Graduate Student Competition: ADSA Dairy Foods Division Graduate Poster Competition

M93 Structural properties of milk protein concentrate (MPC) dispersions and emulsions as influenced by presence of small molecule components. Y. Liang*[1,2], H. Patel[1], L. Matia-Merino[2], A. Ye[3], and M. Golding[2,3], *[1]Fonterra Research Centre, Palmerston North, New Zealand, [2]Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand, [3]Riddet Institute, Massey University, Palmerston North, New Zealand.*

Heat-stable milk-protein-stabilized emulsions with desirable mouthfeel and shelf-life stability are a challenging system. The compositions (i.e., proteins, sugars, and small molecule surfactants) of various emulsion phases affect the stability and the rheological properties of this type of emulsion. This study investigated and elucidated the potential ingredient interactions (i.e., protein—sugar) in protein-based dispersions and emulsion systems. MPC-I and MPC-II were used as model proteins. They had similar protein contents of ~81% and calcium contents of 2230 and 1140 mg/100 g, respectively. They were reconstituted as 10% w/w dispersions at neutral pH and the pHs were adjusted to 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, and 7.4. Model emulsions with 10% w/w protein and 10% w/w oil were prepared at pH 6.8. Sucrose was added at 0—30% w/w. Oscillatory rheology and heat coagulation time (HCT) were measured twice for each sample, and all samples were prepared in duplicate. The presence of sucrose (5—30% w/w) influenced the aggregation process of 10% w/w MPC-I dispersions during a heating and cooling cycle (25 to 90 to 25°C), as shown by an inhibition of the increase in the complex modulus during cooling. The heat stability, which would be expected to have a marked maximum at pH ~6.5 and a minimum at pH ~6.9, of the MPC dispersions was influenced by protein type, pH, and sucrose concentration. MPC-I exhibited a marked maximum at pH 7.2 in the absence of sucrose, which shifted to pH ~6.8 in the presence of 30% sucrose. The HCT of MPC-I was higher in the presence of up to 15% sucrose than in the absence of sucrose and decreased gradually with increased sucrose (>15%) to less than that in the absence of sucrose. In contrast, MPC-II dispersions exhibited a marked maximum at pH 6.4 regardless of the addition of sucrose. The emulsions stabilized by MPC-I and MPC-II, with and without sucrose, had markedly different stabilities and rheological properties. The aggregation state of casein micelles and sugar-induced calcium activity changes affected the heat stabilities and rheological properties of MPC-based dispersions and emulsions. The results help in our understanding of the behavior of milk proteins in systems with high carbohydrate content.

Key Words: milk protein concentrate, emulsion, rheology

M94 Application of bixin as an alternative colorant for Cheddar cheese. X. Li,* T. J. Smith, and M. A. Drake, *North Carolina State University, Raleigh.*

Colorless whey ingredients are desirable in food applications, therefore bleaching is necessary to remove residual annatto (norbixin) colorant from Cheddar cheese whey. Bleaching negatively affects the flavor of dried whey ingredients and new restrictions on chemical bleaching agents have increased the need for bleach or cheese color alternatives. Bixin is a nonpolar form of annatto and is not currently used in the cheese industry. However, because bixin is nonpolar, it may have an increased retention in the cheese matrix with less carryover into cheese whey. The objective of this study was to determine viability of bixin as an alternative cheese colorant and if less bixin was present in cheese whey compared with norbixin. Preliminary studies established minimal homogenization pressure to stably suspend bixin in milk. A concentration of 60 mL bixin /454 kg milk was selected for comparable concentration to norbixin incorporation concentration. Pasteurized milk was cooled to 55°C, then 60 mL bixin/454 kg milk (3.8% wt/vol bixin) was added and homogenized (single stage) at 80 bar. Milk with no colorant and milk with 15 mL/454 kg milk norbixin (3% wt/vol norbixin) were processed analogously as controls. Mass balance was determined by extraction and quantification of bixin and norbixin in milk, whey, and cheese. The experiment was replicated 3 times. Nine percent norbixin (2.1 ppm) was recovered in the unseparated cheese whey compared with 1.3% bixin (0.3 ppm; $P < 0.05$). The amounts of norbixin and bixin both decreased after fat separation of whey, but the decrease was greater for bixin than for norbixin ($P < 0.05$). Whey from cheese with bixin was visibly lighter in color concurrent with decreased b* values compared with whey from cheese with norbixin ($P < 0.05$). These results confirm that less bixin colorant was present in cheese whey compared with norbixin colorant, thus potentially circumventing the need for whey bleaching. Bixin may be a viable alternative to norbixin in the cheese and whey industry.

