

# Graduate Student Competition: ADSA Dairy Foods Oral Competition

**60 Effect of salt replacers and flavor enhancers to reduce sodium in Cheddar cheese on aging and sensory properties.** J. E. Grummer\* and T. C. Schoenfuss, *University of Minnesota, Department of Food Science and Nutrition, St. Paul.*

The objective of this study was to produce 60% reduced sodium Cheddar cheese by using salt replacers and flavor enhancers and investigate the effects on aging and sensory properties. Replacement salts were added at levels to create the same water activity as control. Treatments were KCl only, KCl and one of 4 different flavor enhancers or masking agents (hydrolyzed vegetable protein/yeast extract, a bitter blocker, disodium inosinate (IMP) and disodium guanylate (GMP)), and modified KCl only. In duplicate, salt and salt replacer treatments were applied to create 2 different salt-to-moisture ratios (S/M). Target sodium content was 600 mg/100 g cheese for control and 240 mg/100 g cheese for all reduced-sodium cheeses. In reduced sodium cheeses, KCl was applied at 2.45 times the rate of NaCl (wt./wt.) for the high S/M and 2.16 times for the low S/M. Descriptive sensory analysis was conducted by a trained panel over 4 mo of aging in a replicated Latin square design and analyzed by SAS (SAS Institute, Inc., Cary, NC) software. Gross composition and mineral content (sodium, potassium, magnesium, calcium) were determined, and monthly tests conducted during 4 mo of aging (moisture, pH, water activity, water soluble nitrogen, texture profile analysis, and lactic acid bacteria (LAB) counts). Data was analyzed by XLSTAT (Addinsoft; New York, NY) software using 2-way ANOVA with repeated measures. Umami was higher in cheeses with IMP and GMP. Sulfur and sour dairy notes were higher in reduced-sodium cheeses. Sensory differences were observed between the 2 salt-to-moisture ratios, but the number of significant differences declined during aging. Despite similar moisture levels and water activity between reduced sodium and control cheese, differences in pH, LAB and texture were observed indicating that KCl may not have the same effect on reactions as salt. However, sensory analysis showed that salty, bitter and metallic flavors were not significantly different in reduced-sodium cheeses compared with control.

**Key words:** Cheddar cheese, sodium reduction, salt replacers

**61 The influence of NaCl reduction on the properties of cheddar cheese where moisture contents were kept constant.** K. V. Grant\*<sup>1</sup>, S. Govindasamy-Lucey<sup>2</sup>, J. J. Jaeggi<sup>2</sup>, M. E. Johnson<sup>2</sup>, and J. A. Lucey<sup>1</sup>, <sup>1</sup>*University of Wisconsin, Madison*, <sup>2</sup>*Wisconsin Center for Dairy Research, Madison.*

In producing an acceptable reduced NaCl cheese, it is important to have a good understanding of the effects of reducing NaCl on various cheese properties. We previously studied the properties of Cheddar cheese made with reduced and low NaCl levels without altering the cheese make procedure; which resulted in differences in moisture contents. The objective of this study was to investigate the impact of NaCl reduction on cheese properties independent of moisture content. Duplicate trials were conducted resulting in Cheddar cheese with 3 NaCl levels: normal (~1.7%), reduced (~1.2%), and low (~0.7%). Moisture contents (at 3 wk) and pH (at 4 d) were: 36.7% and 5.01, 37.3% and 5.02, and 38.0% and 5.00, in normal, reduced, and low NaCl cheeses, respectively. Different manufacturing procedures (ripening time, curd size, and salting wait times) were used to correct for the moisture contents. Cheeses were analyzed at 4 d, 3 wk, 5 wk, 3 and 6 mo.

**Key words:** NaCl reduction, Cheddar

**62 Concentration of casein micelles: Changes in renneting functionality in the presence of sodium caseinate.** P. Krishnankutty Nair\*<sup>1,2</sup> and M. Corredig<sup>1</sup>, <sup>1</sup>*Department of Food Science, University of Guelph, Guelph, Ont., Canada*, <sup>2</sup>*Department of Dairy Development, Government of Kerala, India.*

