GRADUATE STUDENT COMPETITION: ADSA DAIRY FOODS DIVISION ORAL COMPETITION

0325 Improving properties of acid skim milk gels by adjusting non-micellar to micellar protein ratio and controlling protein interactions.

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In addition to the protein content, the type and status of milk protein in yogurt milk may affect the final rheological properties of yogurts. In the present study, we investigated the effects of altering non-micellar to micellar casein ratio on the rheological properties and microstructure of acid gels. Model acid gel formulations containing 0, 10, 30, and 60% protein substitution from carbon-dioxide-treated milk proteins (T-MPC) as a source of non-micellar casein and non-fat dry milks (NFDM) as a source of micellar casein were developed. All the samples were standardized to 4% w/w protein and 12% w/w total solids. The pH was adjusted to 6.5 before their pre-heating to 90°C/10 min. Acid milk gels were prepared using Glucono-δ-lactone to obtain final pH4.4 ± 0.05 after 4 h incubation at 30°C. The soluble (serum) phases obtained by centrifugation of heated and unheated milk samples at 25000 g/1h were characterized using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Microstructure and rheological properties of acid gels were characterized using confocal laser scanning microscopy (CLSM) in the fluorescence mode and small amplitude oscillatory rheology (SAOR) (1% strain, 0.1Hz frequency), respectively. Photon correlation spectroscopy was used to study the particle size of the heated milks. ANOVA was used to test the results and statistical significance at $P < 0.05$ was determined, using the statistical software SAS. The SDS-PAGE pattern of model formulations containing T-MPC showed significantly higher ($P < 0.05$) proportion of soluble caseins and disulfide-linked casein-whey protein complexes in the serum phase of unheated and heated milk, respectively. Particle size of formulations containing T-MPC was significantly lower than control samples containing untreated proteins. This can be attributed to the preferential interaction of whey proteins with κ-casein in the soluble phase. SAOR showed a significant increase ($P < 0.05$) in the elastic modulus (G') of acid gels formulated with T-MPC to an optimum level. CLSM images revealed that gels containing treated proteins had smaller, well-connected aggregates with uniform, homogenous pore-sizes, which explained the results of rheological characterization. It can be concluded that the soluble casein-whey protein complexes and optimum non-micellar to micellar casein ratio in the yogurt formulation yielded acid gels with significantly improved rheological properties. Overall, the results suggested that yogurt with varying texture can be made by altering the ratio of non-micellar to micellar casein and by manipulating interactions of milk proteins at soluble and micellar phase. This invention is patent pending.

Key Words: yogurt, carbon dioxide, rheology, microstructure

0326 Controlling the viscosity of milk concentrates through tailored casein-whey protein interactions.

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Heat-induced interactions between caseins and whey proteins form the basis for their functionality in many applications. We have hypothesized that the pH of the milk before preheat-treatment and adjusting preheating temperatures will influence the distribution of casein-whey protein complexes between the micellar and the soluble (serum or continuous) phase of milk and this will affect the viscosity of the continuous phase in concentrated milk. Therefore, the objective of the present study was to investigate the effect of pH and preheating temperatures on the viscosity of skim milk concentrates. Reconstituted milk, 10% w/w total solids (TS), adjusted to pH 6.5, 6.7 (control) and 6.9 was preheated either at 80 or 90°C for 5 min. Following these treatments, the milk was concentrated to final TS of 45 and 50% (w/w) under vacuum at 60°C using a rotary evaporator. Dynamic viscosity of the resulting concentrates was measured at 55°C at a constant shear rate of 100 s^{-1} using a Stresstech Rheometer. Particle size was determined using a Malvern Zeta-sizer-Nano-ZS. The heated and pH adjusted samples were centrifuged at 25,000 g for 1 h to obtain soluble (serum) and micellar phases. The protein interactions in these milk samples were characterized in detail using non-reduced and reduced sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The experiments were repeated at least two times and results were tested by ANOVA and statistical significance at $P < 0.05$ was determined, using the statistical software SAS. Significant differences in the viscosity of milk concentrates were observed ($P < 0.05$) when the milk was pre-adjusted to different pH and pre-heated at different temperatures. The results showed that the concentrates obtained from milk preheated at pH 6.5 and 80°C for 5 min had significantly ($P < 0.05$) lower viscosity compared to that preheated at pH 6.7 and 6.9 at both TS levels studied (45 or 50% w/w). The samples pre-heated at pH 6.5 also showed increase in particle size in contrast to the samples preheated at pH 6.7 and 6.9. Such differences can be explained by differences in the interactions of casein and whey proteins and distribution of casein-whey protein complexes distributed at continuous phase and micellar phase as shown by SDS-PAGE. It can be concluded that adjusting the pH and preheating temperature of milks can be used as levers for controlling viscosity of milk

concentrate for powder manufacturing, which ultimately has an influence on the efficiency of drying.

