

Graduate Student Paper Competition: ADSA Dairy Foods

40 Effects of sucrose on the foaming and interfacial properties of whey protein isolate and egg white protein mixtures. X. Yang^{*1,2}, T. K. Berry^{1,2}, and E. A. Foegeding^{1,2}, ¹North Carolina State University, Raleigh, ²Southeast Dairy Foods Research Center, Raleigh, NC.

Whey proteins form wet foams with properties similar to egg white but lack stability when incorporated in an angel food cake. Sucrose is a major component of cakes; however, its effects on foaming, interfacial and baking properties have not been investigated. In this work, 12.8% (w/v) sucrose (the amount found in angel food cake foams) was added to 10% (w/v) protein solutions. The effects of sucrose on the foaming and interfacial properties of egg white protein (EWP), whey protein isolate (WPI), and mixtures of the two were evaluated. Two types of WPI (one based on ion-exchange separation and the other on membrane filtration) and five EWP/WPI ratios were investigated. Physical properties of foams (drainage 1/2 life and yield stress) and dynamic viscosity of pre-foam solutions were measured. Interfacial rheology was determined by a pending drop method. Normal and no sucrose angel food cakes were prepared and evaluated for volume and structure.

Addition of sucrose caused a 79% increase in drainage 1/2 life of EWP foams, whereas drainage 1/2 life of WPI and EWP-WPI mixtures were increased by no more than 32%. Sucrose significantly ($p < 0.05$) increased dynamic viscosities of all protein solutions but only increased the interfacial dilational elastic modulus of EWP, suggesting that the improved interfacial elasticity of EWP effectively slowed down foam drainage. The yield stress values and interfacial dilational elastic moduli of WPI and EWP-WPI mixtures were decreased with sucrose addition, with a positive relationship established between yield stress and interfacial dilational elastic modulus. The presence of sucrose increased angel food cake volume when EWP proportion was higher than 50%, but showed no improvement in the coarse structure of EWP-WPI mixed cakes. These results indicated that sucrose increased foam stability, interfacial elasticity, and angel food cake volume when combined with egg white. This was not observed in whey proteins and EWP-WPI mixed systems, suggesting that one functional difference between whey and egg white proteins is how they interact with sucrose.

Key Words: Whey Proteins, Sucrose, Foam

41 Rheological and chemopreventive properties of milk fermented with exopolysaccharide-producing lactic cultures. D. H. Purohit^{*}, A. N. Hassan, E. Bhatia, and C. Dwivedi, *South Dakota State University, Brookings.*

The objectives of this research were to evaluate the rheological and chemopreventive properties of milk fermented with different exopolysaccharide (EPS)-producing lactic cultures. Reconstituted (11% wt/vol) skim milk was fermented with single strains of EPS-producing and non-producing cultures. They collected on the surface of undisturbed fermented milk samples and after cutting was measured. All EPS-producing cultures reduced the amount of whey present on the surface of the undisturbed samples, while only three out of five strains reduced syneresis measured after cutting. All EPS-producing cultures except a strain of *Lactobacillus delbrueckii* ssp. *bulgaricus* reduced viscoelastic moduli in fermented milk. In the chemoprevention study, 140 male Fisher rats were divided into 7 groups of 20 each. Rats in 6 groups were fed diets supplemented with fermented milks made with single strains of EPS-producing and non-producing cultures, while rats in

group 7 (control) were fed a diet supplemented with milk acidified with glucono- δ -lactone (GDL). All rats were injected with azoxymethane (15 mg/kg, subcutaneous) at weeks 7 and 8 of age to induce tumors in the gastrointestinal tract and fed their respective diets ad libitum throughout the study. After 30 weeks of initiation, rats were anesthetized with ether, intestinal tissues were isolated, washed with normal saline, and number and size of tumors were recorded in the colon and small intestine. Rats fed diets supplemented with fermented milk made with two EPS-positive and one EPS-negative strains significantly lowered colon tumor incidence and colon tumor multiplicity. Cyclooxygenase-2 (COX-2) enzyme activity (enzyme implicated in colon tumor development) was significantly lower in colon tissue of rats fed diets containing milk fermented with four EPS-producing and one non-EPS-producing cultures than that in rats fed diet supplemented with GDL acidified milk. Differences in the chemopreventive effects among EPS-positive cultures could be due to variations in the type of EPS or other metabolites.