The changes in processing functionality of concentrated milk are caused by several factors, among the most important, the ionic equilibrium and the increase in the interactions between the casein micelles because of their increased volume fraction. This work reports the use of osmotic stressing as a noninvasive method to study concentrated milk, to preserve the ionic balance while reaching very high levels of protein concentration. Objective of the research was to observe the changes in the renneting functionality of casein micelles in the presence of sodium caseinate added either before or after concentration. Protein levels of about 10% were obtained by osmotic concentration for 18h at 4°C, using poly ethylene glycol in permeate. Untreated skim milk with sodium caseinate at 0.2% was concentrated using osmotic stressing. Alternatively, sodium caseinate was added at a similar ratio after concentration. The formation of the rennet-induced gel was followed using rheology and diffusive wave spectroscopy. The amount of soluble casein was quantified using ion exchange chromatography. All experiments were done in triplicate. After 45 min of gelation, the G' were 505 ± 56, 382 ± 41 and 5 ± 5 Pa for control, sodium caseinate added before and after respectively. Similarly, turbidity parameter was 7.26 ± 0.24, 6.21 ± 0.29 and 4.9 ± 0.14 mm<sup>-1</sup> and self diffusion coefficient was 1.34 × 10<sup>-14</sup>, 7.89 × 10<sup>-14</sup> and 2.76 × 10<sup>-13</sup> m<sup>2</sup>s<sup>-1</sup> observed for control, sodium caseinate added before and after respectively. Analysis of the supernatants confirmed a 10% increase in the area of caseins in samples where the sodium caseinate was added after concentration. Interestingly, the soluble casein added before concentration seemed to re-gain renneting functionality, perhaps because of a higher incorporation in the casein micelles during concentration. This research brings new insights on the rearrangements that may occur to casein micelles during concentration, with important consequences for a better understanding of membrane filtration processes and for the use of casein micelles as functional delivery systems.

**Key words:** casein micelles, sodium caseinate, renneting

**63 Impact of transglutaminase on the functionality of micellar casein concentrate in process cheese product applications.** P. Salunke\* and L. E. Metzger, *Midwest Dairy Foods Research Centre, South Dakota State University, Brookings.*

Microfiltration (MF) is used for producing micellar casein concentrate (MCC) from skim milk. MCC is an ingredient that has been evaluated in process cheese product (PCP) manufacture. However, MCC provides inferior functionality relative to rennet casein. A potential method to modify the functional properties of milk proteins is to crosslink them utilizing transglutaminase (TGase, EC 2.3.2.13). The objective of the study was to evaluate the impact of TGase on the functionality of MCC when used in PCP applications. In this study the impact of TGase (Ajinomoto Food Ingredients LLC, Chicago, IL, 100U activity/g) treatment of skim milk before MF as well as TGase treatment after MF was evaluated. Three treatments were utilized and included: TGase treatment (7U/g of protein) before MF (T1); TGase

treatment (7U/g of protein) after MF (T2); and a control. After addition of TGase each of the samples was incubated for 20 min at 50°C, then heated to 70°C and held for 10 min. MF was performed at 20°C using a laboratory scale tangential flow MF system (NCSRT Inc., Apex, NC). A volume concentration factor of 6.2 with diafiltration of 100% of the original skim milk volume was utilized. The experiment was replicated 3 times using 3 different lots of skim milk. After MF each treatment was freeze-dried and subsequently utilized as an ingredient in PCP manufacture. The PCP was formulated to have moisture, fat, salt and protein of 44.0, 25.0, 1.8, and 18.25%, respectively. The ingredients in each formulation were mixed in a Kitchen Aid and PCP was produced using a Rapid Visco Analyzer (RVA). The PCP was analyzed for pH, RVA-Viscosity, Texture profile analysis (TPA) and Dynamic stress rheology. The RVA-Viscosity and TPA-Hardness of PCP made from the TGase treatments was significantly ( $P < 0.05$ ) higher than the control. Additionally the  $G'$ ,  $G''$ ,  $G^*$  and transition temperature of PCP produced from T2 were significantly ( $P < 0.05$ ) higher than T1 and the control. This study demonstrated that TGase treatment modifies the functional properties of MCC when used as an ingredient in PCP.

**Key words:** transglutaminase, micellar casein concentrate, process cheese product

#### **64 Production of a high concentration liquid micellar casein concentrate (18% protein) with a long refrigerated shelf-life.** I. Amelia\* and D. M. Barbano, *Cornell University, Ithaca, NY.*