Key Words: milk concentrate, viscosity, casein-whey proteins, pH.

0327 Partial calcium depletion during membrane filtration impacts gelation of reconstituted milk protein concentrates. H. Eshpari*[1,2], P. S. Tong[3], and M. Corredig[4], *[1]University of Guelph, Guelph, ON, Canada, [2]California Polytechnic State University, San Luis Obispo, [3]Dep. of Dairy Science, California Polytechnic State University, San Luis Obispo, [4]Dept Food Science, University of Guelph, Guelph, ON, Canada.*

Solubilization of colloidal calcium phosphate (CCP) from casein micelles during membrane filtration (e.g., through acidification) may affect the structural organization of these protein particles. The aim of this study was to investigate the effects of addition of glucono delta lactone (GDL) to skim milk during membrane filtration on the structural changes of the casein micelles by studying their functionality after reconstitution of the final powders. In particular, the renneting behavior of the casein micelles was examined, as renneting is affected by both the calcium equilibrium and their supramolecular structure. Milk protein concentrate (MPC) powders were manufactured in duplicate, either by ultrafiltration (65% protein, MPC 65) or by ultrafiltration followed by diafiltration (80% protein, MPC80), using pasteurized skim milk, either at the native milk pH (~ pH 6.6), or after addition of GDL to pH 6.0, followed by spray drying. The amount of total calcium for the MPC80 without and with GDL varied with a significant difference ($p < 0.05$) from 18,449.5 ± 265 to 15,954.5 ± 271 (μg/g), respectively. Samples were reconstituted at a 3.2% (w/w) protein to compare their gelation behavior between treatments. Both reconstituted MPC 65 and MPC 80 treated with GDL showed significantly increased amounts of soluble calcium ($p < 0.05$) and non sedimentable caseins compared to their respective controls, as measured by ion chromatography and SDS-PAGE electrophoresis, respectively. The primary phase of rennet gelation was not significantly different ($p < 0.05$) between treatments, as measured by the amount of caseino-macropeptide released, using reverse phase-high performance liquid chromatography (RP-HPLC). Rheological measurements were performed using a controlled stress rheometer on the reconstituted samples immediately after addition of rennet, both before and after dialysis against skim milk, to ensure similar serum composition for all samples. While reconstituted samples before dialysis showed no gelation (defined as tan d = 1), only control MPC 65 and 80 showed gelation after serum re-equilibration. It was concluded that the gelation properties of reconstituted MPC powders were negatively affected by the presence of soluble casein, and positively affected by the amount both soluble and insoluble calcium present after reconstitution. This work, testing the renneting behavior of various reconstituted MPC samples, clearly demonstrated that decrease in pH to 6.0 during membrane filtration affects the structure of casein micelles with important consequences to their processing functionality.

Key Words: milk protein concentrate, calcium depletion, rennet coagulation

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0328 Utilizing whey protein isolate and polysaccharide complexes to stabilize aerated dairy gels. E. C. O'Chiu* and B. Vardhanabhuti, *University of Missouri, Columbia.*