Key Words: bleaching, norbixin, bixin

M95 Cold enzymatic bleaching of fluid whey and retentate. R. E. Campbell* and M. A. Drake, *North Carolina State University, Raleigh*

Chemical bleaching of fluid whey and retentate with hydrogen peroxide (HP) requires high concentrations (250 to 500 ppm) and is most effective at temperatures greater than 35°C. Off flavors are generated during HP bleaching. Enzymatic bleaching of fluid whey and retentate with lactoperoxidase or Maxibright at cold temperatures (4°C) may be a viable alternative to chemical bleaching. The objective of this study was to compare enzymatic and traditional chemical HP bleaching of fluid whey and retentate at 4°C. Fluid Cheddar whey was manufactured in triplicate from pasteurized whole milk, and subsequently, 80% whey protein retentate was manufactured from the fluid whey. The optimum concentration of HP for enzymatic bleaching at 4°C (10 ppm for fluid whey and 15 ppm for retentate) was determined. In subsequent experiments, bleaching efficacy, sensory characteristics, and volatile compounds were evaluated after 1 h (retentate) or 24 h (fluid whey) bleaching. Controls with no bleaching and traditional HP bleaching (250 ppm HP) were also evaluated. Bleaching efficacy was determined by measuring norbixin destruction compared with the unbleached control, sensory profiles were evaluated by descriptive analysis, and volatiles were measured by gas chromatography mass spectrometry (GCMS). At 4°C, enzymatic bleaching of fluid whey and retentate resulted in higher bleaching efficacy than chemical bleaching with HP alone ($P < 0.05$). Due to concentrated levels of naturally present lactoperoxidase, retentate bleached to completion (>80% norbixin destruction) in less than 10 min. In fluid whey, the addition of Maxibright increased the rate of enzymatic bleaching ($P < 0.05$), from 6 to 2 h for 80% norbixin destruction. Percent norbixin destruction by HP was 11% (after 24h) and 90% (after 1h) in fluid whey and retentate, respectively. Bleached wheys and retentates had decreased sweet aromatic and cooked/milky flavors compared with unbleached controls. Wheys and retentates bleached chemically by HP displayed sulfur flavors not present in enzymatically bleached samples. Volatile compound results were consistent with sensory analysis. These

results suggest that enzymatic bleaching may be a viable and desirable alternative to HP bleaching.

Key Words: bleaching, whey, lactoperoxidase

M96 The effect of milk pasteurization temperature on the bleaching of fluid whey. E. Kang* and M. A. Drake, *North Carolina State University, Raleigh.*