Our objective was to develop a multistage process to produce a high concentration liquid micellar casein concentrate (18% protein-MCC18) with a long refrigerated shelf-life. MCC is a novel milk protein ingredient produced by fractionating skim milk using the filtration technology. To have a long refrigerated shelf-life, the processing of MCC18 was designed to maximize the removal of low molecular weight compounds, e.g., lactose, nonprotein nitrogen (NPN) which can be easily metabolized by microbes for nutrient sources, while minimizing the microbial count in the final product. The production of MCC18 was done over a period of 5 d. The experiment was replicated 3 times with a different batch of raw milk. The raw milk was pasteurized, and skim milk was produced. Skim milk was ultrafiltered to remove more than a half of the lactose and NPN. The UF milk retentate was diluted with RO water and then microfiltered in 4 stages (including 3 stages of diafiltration) to remove approximately 95% of the serum protein and further remove lactose and NPN. The retentate from the last stage of MF was ultrafiltered to concentrate the protein to 18% and batch pasteurized. The final MCC18 contained 18.04% true protein, 0.31% NPN and 0.13% lactose. MCC18 was collected immediately after processing in sterile plastic vials and stored at 4°C. MCC18 at the day of processing contained 100 cfu/mL, 84 cfu/mL, and 190 cfu/mL of total aerobic bacteria and 360 cfu/mL, 62 cfu/mL, and 440 cfu/mL of total spores for replicate 1, 2, and 3, respectively, using the 3M Petrifilm Aerobic Count method. MCC18 was analyzed for the total aerobic bacteria count each week for the 16-week shelf-life at 4°C. The bacterial count didn't change significantly with time (week), however the effect of replicate was significant, as analyzed using the PROC GLM of SAS. The production of MCC could be used to balance excess skim milk supply by concentrating the valuable casein portion, and storing it at refrigeration temperature. This strategy avoids the cost of hauling excess skim milk to a drying plant and the high energy cost of evaporation and drying.

**Key words:** micellar casein, microfiltration, shelf-life

#### **65 Serum protein removal from skim milk with a 3-stage, 3X ceramic Isoflux membrane process at 50°C.** M. Adams\* and D. M. Barbano, *Cornell University, Ithaca, NY.*

Our objective was to quantify the capacity of 0.14  $\mu\text{m}$  ceramic Isoflux microfiltration (MF) membranes to remove serum proteins (SP) from skim milk. A 3-stage, 3X, feed-and-bleed MF study with diafiltration in the latter 2 stages was conducted at 50°C using Isoflux membranes to determine cumulative SP removal percentages after each processing stage. The experiment was replicated 3 times starting with different batches of raw milk. The Proc GLM procedure of SAS was used for statistical analysis. In contrast to 3X MF theoretical cumulative SP removal percentages of 68%, 90%, and 97% after 1, 2, and 3 stages, respectively, the 3X Isoflux MF process removed only 39.5%, 58.4%, and 70.2% of SP after 1, 2, and 3 stages, respectively. Previous research has been published that provides the skim milk SP removal capacities of 3-stage, 3X 0.1  $\mu\text{m}$  ceramic Membralox uniform transmembrane pressure (UTP), 0.1  $\mu\text{m}$  ceramic Membralox graded permeability (GP), and 0.3  $\mu\text{m}$  polymeric polyvinylidene fluoride spiral-wound (PVDF SW) MF systems at 50°C. No difference in cumulative SP removal percentage after 3 stages was detected ( $P > 0.05$ ) between the Isoflux and previously published PVDF SW (70.3%) values, but SP removal was lower ( $P < 0.05$ ) than published GP (96.5%) and UTP (98.3%) values. To remove 95% of SP from 1000 kg of skim milk in 12 h it would take 7, 3, 3, and 7 stages with 6.86, 1.91, 2.82, and 14.24  $\text{m}^2$  of membrane surface area for the Isoflux, GP, UTP, and PVDF SW systems, respectively. The MF systems requiring more stages would produce additional permeate at lower protein concentrations. The ceramic MF systems requiring more surface area would incur higher capital costs. Possible reasons why SP removal with the Isoflux membranes was lower than theoretical include: a range of membrane pore sizes existed (i.e., some pores were too small to pass SP), the selective layer modification and reverse flow conditions at the membrane outlet combined to reduce the effective membrane surface area, and the geometric shape of the Isoflux flow channels promoted fouling of the membrane and rejection of SP by the foulant.