Previous research has shown that heated soluble complexes of whey protein isolate (WPI) with polysaccharides can improve both foam stability and acid-induced gel strength. We utilized these complexes in aerated dairy gels, which could be formulated into novel-textured high-protein desserts. The objective of this study is to determine the effect of polysaccharide charge density and concentration within a WPI-polysaccharide complex on the properties of aerated gels. Three polysaccharides having different degrees of charge density were chosen: low methoxyl pectin (LM-12), high methoxyl type D pectin (HM-D), and guar gum. WPI-polysaccharide complexes were prepared by heating the mixed solutions (8% protein, 0 to 1% polysaccharide) at pH 7. To form aerated gels, 2% glucono-δ-lactone (GDL) was added to the solutions and foam was generated by whipping with a handheld frother. The foam set into a gel as the GDL acidified to a final pH of 4.2. The aerated gels were evaluated for overrun and rheological properties. Stability was determined by measuring drainage (the volume of liquid separated from the aerated gels). Overrun of aerated gel (179% to 14%) significantly decreased as polysaccharide concentration increased due to increased viscosity, which limited air incorporation. Increased concentration was significantly related to increased stability ($P < 0.001$) which could be due to increased viscosity of the pre-foam solutions limiting the mobility of the air bubbles. A negative logarithmic relationship was found between solution viscosity and drainage. However, charge density played an important role on stability. Plot of drainage against solution viscosity revealed that drainage was lowest in samples with high charge density pectin (LM-12) followed by those with low charge density pectin (HM-D). Aerated gels with guar gum (no charge) did not show improvement to stability as separation still occurred even at highest guar concentration. Rheological results showed no significant difference in gelation time among samples; therefore, stronger interactions between WPI and high charge density polysaccharide were likely responsible for increased stability. Rheological results also revealed that aerated gels with LM-12 pectin had the highest final elastic modulus, followed by guar gum, then HM pectin gels. Stable dairy aerated gels can be created from WPI-polysaccharide complexes. High charge density polysaccharides, at concen-

trations that provide adequate viscosity, are needed to achieve stability while also maintaining solution overrun capabilities. This can inform the formulation of dairy-based gels set by acid or calcium such as whipped yogurts and mousses.

Key Words: acid-induced gelation, aeration, whey protein

0329 pH-triggered intragastric gelation of whey protein/alginate and its effect on sucrose release.

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Protein digestion is highly influenced by gastric conditions, protein structures, and the presence of other food components in the gastrointestinal tract. Protein and dietary fibers are common food ingredients; however, the effect of dietary fiber on protein digestion is not fully understood. Our previous study showed that whey protein/pectin mixture formed intragastric gel under simulated gastric conditions, which slowed the degradation of protein and could potentially affect the digestion and release of other nutrients. The objective of this study was to investigate the in vitro gastric behavior of mixed whey protein and alginate, and its effect on the digestion pattern of protein and sucrose release. Mixed solutions of 5% whey protein isolate (WPI), alginate (0.01 to 0.05 alginate to WPI wt. ratio) and 10% sucrose were prepared by heating them together at 85°C for 30 min. Simulated gastric fluid (SGF) consisted of 0.034 M NaCl, 3.2 mg/g pepsin, and pH was adjusted to 1.2, 2, 3, and 4. The in vitro digestion was performed using reciprocating cylinder dissolution apparatus, with 10-g sample added to 78 g SGF (pepsin: protein = 1: 2). Rheological properties and electrophoresis were performed to evaluate the gastric behavior of the mixture, and HPLC was used to measure sucrose release during digestion. At low alginate to WPI ratios, alginate did not significantly affect the degradation of whey protein and the bioavailability of sucrose, as shown by SDS-PAGE and HPLC, respectively. Increasing biopolymer ratio to 0.05 led to extensive intragastric gelation immediately when samples were mixed with SGF at pH 1.2. The mechanism behind intragastric gelation is believed to be the cross-linking between oppositely charged protein and alginate molecules when pH was reduced to lower than the pI of protein. Sucrose was entrapped in the gel network since no sucrose was detected in the digestion media once the intragastric gel was formed. During dissolution, physical movement and proteolysis by pepsin led to slow degradation of the gel, which also resulted in the slow release of sucrose from the matrix in 20 min. Intragastric gelation was only observed in SGF at pH 1.2 and 2.0. This study indicated that at certain conditions whey protein and alginate mixtures could form intragastric gel, which delayed protein digestion and sucrose release from the matrix. These results can potentially lead to formulation of whey protein beverage having lowered postprandial glycemic response.

