Graduate Student Competition: ADSA Dairy Foods Division Oral Competition

80 Norbixin partitioning in full-fat and fat-free Cheddar cheese. T. J. Smith* and M. A. Drake, *North Carolina State University, Raleigh.*

Whey protein is an important commercial product for the dairy industry, and a large portion of it is manufactured from colored Cheddar cheese whey. The Cheddar cheese colorant, annatto, is also present in whey and must be removed by bleaching. A better understanding of the primary colorant in annatto, norbixin, is crucial to produce effective methods for its removal from liquid whey. The objective of this study was to determine norbixin partitioning in cheese and whey from full-fat and fat-free Cheddar cheese. Full-fat and fat-free Cheddar cheeses and wheys were manufactured from colored pasteurized milk in quadruplicate. Three different norbixin levels (7.5, 15, and 30 mL annatto/454 kg milk milk) were used for full-fat Cheddar cheese manufacture and one norbixin level was evaluated in fat-free Cheddar cheese (15 mL annatto/454 kg milk). Norbixin extractions were performed on the milk, the unseparated cheese whey, and the pressed cheese. Norbixin was extracted by solvent extraction and column purification and quantified by high performance liquid chromatography. An average of 9.0% of the norbixin added to the full-fat cheese milk was recovered in the whey and 80.3% was recovered in the cheese. In contrast, 1.2% of the norbixin added to skim milk was recovered in the skim milk cheese whey and 85.2% was recovered in the fat-free cheeses. Level of norbixin addition to cheese milk had no effect on norbixin recovery in cheese or whey ($P > 0.05$) but decreased fat content in cheese milk decreased norbixin content of cheese whey and increased norbixin concentration in the cheese ($P < 0.05$). These results suggest that either norbixin is able to more closely associate with casein when fat is not present or that casein matrix formation and final conformation of fat-free cheese differs from that of full-fat cheese such that a higher percentage of norbixin is entrapped.

Key Words: Cheddar cheese, annatto, norbixin

81 The effect of glucose and citric acid concentration on polymerization of lactose by twin-screw extrusion. A. J. Tremaine* and T. C. Schoenfuss, *University of Minnesota.*

The creation of value-added uses for lactose would benefit dairy processors and enhance whey utilization. Polymerization of lactose to produce oligosaccharides is a promising application. Sugars can be polymerized in the presence of heat and acid, and twin-screw extrusion is a process that could provide a continuous production method. The objective of this study was to investigate the effect of glucose seeding and citric acid levels on the yield of oligosaccharides from lactose. Formulas were blended with a ribbon blender and processed on a Buhler 44-mm twin-screw extruder without a die plate. A high shear profile screw was designed in pretrial experiments, and a feed rate of 15 kg/h and 4 barrel section temperatures of 230°C, 238°C, 238°C, and off, from inlet to outlet were chosen. Treatments included 3 glucose concentrations (0%, 10%, 20%) and 2 citric acid concentrations (1% and 2%), with the remainder of the formula consisting of lactose. Process (die temperature, motor torque and specific mechanical energy) and product responses (color, degree of polymerization (DP), soluble fiber) were measured. DP determined by HPLC indicated profiles were similar between treatments and ranged from DP 1 to DP 15 or higher. Quantification of fiber indicated 26–58% by weight of sample was low and high molecular weight soluble fiber. Citric acid positively affected oligosaccharide yield. These results indicate that oligosaccharides can be created with a continuous process. Further characterization of this product, as well as the development of clean up and concentration methods to remove mono- and disaccharides could lead to the production of a high-value soluble fiber from lactose.

Key Words: extrusion, lactose, oligosaccharides

82 Impact of bleaching on flavor and functional properties of 80% serum protein concentrate. R. E. Campbell*[1], M. Adams[2], D. M. Barbano[2], and M. A. Drake[1], *[1]North Carolina State University, Raleigh, [2]Cornell University, Ithaca, NY.*

