DAIRY FOODS: **TECHNICAL ORAL SESSION: PROTEIN/ POLYSACCHARIDE INTERACTIONS**

0266 Production and purification of whey protein glycate conjugated with low molecular mass dextrans. L. Xu[*1], Y. Gong[1], and J. A. Lucey[2,3], *[1]Department of Food Science, University of Wisconsin, Madison, [2]Wisconsin Center for Dairy Research, Madison, WI, [3]University of Wisconsin, Madison.*

An increasing number of people are suffering from food protein allergies, which have become a growing concern around the world. One possible helpful approach could be to glycate food proteins via Maillard reaction, which may block the IgE binding epitopes of the allergen that are responsible for eliciting an immune response. The goal of this research was to optimize processing conditions for creating and purifying whey protein isolate (WPI) glycate with dextran (DX) of 3 different molecular masses. Purification involved a combination of isoelectric precipitation and ion exchange chromatography. Glycates were characterized using size-exclusion chromatography coupled with multi-angle laser-light scattering (SEC–MALLS), glycoprotein analysis using SDS–PAGE and periodic acid Schiff's glycoprotein staining test with fluorescence laser densitometry. The optimal conjugation condition chosen from these experiments were 10% WPI–30% Dextran (DX), pH 6.5, 62°C for 24h for DX of molecular mass = 10 and 3.5 kDa, and 50°C for 12h for 1 kDa DX. The optimal purification process was performed by ion-exchange chromatography: for G10 (glyate with 10 kDa DX) we used pH 2 running buffer, followed by 0.55M NaCl elution buffer; for G3.5 and G1 (glycate with 3.5 and 1 kDa DX, respectively) we used pH 3 running buffer, followed by 0.52 and 0.5M NaCl running buffer for G3.5 and for G1, respectively. The resulting protein–DX molar ratios were estimated as 1:1.6 and 1:1.8 for G10 and G3.5 with a purity of 91% and 88%, respectively. Future work will focus on examining the allergenicity of the different molecular masses of WPI–DX glycates, using blood sera from cow's milk protein allergic patients.

Key Words: dextran, whey protein isolate, Maillard reaction

0267 Impact of Maillard modification on the in vitro carbohydrate digestibility of WP-dextran glycates. Y. Gong[*1], L. Xu[1], and J. A. Lucey[1,2], *[1]Department of Food Science, University of Wisconsin, Madison, [2]Center for Dairy Research, University of Wisconsin, Madison.*

Whey protein (WP) conjugation with dextran (DX) by the Maillard reaction might provide an alternative approach to decrease the immunogenicity of milk proteins. Protein digestibility of whey protein-dextran (WP-DX) glycates has recently been investigated by our group using an in vitro infant digestion model. The digestibility of carbohydrate part of the glycates is unknown. According to human colonic fermentation models, DX can be entirely degraded by the colonic microflora. In this study, we investigated the in vitro carbohydrate digestibility to find out the impact of conjugation on the glucose release of dextran from WP-DX glycates compared with maltodextrin and a mixture with dextran and whey protein isolates (WPI). WP-DX glycates were made with dextran (molecular weight 10 kDa) and WPI via our patented aqueous Maillard reaction method. The glycates were separated and purified by chromatography. The glycates were digested at 37°C by in vitro model using an enzyme mixture including pepsin, pancreatin and amyloglucosidase, which represent enzymes in the digestion system of animals and humans. The free glucose was measured at 0, 10, 20, 60, 120, 180 min and 24 h by HPLC with ion-exchange chromatography and RI detector. The percentage glucose release of dextran and maltodextrin were 6.3 and 64.5% (g glucose/100 g carbohydrates) respectively, during the first 10 min. The glucose release from WP-DX glycates was 9.5, 13.2 and 16.1% at 60, 120, and 180 min, respectively. The glucose release of glycates were lower ($p < 0.05$) than that of dextran alone at 60, 120, and 180 min of digestion. However, no difference was observed in glucose release rate between WP-DX glycates and dextran alone at 0, 10, and 20 min of digestion. The results indicated that both dextran and WP-DX glycates can be digested into glucose by this mixture of digestion enzymes. The rate of glucose release and extent of release were lower for DX compared to maltodextrin. The conjugation of dextran and WP slowed down the glucose release of dextran. Slower digestion of dextran in conjugates might help maintain a possible protective impact of the polysaccharide for reducing WP allergenicity during digestion.

