (−62%) while skim milk HDOL increased it (+42%). In addition, the growth of casein aggregates was rapidly stopped in presence of buttermilk HDO fraction. This suggests that components in this fraction from buttermilk interfere with the aggregation of renneted casein micelles and more investigation is underway to determine the role of MFGM components.

Key Words: buttermilk, MFGM, rennet-induced coagulation