Chemical bleaching is a commonly applied unit operation in whey protein processing that negatively influences whey protein flavor. Recent studies have shown that native milk lactoperoxidase (LP) is active in cheese whey and can be used to bleach annatto colored fluid whey. A low concentration of hydrogen peroxide (10–50 ppm) is required for LP activity. When excess concentrations of HP (>100 ppm) are applied, LP is inactivated and HP chemical bleaching occurs. Fluid milk can be subjected to a wide range of heat treatment before cheese manufacture and heat treatment may influence subsequent whey bleaching efficacy. The objective of this study was to investigate the effect of milk pasteurization temperature on the bleaching efficacy of fluid whey. Milk was pasteurized at 63°C for 30 min (LHT) or 79°C for 20 s (HHT) and colored Cheddar whey was produced and fat separated. LP activity (LHT) or inactivation (HHT) was determined by a colorimetric assay. Hydrogen peroxide was then added at 250 ppm (chemical bleaching) or 25 ppm (LP bleaching) at 35 or 50°C and aliquots were collected after 10, 20, or 60 min. Unbleached colored wheys was used as a control. Norbixin extraction and quantification as well as b* values were measured to determine bleaching efficacy. The norbixin content of HHT whey was 82% lower than that of LHT ($P < 0.05$), and the protein content was decreased by 22% for HHT whey compared with LHT whey. Whey from LHT had a higher bleaching efficacy ($P < 0.05$) from 25 ppm HP (80% norbixin destruction) than 250 ppm HP (10% norbixin destruction) at 35°C and 50°C, consistent with LP activity. Bleaching of whey from HHT was lower than LHT in all treatments and there were no differences ($P > 0.05$) between 25 and 250 ppm HP (1.2% norbixin destruction). Regardless of milk pasteurization temperature, greater bleaching was observed with increased time. These results demonstrate the influence of milk heat treatment on bleaching efficacy and also suggest differences in norbixin association with whey components with different milk heat treatment.

Key Words: bleaching, lactoperoxidase, pasteurization

M97 The effect of acidification of retentate on the flavor of spray-dried whey protein concentrate. C. W. Park*[1], E. Bastian[2], B. Farkas[1], and M. A. Drake[1], *[1]North Carolina State University, Raleigh, [2]Glanbia Nutritionals, Twin Falls, ID.*

Off-flavors in whey protein negatively influence consumer acceptance of whey ingredient applications. Clear acidic beverages are a common application of whey protein and recent studies have demonstrated that beverage processing steps, including acidification, enhance off flavor production from whey protein. The objective of this study was to determine the effect of pre-acidification of whey protein retentate before spray drying on flavor of dried whey protein concentrate (WPC). Cheddar cheese whey was manufactured, fat-separated, pasteurized, bleached (250 ppm hydrogen peroxide), and ultrafiltered (UF) to 80% protein retentate, 13% solids (wt/wt). The liquid retentate was then acidified using a blend of phosphoric and citric acids to the following pH values: no acidification (pH 6.5), pH 5.5, or pH 3.5. UF permeate was added to pH 5.5 and pH 6.5 retentates to dilute the protein to the same level as the pH 3.5 retentate (74% protein retentate, 13% solids (wt/wt)). The retentates were then spray dried. The experiment was replicated 3 times. Flavor and volatiles of the WPC74 were evaluated by sensory and instrumental analyses, respectively. Each WPC74 (n = 9) was rehydrated to 10% solids (wt/vol) and adjusted to each of the pH values (6.5, 5.5, or 3.5). Both main effects (pH 6.5, 5.5, and 3.5 before spray drying) and interactions between treatments and final pH when evaluated were investigated. Pre-acidification to pH 3.5 resulted in decreased cardboard flavor and aroma intensity and an increase in soapy flavor ($P < 0.05$) with decreased concentrations of lipid oxidation products hexanal, nonanal, decanal, as well as the protein degradation products dimethyl disulfide and dimethyl trisulfide ($P < 0.05$). Adjustment to pH 5.5 before spray drying increased cabbage flavor ($P < 0.05$) and increased the concentration of the protein degradation product dimethyl trisulfide ($P < 0.05$). The effects of pre-acidification were consistent regardless of the pH the solutions were adjusted to after spray drying. These results demonstrate that acidification of WPC80 retentate before spray drying decreases off flavors compared with acidification of spray dried WPC.