Key Words: in vitro, digestion, WP-DX glycates

0268 Effects of mineral salts and calcium chelating agents on the functionalities of milk protein concentrate prepared by ultrafiltration. X. Luo*, L. Ramchandran, and T. Vasiljevic, *Victoria University, Melbourne, Australia.*

Functionality of milk protein concentrates can be tailored by modifying state of casein micelles through manipulation of processing conditions including temperature, pH and/or addition of calcium chelators. The objective of this study was to investigate the effect of calcium and calcium chelating agents (EDTA and citrate) on the performance of membrane ultrafiltration (UF) process and the functionalities of resulting milk protein concentrates (MPC). Skim milk adjusted to pH 5.9 was pre-treated with EDTA or citric acid (10, 20 or 30 mmol) and ultrafiltered using a polyethersulfone (PES) membrane at 15°C to five times concentration factor. The membrane performance was measured by the permeate flux during UF process. Used membranes were examined using scanning electron microscopy (SEM). The MPC samples were freeze dried and powders were assessed for physical functionalities including solubility, heat stability and emulsification.Addition of chilators led to a shift in a protein-mineral equilibrium and calcium dissociation from the casein micelle. The total calcium in the final MPC was reduced ($p < 0.05$) from 191 (control) to 131 mM or 135 mM for skim milk pre-treated with 30 mmol of EDTA or citrate, respectively. The casein micelle particle size was subsequently reduced ($p < 0.05$) from 200 nm (control) to 28 nm or 24 nm for the milk pre-treated with EDTA or citrate at concentrations equal to or greater than 20 mmol. Consequently, solubility of the MPC increased ($p < 0.05$) from 92% (control) to 98% (ETDA, $\geq$ 20 mmol) or 98.9% (citrate, 30 mmol); heat stability was also enhanced ($p < 0.05$) from 78% (control) to 83% (EDTA, 20 mmol) or 87% (citrate, $\geq$ 20 mmol). The emulsion capacity has increased from 1170 (control) to 1392 or 1459 (g oil/g protein) ($p < 0.05$) when 30 mmol of EDTA or citrate were added, respectively. Addition of EDTA or citrate hindered the membrane performance as observed by reduced permeate flux from 10.5 kg/h.m^2 (control) to 7.9 kg/h.m^2 (EDTA, $\geq$ 20 mmol) and 8.6 kg/h.m^2(citrate, $\geq$ 20 mmol) at the start of UF. Consequently UF processing time increased from 5 h (control) to 7 h (EDTA) or 6 h (citrate). This work has provided new insights into the relationship between calcium, calcium chelators and their influence on the casein micelle size and the physicochemical properties of MPC produced using UF, and also demonstrated the potential of using EDTA and citrate acid to manipulate MPC product functionality using UF.

Key Words: milk protein concentrate (MPC), functionality, ultrafiltration (UF), membrane, calcium chelator, casein micelle

0269 Storage stability of sodium caseinate stabilized oil-in-water emulsions as affected by severe heat treatment and storage temperatures. Y. Liang*[1], G. Gillies[2], H. G. Patel[3], L. Matia-Merino[1], A. Ye[4], and M. Golding[1,4], *[1]Massey University, Palmerston North, New Zealand, [2]Fotnerra Research and Development Centre, Palmerston North, New Zealand, [3]South Dakota State University, Brookings, [4]Riddet Institute, Palmerston North, New Zealand.*