Key Words: Fermented Milk, Rheology, Chemoprevention

42 Effect of different types of emulsifiers on the functional properties of low-fat process cheese. E. M. Salim^{*1}, S. Govindasamy-Lucey², M. E. Johnson², and J. A. Lucey¹, ¹University of Wisconsin, Madison, ²Wisconsin Center for Dairy Research, Madison, WI.

In this study, the impact of different types of emulsifiers (EM) on the textural and rheological properties of low-fat process cheese (LPC) was investigated. After a preliminary screening of 9 different types of EM, small lab-scale LPC were prepared from fat-free cheese base (that was made by direct acidification of skim milk to pH 5.6 using citric acid), and the addition of 5 different EM at 4% level; citric acid esters (CAE), diacetyl tartaric acid esters of monoglycerides (DATEM) (Anionic), N-cetyl-N,N,N trimethylammonium bromide (CTAB) (Cationic), distilled monoglycerides (DM), and mono-diglycerides (MD) (Non-ionic). No citrate or phosphate-based salts were used for LPC manufacture. Control nonfat process cheese was made without any EM. Moisture content and pH of LPC were kept constant at 63.0% and 5.5, respectively. Texture profile analysis was used to determine adhesiveness and hardness at 40 and 80% compression levels (CL). Meltability was measured by UW Melt-Profiler as the degree of flow (DOF). Small amplitude oscillatory rheometry determined storage modulus (G' or stiffness) and loss tangent (LT; a meltability indicator) during heating from 5 to 85°C. LPC with EM were softer than control at 40% CL but harder than control (except for DATEM and CTAB) at 80% CL. LPC made with DATEM and CTAB had similar properties except that DATEM exhibited fracture during compression while CTAB cheese was sticky. LPC with DM, MD, and CAE were generally similar and had higher DOF than control. The only major difference between these cheeses was that MD had similar G' values at 8°C to control whereas DM and CAE had higher G' values. The addition of all types of EM produced cheeses with lower maximum LT and higher G' values at 85°C relative to control cheese. These results demonstrate that different types of EM can be used to modify both low temperature properties (hardness, fracture) and high temperature properties (melt, flow) of LPC, which would be useful for various applications like slices, blocks and shreds. These LPC cheeses are currently being scaled-up to further investigate their physicochemical properties.

Key Words: Low-Fat Process Cheese, Emulsifiers, Functionality

43 Manufacture and characterization of whey protein concentrate from microfiltration of milk. H. S. Somni* and V. V. Mistry, *South Dakota State University, Brookings.*

Whey protein concentrates (WPCs) are widely used in the dairy industry due to their nutritive and functional properties. There is a high degree of variability in composition, functional, and sensory properties of WPCs. Commercial WPCs can also develop stale flavor due to the presence of lipid and protein impurities. These attributes limit the extent of their use in the food industry. Whey proteins from microfiltration (MF) permeate are unique; they have not been subjected to cheese making process, are in native state, its composition is similar to cheese whey, practically free of bacteria and impurities of fat and protein. Such whey proteins have the potential of providing useful applications in various products. Raw skim milk was microfiltered at 50-55°C to separate whey protein (MWP). MWP was further concentrated by UF and spray drying to obtain MWP concentrate (MWPC). At no stage were any of the fractions in the stream pasteurized or diafiltered. The average composition (4 replicates) of MWPC powder was; 49% protein, 4.5% ash, 0.6% fat and 6.1% moisture. The functional properties of the product were compared with two commercial WPCs (samples A and B) at pH 7. The minimum concentration to gel at 80°C/30 min for all the samples was 8% protein. For emulsion stability, 33% fat emulsion was prepared in 100 ml of 2% protein solution and the fat content in bottom 50 ml was measured after 2 hrs. The stability was expressed as percent ratio of fat in bottom layer to total fat. The emulsion stability was 61.4, 68.2 and 62.1% respectively, for MWPC, sample A and sample B. The foaming properties were expressed as % overrun and foam stability. The average overrun for NPWC and sample A was 963.1 and 486.8 respectively. Only 44% foam collapsed after 30 min whereas for sample A the foam collapsed in less than 5 min. For sample B the foam was very unstable to measure even overrun