Whey proteins that have been removed before the cheese making process are referred to as "native" whey proteins or milk serum proteins. Chemical bleaching of residual annatto has a detrimental effect on whey protein flavor and functional properties. Serum protein allows direct investigation of these effects without the confounding influence of cheesemaking parameters. The objective of this study was to characterize and compare the sensory and functional properties of 80% milk serum protein concentrate (SPC80) produced from bleached and unbleached microfiltered (MF) skim milk permeate with and without added annatto color. Colored and uncolored MF permeates were bleached with benzoyl peroxide (BP) or hydrogen peroxide (HP), ultrafiltered, diafiltered and spray-dried. SPC80 from unbleached colored and uncolored MF permeates were manufactured as controls. All treatments were manufactured in triplicate. Flavor and functional properties of SPC80 were evaluated by sensory and instrumental analyses. The HP bleached SPC80 was higher in lipid oxidation compounds than other bleached or unbleached SPC80, specifically hexanal, heptanal, nonanal, decanal, and 2,3 octadienone($P < 0.05$). Consistent with instrumental results, HP SPC80 were also higher in aroma intensity, cardboard and fatty flavors compared with other SPC80 ($P < 0.05$). The BP SPC80 had lower norbixin concentrations compared with SPC80 bleached with HP ($P < 0.05$). Functionality tests demonstrated that HP SPC80 had more soluble protein after 10 min of heating at 90°C at pH 4.6 and pH 7 than the no bleach and BP treatments regardless of additional color. SPC80 foams generated from bleached samples were more stable than those from unbleached samples ($P < 0.05$). SPC80 bleached with HP were lower in yield stress than other samples ($P < 0.05$). HP bleaching caused more lipid oxidation products and subsequent off flavors than BP bleaching. Bleaching also altered heat and foam stability and this effect was more pronounced with HP compared with BP. These results demonstrate that chemical bleaching alters flavor and functional properties of milk serum proteins. Optimized bleach concentrations and conditions should be determined for whey or chemical bleaching alternatives established.

Key Words: serum proteins, bleaching, functionality

83 Study of the heat-induced interaction pathway between whey protein and buttermilk components. M. Saffon*[1], R. Jiménez-Flores[2], M. Britten[3], and Y. Pouliot[1], *[1]STELA Dairy Research Center, Institute of Nutraceuticals and Functional Foods (INAF), Université Laval, 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 showed that it was possible to form mixed aggregates by heating whey protein in the presence of buttermilk components. It

was hypothesized that caseins and milk fat globule membrane proteins act as a nucleus in the process of whey protein aggregation. Buttermilk powders were obtained by spray drying after ultrafiltration (UF) and diafiltration (DF) of regular buttermilk and whey buttermilk. A portion of these powders was treated by supercritical fluid extraction (SFE) to remove non-polar lipids. Powders were mixed with UF/DF concentrated whey, and the mixtures were heated to 90°C for 5 min at pH 4.6. The dispersions were heated with or without thiol blocking N-ethylmaleimide (5 mM). The liberation of free –SH and the protein profiles were determined every 5 min respectively with the Ellman's reagent and one dimensional PAGE technique (SDS-PAGE) under non-reducing and reducing conditions. 3D images were taken at 100× with a confocal laser-scanning microscope. All experiments were performed in triplicate and the concentrations of free –SH in the mixtures were compared with control (whey) using T tests. Addition of buttermilk significantly decreased the liberation of free –SH during heating. SDS-PAGE gels under non-reducing conditions showed a decrease in native whey and buttermilk proteins during heating, and large aggregates were evident. These large aggregates were also found under reducing conditions and in the presence of N-Ethylmaleimide (NEM). Three-dimensional (3D) images confirmed the interaction between protein and fat globule membrane even in presence of NEM. Overall, our results suggest that the MFGM (either protein or phoshpolipids) could act as an initiator during the formation of mixed aggregates with whey proteins.

Key Words: heat-induced interaction, milk fat globule membrane, whey protein

84 Effect of milk processing on the anticarcinogenic capacity of the milk fat globule membrane. R. Zanabria*[1], A. Tellez[2,1], M. 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), the surface-active material surrounding fat globules in milk, has been shown to have potential anticarcinogenic properties by inhibiting the proliferation of cancer cells through cytotoxic and apoptotic effects. Though the effect of milk processing on the MFGM functional properties is known, the effects over its bioactivity are still lacking. Objective of this work was to determine if processing (i.e., heat treatment) has an effect over the MFGM bioactivity. Two representative colon cancer cell models (HT-29 and Caco-2) were used to test the antiproliferative capacity of MFGM isolates obtained from raw and heat-treated milk. Raw MFGM material was obtained by washing the cream twice with endotoxin free water, subsequent freeze-thawing and ultracentrifugation. Bacterial contamination and lipopolysaccharide (LPS) presence was quantified and kept at minimum by collecting the milk with a catheter and working under sterile conditions. For the heat treated samples, raw milk was batch-treated at 80°C for 10 min (HT80), 74°C for 3 min (HT74) or 63°C for 30 min (HT63) and then the MFGM was extracted. Cell proliferation was studied by the BrdU colorimetric test. Results showed a similar dose-dependent DNA synthesis decrease in both cell lines exposed to 6.25–200 μg protein raw MFGM/mL, although Caco-2 cells seemed to be more resistant. The EC50 values were 111 and 88 μg protein MFGM/mL for Caco-2 and HT-29 cells, respectively. Ceramide (20 μM) used as positive control showed a similar effect, supporting the hypothesis of the sphingolipids as one of the possible compounds responsible for the bioactivity. When the heat treated samples where used, this bioactivity was significantly affected. The HT80 highest dose only produced a 10% reduction in cell proliferation while the lowest concentrations caused the opposite effect by incrementing cell DNA synthesis up to 35%. Similar trends, yet improved, were observed with the HT74 and HT63 fractions: 100 μg protein MFGM/mL caused a cell division decrease of 30 and 40% respectively. These results will be discussed in light of the physico-chemical changes occurring to the MFGM during heating, and they raise new questions related to which active components play a role in the antiproliferative effect observed with the colon cancer cell lines.