Oil-in-water emulsions are an important basis of many food products such as soup, sauces, salad dressing, processed cheese and whipped cream. In many cases, liquid emulsions are processed at high temperature (e.g., retort or UHT processing) and may be stored at different temperatures. There is little information on how high heat treatment and storage temperatures influence the creaming stability of caseinate-stabilized emulsions. In this study, we investigated the effects of heating and storage conditions on the structural, mechanical and rheological properties of caseinate-stabilized emulsions. The stock emulsion was prepared by mixing a reconstituted sodium caseinate solution (2% w/w) with 60% w/w oil and subjecting it to a high pressure homogenization. Caseinate solutions of different concentration were heated separately at 120°C as a function of time up to 60 min. These heated caseinate solutions were then mixed with the stock emulsion in different ratios to form the model emulsions with 1−8% protein. The creaming stability of unheated emulsions was determined between 20 and 60°C. All experiments were performed at least in duplicate. The creaming kinetics determined by Turbiscan showed that the phase separation of model emulsions was markedly dependent on the duration of the heat treatment. The differences between unheated and heated emulsions were attributed to the heat-induced physicochemical changes in sodium caseinate nanoparticles. At low and moderate caseinate concentrations (2% and 4% respectively), the droplet-droplet interactions were weakened while the droplet-droplet interactions increased at high Na-CN concentration (6%) by the addition of heated sodium caseinate. It seems that the former structural change is predominantly due to reduced depletion attraction, whereas both reduced depletion attraction and decreased continuous phase viscosity influenced the later structural change. Unheated emulsions stored at higher temperature (60°C) resulted in an accelerated phase separation compared to those stored at lower storage temperatures. The main cause was attributed to the weakened depletion energy and decreased viscosity at accelerated temperatures. Both changes lead to a rapid droplet network formation and rearrangement.

Key Words: emulsion, heat-induced degradation, depletion flocculation

0270 Understanding mechanisms of the plasmin-induced dissociation of the casein micelle. H. Bhatt*[1], A. Cucheval[1], C. Coker[1], H. G. Patel[2], A. Carr[3], and R. Bennett[3], [1]*Fonterra Research & Development Centre, Palmerston North, New Zealand,* [2]*South Dakota State University, Brookings, SD,* [3]*Massey University, Palmerston North, New Zealand.*

Bovine plasmin is a highly heat-resistant enzyme that is naturally present in milk. Plasmin can survive severe heat treatments such as UHT and may act on casein during the storage of milk products and lead to proteolysis, gelation, and bitterness. We explored the plasmin-induced dissociation of the casein micelle to achieve a better understanding of gelation and sedimentation mechanisms in different milk products. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and reverse phase high performance liquid chromatography (RP-HPLC) were used to monitor the extent of hydrolysis of casein. The particle size, turbidity, mineral level in the serum phase, and sedimentation were also analyzed and were correlated with different extents of hydrolysis. The particle size and turbidity decreased during the initial hydrolysis, but increased dramatically towards the end of hydrolysis. This indicated that casein micelle dissociation occurred during early stages and that aggregation of hydrolyzed peptides occurred towards the final stages of hydrolysis. The total calcium and phosphorus level in the serum phase increased linearly with an increase in the extent of hydrolysis, suggesting the release of peptides containing colloidal calcium phosphate from the casein micelle. The SDS-PAGE and RP-HPLC results indicated that hydrophilic peptides, e.g., proteose peptones, were the first to dissociate from the casein micelle on plasmin-induced hydrolysis; hydrophobic peptides, e.g., γ-caseins, dissociated slowly and with dissociation patterns that were identical to those of κ-casein, suggesting that, even after breakage of the anchor points, the release of κ-casein from the micelle was too slow to cause gelation. These results provide new insights into the dissociation pattern of the casein micelle and how this relates to plasmin-induced sedimentation or gelation of UHT milk systems.