**Key words:** microfiltration, serum protein, ceramic membrane

#### **66 The manufacture of linoleic acid-modified chitosan/ $\beta$ -lactoglobulin nanoparticles as a delivery system of quercetin.** H.-K. Ha\*, M.-R. Lee, and W.-J. Lee, *Division of Applied Life Sciences (Institute of Agriculture and Life Science), Gyeongsang National University, Jinju, Korea.*

The nutritional delivery and absorption of poorly bioavailable materials, such as quercetin, can be improved by entrapping such nutrients inside of nanoparticles. The hypothesis of this study was that attractive forces between chitosan and  $\beta$ -lactoglobulin ( $\beta$ -lg) may play a critical role in the formation and physicochemical properties of linoleic acid-modified chitosan (CS-LA)/ $\beta$ -lg nanoparticles containing nutrients with low bioavailability, such as quercetin. The objective of this research was to investigate how manufacturing variables, such as degree of substitution (DS) of CS-LA and incubation temperature, affect the formation and physicochemical properties of CS-LA/ $\beta$ -lg nanoparticles. CS-LAs with different DS (2.7, 4.6, and 8.4%) determined by  $^1\text{H}$  NMR were synthesized via carbodiimide-mediated coupling reaction. CS-LA/ $\beta$ -lg mixtures at pH 4.4 were incubated at 5, 10, 15, and 20°C for 30 min. The morphological and chemical properties of CS-LA/ $\beta$ -lg nanoparticles were determined by atomic force microscopy and electrophoretic light scattering spectrophotometer. Encapsulation efficiency of quercetin was determined by high perfor-

mance liquid chromatography. In atomic force microscopy images, spherically-shaped particles with a diameter from 170 to 350 nm were observed indicating that nanoparticles were successfully formed. Zeta-potential value of nanoparticles was  $\sim +18$  mV. As DS was increased from 2.7 to 8.4%, which may enhance hydrophobic attractions between chitosan and  $\beta$ -lg, the size of CS-LA/ $\beta$ -lg nanoparticles was significantly ( $P < 0.05$ ) increased from 275 to 351 nm and encapsulation efficiency of quercetin was significantly ( $P < 0.05$ ) increased from 52 to 56%, respectively. As incubation temperature was increased from 5 to 20°C, a significant ( $P < 0.05$ ) increase in the size of CS-LA/ $\beta$ -lg nanoparticles from 183 to 319 nm was observed while encapsulation efficiency of quercetin was significantly ( $P < 0.05$ ) decreased from 56 to 53%. In conclusion, DS and incubation temperature were the major key-parameters determining the size of nanoparticles and encapsulation efficiency of quercetin.

**Key words:**  $\beta$ -lactoglobulin, chitosan, nanoparticles

**67 Alternative bleaching methods for 80% whey protein concentrate.** E. J. Kang\* and M. A. Drake, *North Carolina State University, Raleigh.*

Whey protein concentrate (WPC) is an important ingredient in the food industry due to its nutritional and functional properties, including a bland flavor and color. To remove residual annatto colorant, fluid whey is bleached during processing to WPC. Recent studies have shown that the 2 approved bleaching agents in the United States, hydrogen peroxide (HP) and benzoyl peroxide, remove color but negatively impact flavor. The objective of this study was to evaluate alternative methods for bleaching WPC80: UV radiation (UV), acid-activated bentonite (BT) and Maxibright (MB). Cheddar cheese colored with annatto (15mL/454kg milk; 3% norbixin content) was manufactured following standard procedures and liquid whey was collected. Following pasteurization and fat separation, liquid whey was subjected to one of 5 treatments: control (CT) (no bleaching; 50°C, 60 min), HP (250 mg/kg; 50°C, 60 min), UV (1 mL/min exposure; 50°C), BT (0.5% w/w; 50°C, 60 min), or MB (2 dairy bleaching units/mL with 0.5mM HP; 35°C, 30 min). The treated whey was then ultrafiltered, diafiltered at 50°C, and spray-dried to make WPC80. The entire experiment was replicated 3 times. Color (norbixin extraction), descriptive sensory and instrumental volatile analysis were conducted on WPC80. Norbixin recovery rates were 73, 61, 21, and 10% for HP, UV, BT and MB treatments, respectively. Bleaching reduced sweet aromatic flavor in WPC80 compared with CT WPC80 regardless of bleaching agent ( $P < 0.05$ ). The HP WPC80 had higher cardboard and fatty flavors compared with CT WPC80 while the UV and MB WPC80 displayed distinctive mushroom/burnt or potato flavor, respectively. Consistent with sensory results, guaiacol (smoky/burnt) and methional (potato) were detected, respectively, in the UV and MB WPC80. Volatile lipid oxidation products were higher in HP, UV and MB WPC80 compared with BT and CT WPC80, respectively ( $P < 0.05$ ). Based on bleaching efficacy and flavor profiles of WPC80, BT and MB may be potential alternatives to HP for bleaching whey.