Key Words: MFGM, bioactivity, anticarcinogenic

85 Heat stability of micellar casein concentrate (MCC) as affected by temperature and pH. A. Sauer* and C. I. Moraru, *Cornell University, Ithaca, NY.*

The increasing interest in using MCC obtained by membrane separation in manufacturing shelf-stable high protein products creates a need to understand the effect of sterilization treatments on the stability of this ingredient. The goals of this work were to: 1) elucidate the effects of pH and sterilization temperatures on the mineral distribution and dissociation of caseins; and 2) use the generated knowledge to develop solutions for stabilizing the MCC during sterilization treatments. MCC powders were reconstituted with water at 8% concentration, and the resulting dispersions were adjusted to pH values of 6.5–7.3. Subsequently, the 8% MCC samples were heated in an oilbath to 110°C to 150°C, in 10°C increments. The come-up time was identical for all heat treatments. The treated samples were evaluated for particle size, soluble minerals and casein dissociation. Measurement of soluble minerals and casein dissociation (by LC-MS/MS) were performed on supernatants obtained after ultracentrifuging the samples for 60 min at 100,000 × g. The study was conducted in triplicate and the data analyzed statistically. At pH 6.5 and 6.7, all heat treated samples were visibly aggregated or coagulated. At pH 6.9, higher temperatures lead to increased particle size, while at pH > 6.9 little or no changes were observed after heat treatment. Casein dissociation increased with increasing pH for all 4 caseins, at all temperatures, with dissociation of κ-CN and β-CN being most significant ($P \leq 0.05$). At higher pH, the levels of dissociated αs-CN decreased after heat treatment, suggesting aggregation of αs-CN due to the presence of calcium and lost protection by κ-CN. It was concluded that increased stability of MCC requires increasing the pH and lowering the processing temperature. MCCs were then submitted to sterilization after targeted modifications: increased pH and lower processing temperature, at equivalent lethality. A significant reduction ($P \leq 0.05$) in particle size was obtained and no coagulation or aggregation occurred after retorting or UHT. The generated knowledge will allow the effective stabilization of MCC in practical applications, such as the production of novel, shelf-stable protein beverages.

Key Words: micellar casein concentrate (MCC), sterilization, stability

86 Development of a model system to understand the mechanisms of instability and to predict the shelf-life of oil-in-water emulsions. 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.*

Emulsion systems are generally very complex depending on the type and the amount of protein or surfactant present in the various emulsion phases; it is difficult to understand the mechanism of instability. The objective of this study was to design a simple model system to characterize the role of the proteins and surfactants present in the various phases of an emulsion and to probe the exact mechanisms of instability. Four

possible oil-in-water model emulsion systems (pH 6.8 to 7.0) that represented potential interactions between protein-coated or surfactant-coated emulsion droplets and non-adsorbed proteins present in the continuous phase were designed. The model emulsions were prepared by 2 different methods: (a) by mixing stock emulsions [20% w/w oil-in-water emulsions stabilized by 1.0% w/w sodium caseinate (Na-CN) or Tween-20] with solutions of milk protein concentrate (MPC), whey protein concentrate (WPC) and hydrolyzate (WPH) to yield emulsions with a range of protein concentration and (b) by using Na-CN, WPC, and MPC as the sole source of emulsifier for a comparison study. Model emulsions were characterized for particle size, creaming stability, microstructure, rheology, and PAGE (PAGE). Depletion flocculation was observed in emulsions prepared with MPC when the protein concentration in the continuous phase was above 3% w/w and the oil droplets were stabilized primarily by either Na-CN or Tween-20. The state of aggregation and the polydispersity of non-adsorbed casein micelles in the continuous phase determined the extent of depletion flocculation and the shear flow behavior. The caseinate-stabilized droplets were incorporated in the heat-induced protein gel network in model emulsions containing excess non-adsorbed WPC, but Tween-20 stabilized droplets were repelled from the gel. PAGE analysis of various emulsion phases and the addition of EDTA to the systems confirmed competitive adsorption and surface displacement of caseins by whey proteins. The unique model systems approach improved our understanding of the influence of heat-induced ingredient interactions in the system and was success in describing the potential interparticle interactions, which can be used to improve the stability of protein-stabilized emulsions.