Key Words: plasmin, UHT milk, gelation

0271 Heat-induced changes in milk proteins in high-carbohydrate media. T. Huppertz*[1,2] and H. G. Patel[2], [1]*NIZO food research, Ede, Netherlands,* [2]*South Dakota State University, Brookings.*

Casein micelles in milk are unique association colloids, sterically stabilized by a polyelectrolyte brush consisting of κ-casein, and are crucial structure elements in the creation of desirable texture and stability in dairy products such as cheese and yogurt. Casein micelles show remarkable colloidal stability, but when treatment conditions extreme or solvent quality is reduced, the micelles lose their colloidal stability and aggregate. This study investigated the colloidal stability of casein micelles and whey protein at high temperature and in the presence of a high level of carbohydrate, conditions commonly encountered in caramels, sweetened condensed milk and Dulche de Leche. Adding 10−50% carbohydrate to milk reduced their colloidal stability of casein micelles. These effects are more extensive for carbohydrates of lower molar mass. Heating milk at > 110°C increased casein micelle size and turbidity as a result of the aggregation of casein micelles, with contributions from heat-induced denaturation and aggregation of whey proteins. Heat-induced increases in particle size and turbidity were more extensive at higher heating intensity, with increasing carbohydrate concentration and decreasing molar mass of the carbohydrate. The presence of a free aldehyde group in reducing sugars also strongly influence the heat stability of milk through Maillard reaction products. The positive effect of Maillard reaction products on the heat stability of milk was derived from the formation of reductones, which can facilitate the covalent cross-linking of milk proteins and hence increase the heat stability. Whey protein denaturation was impaired by the presence of carbohydrates. Denaturation temperature increased with increasing carbohydrate content, effects being larger for low-molecular-mass carbohydrates. The size of heat-induced whey protein aggregates could be tailored by a combination of carbohydrate type and concentration. The results presented facilitate the extension of our understanding of the behavior of milk proteins in environments strongly deviating from natural physiological conditions. Such insights can be applied to understand and tailor the behavior of milk proteins in environments of high carbohydrate content, e.g., caramels, sweetened condensed milk and Dulche de Leche, and facilitate the design of rules for attaining maximum milk protein functionality in these systems.

Key Words: casein, whey protein, heat, carbohydrate, heat stability, denaturation

0272 Effects of pH on the morphology and mechanical property of heat-induced whey protein aggregates. C. W. Y. Lam* and S. Ikeda, [1]*University of Wisconsin, Madison.*

Whey proteins denature and aggregate when they are heated in aqueous environments. It is known that the morphology of resulting aggregates varies depending on pH. However, the structure-property relationship of heat-induced whey protein aggregates has not been fully understood. The objectives of this study were to study the effect of pH on the morphology of heat-induced whey protein aggregates and to seek correlations between their morphologies and mechanical properties. Whey protein was dissolved in deionized water adjusted to pH 3−7 and heated at 80 ± 0.2°C. Subsamples were taken at pre-specified time intervals and quenched in a 0°C water bath. The sample solutions were diluted to a protein concentration of 10-100 ppm, deposited on to freshly cleaved mica