Key Words: whey protein, flavor, acidification

M98 Sensory properties and composition of permeate and permeate fractions. K. Frankowski* and M. A. Drake, *North Carolina State University, Raleigh.*

One of the major contributing factors to hypertension in the US is from the amount of sodium in the American diet. Many food companies are trying to limit the amount of sodium in their products. Permeate, the liquid remaining after whey or milk is ultrafiltered, has been suggested as a salt substitute. It has not been established what component(s) of permeates contribute to sodium replacement or if permeates exhibit salty taste. The objective of this study was to determine the sensory properties of permeates and their composition with a specific focus on organic acids and mineral composition. Eighteen milk or whey permeates and permeate fractions were obtained in duplicate from commercial facilities. Proximate analyses and specific mineral contents were determined. Descriptive analysis of permeates, fractions and model solutions was conducted using a trained sensory panel. Organic acids were extracted and separated and quantified by high performance liquid chromatography (HPLC). Milk and whey permeates were characterized by cooked/milky and brothy flavors, sweet taste and low salty taste. Permeates with lactose (DLC) removed were distinctly salty. Sensory tests with sodium chloride solutions confirmed salty taste of DLC and the low salty taste of permeates were not solely due to the sodium concentration present. Lactic and citric acids and minerals were higher in DLC compared with milk or whey permeates, concurrent with increased salty taste. These results demonstrate that organic acids and minerals enhance salty taste in permeates.

Key Words: permeate, lactose, sodium reduction

M99 Effect of SO-TEC clear whey on physico-chemical characteristics of Cheddar cheese and its whey. A. C. Biswas* and L. E. Metzger, *Dairy Science Department, Midwest Dairy Foods Research Center, South Dakota State University, Brookings.*

Annatto is a color extract that is widely used to produce colored cheddar cheeses. Approximately 15 to 20% of the annatto added to the cheese milk partitions into the whey, which gives it an undesirable yellow color that is bleached chemically or enzymatically. However, bleaching can generate off flavors and a reduction in the nutritive and functional value of proteins. Recently, SO-TEC Natural Cheese color NCC22000, a patent-pending proprietary color formulation, was developed. This cheese color is prepared by encapsulating a blend of red and yellow

fat-soluble carotenoid colors of paprika and β-carotene. This color is claimed to be selectively entrapped within the cheese curd with none of the color being carried into the whey stream. Consequently, using this color, cheddar cheese can be produced while generating an uncolored whey stream. The objective of this study was to characterize the physico-chemical properties of cheddar cheese and its whey manufactured using SO-TEC cheese color. Three replicates of 3 treatments of cheddar cheese were produced in this study. The treatments were: a control with no color addition (NC); annatto color (AC) addition at 0.007% of milk weight; and SO-TEC NCC22000 color (STC) at 0.056% of the milk weight. The whey from each treatment was separated, pasteurized, and analyzed for color and compositional analyses. There were no significant differences ($P > 0.05$) in whey composition (fat, protein, total solids, and ash) or cheese composition (fat, protein, total solid, ash, and salt) among the treatments. The color of the STC cheese and AC cheese were similar. The L*, a*, and b* values were 74.27, 14.92, and 56.05, respectively, for the STC cheese and 74.34, 13.16, and 50.89, respectively, for the AC cheese. There were no significant ($P > 0.05$) differences in the color of the STC whey and the NC whey and both of these treatments had a significantly ($P < 0.05$) higher L* value and a significantly ($P < 0.05$) lower a* and b* value compared with the AC whey. The whey produced from SO-TEC Natural Cheese color NCC22000 has a color identical to uncolored cheese whey and would not need to be bleached to remove undesirable yellow/orange color.

Key Words: cheese color, no-color whey, cheddar cheese

M100 Effectiveness of ultrasonication in inactivating spores of *Bacillus* spp. in skim milk. S. Khanal*[1], S. Anand[1], and K. Muthukumarappan[2], [1]*Midwest Dairy Foods Research Center, Dairy Science Department, South Dakota State University, Brookings,* [2]*Agricultural and Biosystems Engineering Department, South Dakota State University, Brookings.*