Key Words: milk protein, oil-in-water emulsion, instability

87 Shear stabilized micro-phase-separated dairy gels containing significant concentrations of β-glucan. N. Sharafbafi*[1], S. M. Tosh[2], and M. Corredig[1], *[1]University of Guelph, Guelph, Ontario, Canada, [2]Agriculture and Agri-Food Canada, Guelph Food Research Center, Guelph, Ontario Canada.*

Incorporation of nutritionally significant concentrations of soluble fiber (β-glucan) into dairy food products is challenged by thermodynamic incompatibility between polysaccharide molecules and milk proteins. It was hypothesized that gelation of milk proteins under shear would increase the connectivity between the β-glucan phase separated domains, and result in dairy matrices for the delivery of nutritionally significant concentrations of dietary fiber. Rennet induced gelation was the method of choice as it possesses minimal effect on ionic condition of mixed systems. Different shear rates (50 and 100 s^{-1}), shearing times (17, 20, 25 min), and different concentrations of milk proteins (2.78, 5.57, and 8.36%, wt/wt) and β-glucan (0.2 and 0.4%, wt/wt) were tested, to evaluate possible differences in structure of the gels formed. The mixtures were continuously sheared (using rheometer) until approximately 80% of the caseino-macro-peptide was released. Shearing for a longer time resulted in an increase in the viscosity of the mixtures and breakage of the bonds formed between renneted casein micelles. At a constant shear rate and β-glucan concentration, increase in protein concentration significantly increased the stiffness of the gel. At constant protein concentration and low concentrations of β-glucan (≤0.4%), an increase in shear rate reduced the time of gelation and the stiffness of the gel, while it resulted in a faster gelation time and higher gel modulus at higher concentrations of β-glucan (≥0.4%; $P \leq 0.5$). Micro-structural analysis of gels after 2 h of renneting revealed high connectivity between the phase separated domains at high concentrations of milk proteins and β-glucan. At these concentrations, due to formation of bi-continuous structures upon mixing, increase in shear rate resulted in lower viscosity and formation of droplet like domains, which may have resulted in self supporting gels. It was concluded that controlling shear during gelation of milk proteins mixed with β-glucan makes it possible to create novel microstructures in dairy products that provide nutritionally significant (≥0.3%, wt/wt) concentrations of soluble fiber.

Key Words: phase separation, shear, gelation

88 Streamlining the product development process: Use of the preferred attribute elicitation technique to extract key texture attributes influencing consumer liking of dairy yogurts. A. Grygorczyk*[1], M. Corredig[1], I. Lesschaeve[2], and L. Duizer[1], *[1]University of Guelph, Guelph, ON, Canada, [2]Vineland Research and Innovation Centre, Vineland Station, ON, Canada.*

Understanding the key attributes affecting consumer liking is very important for guiding the product development process. While conventional profiling methods employing trained panelists provide a detailed description of products, they cannot generate insight into importance of attributes as they do not engage consumers. The method examined in this study is a novel method, which we will refer to as preferred attribute elicitation (PAE). The method derives important attributes by asking a panel of consumers to agree to a set of attributes which stand out most to them, to rank them according to importance and then to rate the products based on those attributes. As this a novel technique there is a need to examine the method further to understand its strengths and weaknesses, as well as its place among sensory methodologies. The principal components plots generated by the PAE approach and conventional profiling were compared using multiple factor analysis and the RV coefficient. The results for texture characterization of yogurts were found to be highly correlated between the 2 methods (RV coefficient = 0.877). The PAE method was repeated in 4 sessions and a comparison of sessions employing the same set of products generated significant RV coefficients, indicating that the approach generated reproducible data for this set of products. The results confirmed the importance of texture to consumer liking and found that runny texture and gritty mouthfeel detracted from liking. Nearly all attributes generated by consumers explained a significant portion of the variability in the study, indicating that consumers were capable of characterizing products in a meaningful way. It is expected that the method would be most useful in the early stages of product development when only a general product characterization is required and a detailed description may generate confusion.

Key Words: yogurt, rapid profiling, texture