surfaces, air-dried, and imaged using atomic force microscopy (AFM) operated in peak-force tapping mode in air. Further mechanical tests were done with AFM force spectroscopy, where the whey protein aggregates were indented directly to obtain interaction forces. These force curves were analyzed where the Young's modulus (E) of the samples can be fitted and calculated using the Hertzian model. The data were used to verify the mechanical and surface properties of the samples with different pH obtained with AFM imaging. At pH 3, a relatively small fraction of protein aggregates revealed fibrillar morphologies, while the majority of aggregates appeared to be particulate. At other pHs, only particulate aggregates were observed. All of these particulate aggregates were composed of smaller elementary particles, suggesting that the heat-induced aggregation was a two-step process regardless of pH, consisting of the formation of primary aggregates, followed by the secondary aggregation between primary aggregates. The Feret's diameter, representing the diameter of the smallest circle that entirely covers an individual whey protein aggregate, became more dependent of the aggregate size with increasing pH, indicating that whey protein aggregates became more extended, coarse, or anisotropic with increasing pH. Furthermore, the surface roughness evaluated based on the cross-sectional height data decreased by a factor of 2 with increasing pH from 5.5 to 7. This suggested that the protein aggregates collapsed, meaning that the primary aggregates were denser and more tightly packed within the aggregate. From the force spectroscopy analysis, the samples prepared at pH 7 showed larger E values than the samples prepared at pH 5.5. This suggested that the samples at pH 7 were stiffer, which conformed with the previous morphological results.

Key Words: whey protein, heat-induced aggregation, AFM

0273 Strengthening interfacial whey protein films by conjugation with gellan. B. Cai* and S. Ikeda, *The University of Wisconsin, Madison.*

Whey protein can be used as an emulsifier that forms nanometer-thick rigid layers at hydrophobic-hydrophilic interfaces in dispersed systems and provides stability against coalescence of the dispersed phase. If another surfactant is added, however, whey protein will be displaced competitively from the interface, leading to a loss of stability of the dispersed system. Gellan is a network-forming polysaccharide widely used in the food industry. Whey protein-gellan conjugates are expected to show enhanced resistance against surfactant-induced competitive displacement because gellan is considered to form additional networks at the interface. The objectives of this study were to conjugate whey protein either covalently or electrostatically with gellan and to investigate the effect of the different conjugation methods on interfacial structure and resistance to the surfactant-induced competitive displacement from the interface. Whey protein was conjugated either covalently or electrostatically with gellan. The conjugate was dissolved in water and spread on the air-water interface formed on a Langmuir trough. Food-grade nonionic surfactant Tween 20 was then injected into the aqueous phase to induce competitive displacement. Langmuir-Blodgett interfacial films were sampled at pre-specified surface pressures by dipping a freshly cleaved mica sheet in and out through the interface. The interfacial films thus transferred on the mica surface were imaged using atomic force microscopy. Both covalent and electrostatic conjugates formed close-packed interfacial films at the air-water interface. String-like structures of gellan attached to globular protein molecules were also evident. The thickness and surface pressure of the interfacial films were approximately 0.2-0.4 nm and 8-12 mN/m, respectively. Upon the addition of Tween 20, the surface pressure increased further due to the adsorption of the surfactant to the interface. Nanometer-sized surfactant domains first appeared at a surface pressure around 20 mN/m, and expanded their areas with increasing surface pressure. The thickness of protein domains increased with increasing surface pressure, consistent with the previously proposed orogenic displacement mechanism. At a certain surface pressure (e.g., 23 mN/m), the covalent conjugate occupied a larger interfacial area (83%) than both electrostatic conjugate (74%) and the WPI control (61%), The interfacial area occupied by the covalent conjugate displaced from interface less rapidly than electrostatic conjugate demonstrating a more resistant interfacial structure. These results suggest that covalent conjugation of whey protein with gellan is a more effective approach than electrostatic conjugation in strengthening interfacial protein layers and enhancing their resistance against surfactant-induced competitive displacement from the interface.

Key Words: whey protein, gellan, interface

0274 Enhancement of radical quenching ability of sweet whey and casein hydrolyzate: mutual supplementation with thermally generated maillard reaction products. Z. Z. Haque* and D. Mukherjee, *Food Science, Nutrition & Health Promotion, Mississippi State University, Mississippi State.*