Key Words: intragastric gelation, digestion, sucrose

0330 Evaluation of an adsorbent for the removal of aflatoxin M1 from contaminated milk.

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Lactating cows that consume aflatoxin B_1 (AFB_1) contaminated feed containing approximately 20 parts per billion (ppb) may produce aflatoxin M_1 (AFM_1) contaminated milk that exceeds the FDA maximum allowable limit of 0.5 ppb. Current detoxification methods for the reduction of AFM_1 include the use of sequestering agents added to feed. The sequestering agents act as an enterosorbent to ameliorate the toxicity of AFB_1 by reducing intestinal absorption. However, not all AFB_1 is bound and the residual can be metabolized to AFM_1. Once this tolerance level of 0.5 ppb AFM_1 is surpassed, the milk must be discarded because it cannot be used for human consumption resulting in economic losses. The current study examines the proficiency of an adsorbent, powdered activated carbon (PAC) to bind AFM_1 in various milk types as PAC has excellent adsorption properties in an aqueous environment. A total of 24 samples ($r = 3$) contained artificially spiked AFM_1 (0.5 ppb) and 0.1%, 0.25%, and 0.4% PAC in whole, skim, and raw milk. Samples were shaken, extracted using Agilent QuEChERS extraction salts, and analyzed via liquid chromatography with mass spectrometry detection. A concentration of 0.5 ppb AFM_1 was spiked into 10 mL to yield a final concentration in whole (0.54 ± 0.07 ppb), skim (0.46 ± 0.01 ppb), and raw milks (0.56 ± 0.03 ppb). The highest concentration of PAC (0.4%) resulted in a significant decrease in AFM_1 contamination ($p < 0.05$) with a reduction of 65% (0.18 ± 0.08 ppb), 91% (0.05 ± 0.01 ppb), and 52% (0.24 ± 0.03 ppb) of AFM_1 from the whole, skim, and raw milks, respectively. No milk showed any significant difference in percent protein, lactose, or total fat relative to their milk blanks ($p > 0.05$) suggesting that PAC has no effect on milk constituents. Preliminary results show that the use of PAC can reduce the amount of AFM_1 below the FDA safety limit and, as a result, prevent the dumping of milk.

Key Words: AFM_1, activated carbon, milk

0331 Application of FT-IR and flow cytometry to evaluate the effect of sodium chloride on probiotic bacteria.

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The aim of the study was to investigate the effect of varying sodium chloride concentrations on cell membrane, viability and proteolytic activity of probiotic bacteria. Reconstituted skim milk was inoculated with *Lactobacillus acidophilus* at varying salt concentrations (0-10% NaCl) and pH levels (4.0, 5.0 and 6.0) and ACE-inhibitory activity and proteolytic activity were determined. Additionally, the effects of NaCl reduction and its substitution with KCl on cell membrane of certain probiotic bacteria (*Lb. acidophilus*, *Lb. casei* and *B. longum*) and

a pathogenic bacterium, *Escherichia coli* were investigated using Fourier transform infrared spectroscopy (FT-IR). A critical NaCl concentration that inhibited the growth of *E. coli* without significantly affecting the growth of probiotic bacteria was determined by monitoring cell growth and FT-IR spectra. To evaluate the effect of substitution of NaCl with KCl, substitution was performed at critical total salt concentration at varying concentrations (0%, 25%, 50%, 75% and 100% KCl). Furthermore, the effects of varying NaCl concentrations on viability, membrane integrity and metabolic activity of these probiotic bacteria were studied using conventional technique and flow cytometry. The findings revealed that in *Lb. acidophilus* degree of proteolysis increased with higher salt concentration at pH 5.0 and 6.0 and ACE-inhibitory activity was highest at pH 5.0 at all salt concentrations. Fourier transform infrared spectroscopy results demonstrated significant shifts occurring in amide-I and amide-III regions when *Lb. acidophilus* was subjected to varying salt concentrations. Further, the conventional technique revealed that 2.5% was the critical level of NaCl to inhibit the growth of *E. coli* without significantly affecting the growth of most probiotic bacteria. The FT-IR analysis also highlighted the changes that occurred mainly in amide regions on increasing NaCl concentration from 2.5 to 3% in most bacteria. The findings suggest that 50% substitution of NaCl with KCl at 2.5% total salt could inhibit *E. coli*, without affecting the probiotic bacteria. Lastly, the observations from conventional culture technique were compared with the findings from flow cytometric analysis on metabolic activities of the cells and it was revealed that there was a correlation between culturability and dye extrusion ability of *Lb. casei* and *B. longum*. However, a certain population of *Lb. acidophilus* was viable as per the plate count method but the efflux activity was compromised. The metabolic activity of *Lb. casei* was found to be highest among the three probiotic bacteria.