Bacterial spores are resistant to pasteurization and may affect quality of milk products. They are known to form biofilms within dairy processing environments. Non-thermal processes such as high pressure, and pulse electric field have been tried to inactivate spores but with a limited success. In this investigation, effect of ultrasonication has been studied on spores of 3 *Bacillus* species, commonly isolated from thermally processed dairy products. Spores of *Bacillus coagulans* (ATCC 12245), *B. licheniformis* (ATCC 6634), and *G. stearothermophilus* (ATCC 15952) were produced by 1 to 2 wk of incubation on modified Brain Heart Infusion agar plates. Spores were harvested from plates by washing and centrifugation, followed by heating at 85°C for 10 min to inactivate the vegetative cells. Sterile skim milk samples, spiked with individual spores at an average level of log 5 cfu/mL, were subjected to ultrasonication through a 13 mm probe using a 20 KHz-VC 505 sonicator (Sonics and Material, USA), at 80 and 100% amplitude for 1, 5, and 10 min. Samples were kept in an ice bath during the treatments for temperature control. Ultrasonicated samples were batch pasteurized at 63°C for 30 min to study the combined treatment effect. Experiments with 3 replicates each were repeated twice. Spore counts were compared before and after respective treatments and were found to be significantly different ($P < 0.05$). A maximum of 35, 33, and 49% of *B. coagulans*, *B. licheniformis*, and *G. stearothermophilus* spores were inactivated respectively, by the ultrasonication treatment of 80% amplitude for 10 min. The combined ultrasonication and pasteurization resulted in 50, 40, and 65% reduction of these spores, respectively. No changes were observed in spore morphology or dimensions, as visualized under the scanning electron microscope. From this research, it is evident that the ultrasonication has the limited ability to destroy *Bacillus* spores. The inactivation percentage was increased up to 65 by combining pasteurization with ultrasonication. Among the spores studied, *B. licheniformis* and *G. stearothermophilus* were the most and least resistant, respectively

Key Words: ultrasonication, spores, amplitude

M101 Screening of different enzymes for modification of the enzyme cleaning step of an existing membrane CIP protocol. D. Singh* and S. Anand, *Dairy Science Department, Midwest Dairy Foods Research Center, South Dakota State University, Brookings.*

Our previous studies revealed that the *Bacillus* species were observed to be most resistant against the cleaning protocol being followed for the entire constitutive microflora isolated from commercial whey concentration membranes. The present investigation was conducted to degrade biofilms by screening of different enzymes (protease, lipase, lactase, α-glucosidase, β-galactosidase) against 24-h-old in vitro biofilms developed by a *Bacillus* isolate using a CDC bioreactor. Data were statistically analyzed using PROC GLM of SAS program with treatment being a fixed effect. The enzyme β-galactosidase was observed to be most effective as it resulted in reductions of 1.71 logs and 0.88 logs under static and dynamic conditions, respectively. Protease and lipase were also found to be effective against embedded cells to some extent. For further evaluations, 3 different approaches were applied to modify the existing CIP protocol. Replacement of existing enzyme cleaning step with only β-galactosidase enzyme (CIP-1) as first modification, against single and mixed species biofilms did not result in any improvement in the effectiveness of the existing CIP protocol. Second approach with modified cleaning conditions (CIP-2) using a narrower range of pH for all the steps, and extended time (60 min) for the enzyme cleaning (step 4) followed by surfactant step (20 min) resulted in greater cleaning efficacy. Third approach (CIP-3) applied a combination of enzymes; protease, lipase, and β-galactosidase to replace the enzyme cleaning step along with modified cleaning conditions as given above. Results revealed increased effectiveness of CIP-3 against *Bacillus* biofilms with cumulative reductions of 3.21 logs as compared with 3.09 logs of existing CIP protocol. Similarly, treatment with CIP-3 against mixed species biofilms resulted in a reduction of 3.86 logs, as compared with the existing CIP reductions of 3.56 logs. Application of CIP-3 on other single and mixed species biofilms also showed a similar trend of effectiveness. It is concluded that the CIP-3 protocol may serve as an effective replacement for the existing CIP protocol.

Key Words: biofilms, CIP, dynamic