Copiously available sweet whey is a source of nutritious proteins, peptides, free amino acids, and lactose. Abundant N-terminal, ε-amino-groups and the reducing sugar, allows thermal generation of Maillard reaction products (MRPs) on heating. With an aim to develop powerful natural antioxidative foods, this research investigates the enhancement of short (Antioxidative Activity; AA) and long term (Antioxidative Persistence; AP) ROS quenching ability of two types of sweet whey, Cheddar (ChWPC) and Edam (EWPC) whey and enzymatic hydrolyzate of casein (CH) through mutual supplementation with freshly heat-generated MRPs. Whey and CH dispersions (3 and 2%, respectively, w/v) were heated for 0 to 4 h at 90°C in McIlvaine's iso-ionic buffer (pH 7.0), with and without

added lactose (1%, w/v), to generate MRPs. AA and AP were determined from luminol-induced chemiluminescence (CL) caused by unquenched hydroxyl radicals generated by pyrolysis of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (ABAP). Decrease in CL, measured as relative light units (RLU), compared to control (without the test materials) at maximal radical generation within one and 2 h, were respectively measures of AA and AP. Thermal generation of MRPs tended to enhance AA and AP in both sweet wheys though this effect was more dramatic for ChWPC. This was conceivably due to its greater peptide content. CWPC and EWPC heated for 1 h exhibited radical induced CL maxima of 99 and 141 RLU, respectively. Whereas values for ChWPC + lactose and EWPC + lactose were 90 and 140 RLU, respectively. Furthermore, a direct correlation was observed between added lactose induced MRP formation and AA of ChWPC, though this was not so clear for EWPC. AA of CH after heating for 4 h showed a CL of 79 RLU. However, this decreased when lactose, ChWPC and EWPC were added to CH as seen from increasing CL of 164, 107, and 121, respectively. The AP values for the same treatments were 53, 87, 57, and 62 RLU, respectively. The study not only indicated the variable effect of MRPs on antioxidative properties of the sweet wheys and CH, but also depicted the dramatic time-dependent thermal enhancement of AA and AP of ChWPC on MRP formation with added lactose. These data can potentially lead to the development of powerful new antioxidants to alleviate the detrimental effects of cellular oxidative stress.

Key Words: antioxidants, free radicals, reactive oxygen species.

0275 Impact of heat treatments on the functionalities of milk protein concentrate 80. R. M. Horak*, J. A. Lucey, and M. Molitor, *University of Wisconsin, Madison.*

Processing conditions impact the properties and functionality of milk protein concentrate 80 (MPC80). Nonfat dry milk is categorized according to the processing temperatures (low, medium, high) as these treatments greatly influence its functionality and end use. It is unknown how MPC80 properties will be affected by increased pasteurization temperatures of skim milk (before concentration). Increased pasteurization temperatures will denature more whey protein, which could impact solubility, foam stability, and viscosity of the reconstituted powder. Raw skim milk received heat treatments of 72°C for 16 s or 77, 82, or 87°C for 1 min, immediately before ultra- and diafiltration. The retentate (23% TS) was spray dried and outlet temperature was adjusted to maintain consistent moisture content between powders (~4.4%). The powders had similar composition including fat contents (< 2%). Functionality testing included solubility, foaming, tapped bulk density, and reconstituted viscosity. Tests were conducted within 1 wk of production. Powders were also stored at 30°C for 6 mo with functionality tested every 30 d. SDS-PAGE results show higher heat treatments produced more di-sulfide linked aggregates. For all powders, solubility slightly decreased during storage, but higher heating temperatures did not have a significant impact on solubility. Initial foaming experiments indicated that heat treatments of 82°C or 87°C for 1 min produced foams that were stiffer and more stable than samples treated at temperatures of 77°C for 1 min. Bulk density decreased over storage but was not significantly affected by heat treatment. Experiments investigating the viscosity of reconstituted powders are ongoing; however, initial results indicate that viscosity slightly increased with an increase in heating temperature. Based on preliminary results, it was concluded that increased heat treatments had the greatest impact on the foaming properties of MPC80 but has less impact on other functional properties.

Key Words: milk protein concentrate, processing, functionality