Key Words: FTIR, flow cytometry, probiotic bacteria

0332 Genomic insights into high exopolysaccharide-producing dairy starter bacterium *Streptococcus thermophilus* ASCC 1275. N. Shah, Q. Wu* and H. M. Tun, *The University of Hong Kong, Hong Kong.*

Streptococcus thermophilus ASCC 1275 (ST 1275) is a typical dairy starter bacterium and produces the highest known amount (~1,000 mg/L) of exopolysaccharide (EPS) in milk within this species. This organism produces both capsular and ropy EPS and possesses textural modifying properties for yogurt and cheese. In this study, de novo shotgun paired-end pyrosequencing was applied to complete the whole genome of ST 1275. The genome size of ST 1275, a plasmid-free bacterium, was ~ 1.85 Mbp with an average GC content of 39.1%. A novel *eps* gene cluster for EPS assembly containing two-pair genes of *eps*C-*eps*D for determining the chain length of EPS was found in ST 1275 genome, which confirms that ST 1275 produces two types of EPSs as found in our previous studies. Compared with other sequenced *S. thermophilus* strains, ST 1275 possessed the lowest numbers of 5 rRNA operons and 55 tRNAs suggesting that this organism may have a more effective protein synthesis machinery. The highest number of four separate CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) loci was found in ST 1275 genome indicating that this organism may have a better adaptive immunity against various bacteriophage infections. Further analysis including carbohydrate utilization, effective proteolytic system, sophisticated stress response systems and defense systems in ST 1275 was performed to provide genomic insights into its adaptation to milk and as a cell factory for EPS production during milk fermentation. The elucidation of ST 1275 genome makes this organism as a model dairy starter bacterium for the research of high EPS yield and capsular/ropy EPS producer from the species of *S. thermophilus*.

Key Words: genome sequencing; EPS biosynthesis; *Streptococcus thermophilus*

0333 Effectiveness of pulsed light treatment on the inactivation of pathogenic and spoilage bacteria on cheese surface. J. Proulx*[1], L. Hsu[1], B. Miller[1], G. Sullivan[1], K. Paradis[2], and C. I. Moraru[1], [1]*Cornell University, Ithaca, NY,* [2]*McGill University, Montreal, QC, Canada.*

Cheese products are susceptible to post-processing cross-contamination that can lead to both food safety issues and significant losses due to spoilage. Pulsed Light (PL) treatment, consisting of short, high-energy light pulses, could represent a solution to address this issue since it is a nondestructive technology that can effectively inactivate microorganisms on surfaces. This study examined the effectiveness of PL on the inactivation of the spoilage microorganism *P. fluorescens* and the pathogen surrogates *E. coli* ATCC 25922 and *L. innocua*. The effect of inoculum level, cheese surface topography, and the presence of clear polyethylene packaging were evaluated in a full factorial experimental design. The challenge microorganisms were grown to stationary phase: *P. fluorescens* 1150 was grown at 30°C in tryptic soy broth (TSB) while *E. coli* ATCC 25922 and *L. innocua* FSL C2–008 were grown at 37°C in TSB and brain heart infusion (BHI), respectively. White cheddar and processed cheese, chosen for their different surface topography, were cut into 2.5 cm × 5 cm slices. The samples were then spot inoculated using ten droplets of 10 μL per slice, resulting in an initial concentration of either 5 or 7 log CFU/slice. Inoculated samples were dried overnight at 4°C. For treatments through packaging, sterile UV-transparent low-density polyethylene packaging was placed on top of the inoculated cheese samples immediately before the PL treatment. Cheese samples were then exposed to PL doses of 1.1 to 13.2 J/cm^2. PL-treated samples were stomached for 2 min in Butterfield Phosphate Buffer, the extract then plated on selective media and survivors enumerated by standard plate

counting (SPC). When survivor counts fell below the SPC detection limit, the most probable number was used. Experiments were performed in triplicate and data were analyzed using a general linear model. PL was most effective against *E. coli*, achieving a maximum log reduction of 5.4 ± 0.3, at a dose of 13.2 J/cm^2. For *P. fluorescens*, a maximum log reduction of 3.7 ± 0.9 and for *L. innocua* a maximum log reduction of 2.9 ± 0.8 at 13.2 J/cm^2 were obtained. The process parameter effects tested showed varying statistical significance when used in different combinations, but PL treatments through packaging and without packaging consistently resulted in similar inactivation levels. This study suggests that PL has strong potential for decontamination of cheese surface.

Key Words: pulsed light, cheese, pathogenic and spoilage bacteria