**Key words:** WPC80, bleaching, flavor

**68 Impact of bleaching whey on the sensory and functional properties of 80% whey protein concentrate.** S. M. Jervis\*<sup>1</sup>, R. E. Campbell<sup>1</sup>, K. Wojciechowski<sup>2</sup>, D. M. Barbano<sup>2</sup>, and M. A. Drake<sup>1</sup>, <sup>1</sup>North Carolina State University, Raleigh, <sup>2</sup>Cornell University, Ithaca, NY.

Whey is a highly functional food that has found wide-spread use in a variety of food and beverage applications. Whey proteins used in such applications are largely from annatto colored Cheddar cheese, where the resulting color is undesirable and must be bleached. The objective of this study was to compare 2 commercially approved bleaching agents, benzoyl peroxide (BP) and hydrogen peroxide (HP), and their effects on the flavor and functionality of whey protein concentrate 80 % (WPC80). Colored and uncolored liquid whey were bleached with BP or HP, ultrafiltered, diafiltered and spray-dried. WPC80 from unbleached colored and uncolored Cheddar whey were manufactured as controls. All treatments were manufactured in triplicate. WPC80 were evaluated by sensory, instrumental analyses, functionality, color, and proximate analysis. HP bleached WPC80 were higher in lipid oxidation compounds than other bleached or unbleached WPC80, specifically hexanal, heptanal, octanal, nonanal, decanal, dimethyl disulfide, and 1-octen-3-one ( $P < 0.05$ ). HP treatments were higher in fatty and cardboard flavors compared with the unbleached and BP bleached samples ( $P < 0.05$ ). WPC80 bleached with BP had lower norbixin concentrations compared with WPC80 bleached with HP ( $P < 0.05$ ). Hunter CIE Lab color values ( $L^*$   $a^*$   $b^*$ ) of WPC powders were distinct on all 3 color scale parameters and HP bleached WPC80 had the highest  $L^*$  values. Iron concentration was lower in the HP-bleached WPC80 ( $P < 0.05$ ), all other mineral and proximate values were not different among treatments ( $P > 0.05$ ). HP treatments had more soluble protein after 10 min of heating at 90oC at pH 4.6 and pH 7 than the unbleached and BP treatments. Overall, HP bleaching caused more lipid oxidation products than BP bleaching but enhanced the solubility of the WPC80.

**Key words:** whey protein, bleaching, flavor

**69 The complete genome sequence of *Bifidobacterium animalis* ssp. *animalis* ATCC 25527<sup>T</sup> and analysis of growth in milk.** J. R. Loquasto\*<sup>1</sup>, R. Barrangou<sup>2,1</sup>, E. G. Dudley<sup>1</sup>, and R. F. Roberts<sup>1</sup>, <sup>1</sup>The Pennsylvania State University, University Park, <sup>2</sup>Danisco USA Inc., Madison, WI.

Bifidobacteria are putative probiotic organisms commonly added to fermented dairy products. The number of complete bifidobacterial genomes is increasing and analysis of these genomes has provided important insight into the physiology of these organisms. The objective of this work was to sequence the genome of *B. animalis* ssp. *animalis* ATCC 25527<sup>T</sup> with the aim of providing insight into the genetic diversity responsible for phenotypic differences reported between *B. animalis* ssp. *animalis* (Baa) and *B. animalis* ssp. *lactis* (Bal). The genome of ATCC 25527<sup>T</sup> was shotgun sequenced using 454 technology. After contig assembly, alignment and several rounds of gap closing, the complete 1,932,963 bp genome was determined and verified by comparison to a *KpnI* optical map. The genome was annotated using Rapid Annotation using Subsystems Technology (RAST) and at NCBI. Comparative analysis of the Baa ATCC 25527<sup>T</sup> and Bal DSMZ 10140<sup>T</sup> genomes revealed high degrees of both synteny and homology. Comparison of the Baa and Bal genomes for differential content revealed 108 and 121 genes that were unique to and absent in, the BAA genome, respectively. Unique genes were identified as having less than 10% amino acid identity between protein sequences of both genomes, as detected by RAST. Among the differential gene content are a set of unique CRISPR-associated genes and a novel CRISPR locus containing 31 spacers in the genome of Baa. Although previous research has suggested one of the defining phenotypic differences between Baa and Bal is the ability of Bal strains to grow in milk and milk-

based medium, no obvious differences in gene content responsible for this phenotype were identified between the 2 genomes. Furthermore, growth and acid production in milk and milk-based medium did not differ significantly in experiments examining Bal (DSMZ 10140<sup>T</sup> and

Bl04) and Baa (ATCC 25527<sup>T</sup>). These data suggest that this widely accepted defining phenotypic trait may not distinguish the subspecies.

**Key words:** bifidobacteria, probiotics, genome sequencing