Key Words: Whey Protein Concentrate, Functional Properties

44 Transport of glucose by *Bifidobacterium animalis* ssp. *lactis* occurs via facilitated diffusion. E. P. Briczinski*, A. T. Phillips, and R. F. Roberts, *The Pennsylvania State University, University Park.*

Bifidobacterium animalis ssp. *lactis* strains are often studied as potential probiotics. Colonization by and survival of probiotics in the large intestine depend on utilization of fermentable carbohydrates not absorbed or metabolized by the host. Although carbohydrate utilization is critical to bifidobacterial activity in the gut, relatively little is known about carbohydrate transport in this genus. While most strains of bifidobacteria are able to ferment glucose, glucose non-fermenting strains (GNF) have been reported. The objective of this research was to develop an understanding of the difference in glucose utilization between strains that grow on glucose compared to GNF strains. Two closely related strains of *B. animalis* ssp. *lactis*, DSMZ 10140 (type strain) and RB 4825 (obtained from a commercial starter culture manufacturer), were compared. DSMZ 10140 (glucose-positive) and RB 4825 (glucose-negative) were indistinguishable using nucleic acid-based techniques (PFGE, RAPD-PCR, gene sequencing). A low-affinity facilitated diffusion glucose transporter was identified in DSMZ 10140 by performing uptake assays with D-[U-¹⁴C] glucose. Based on kinetic analyses, mean values for K_t and V_{max} were 14.8±3.4 mM and 0.13±0.03 μmole/min/mg cell protein, respectively. When competitor carbohydrates were included in uptake assays, stereospecificity was exhibited, with greater competition by methyl-β-glucoside than methyl-α-glucoside. There was no inhibition

of glucose uptake by sodium fluoride, iodoacetic acid, sodium arsenate, sodium azide, 2,4-dinitrophenol, monensin, or valinomycin, which often block active transport; however, significant inhibition (>30%) was observed with phloretin, an inhibitor of facilitated diffusion of glucose. Glucose uptake by four additional commercial strains of *B. animalis* ssp. *lactis* was also inhibited by phloretin, indicating the activity of facilitated diffusion glucose transporters. The glucose transporters characterized in this work are the first identified in *B. animalis* ssp. *lactis* and the first identified facilitated diffusion transporters of glucose in actinobacteria.

Key Words: *Bifidobacterium animalis* ssp. *lactis*, Probiotics, Glucose Transport

45 Characterizing stress responses of bifidobacteria strains of industrial importance. A. K. Abdalla*^{1,2}, M. A. Mohran¹, S. C. Ingham², J. R. Broadbent³, and J. L. Steele², ¹Assiut University, Assiut, Egypt, ²University of Wisconsin, Madison, ³Utah State University, Logan.

Delivery of probiotic bifidobacteria in foods would benefit from the development of technologies to enhance their survival in foods and during gastric passage. This research aims to better understand how bifidobacteria respond to stress and identify potential strategies to enhance their survival. Intrinsic resistance to acid (HCl) for three strains each of *Bifidobacterium animalis* ssp. *lactis* and *Bifidobacterium longum* were screened at pHs ranging from 2.0 to 6.5. In general, *B. lactis* strains had a higher intrinsic acid resistance than *B. longum*. For example, while no significant reduction in viability of the *B. lactis* strains was observed after 2h at pH 3.5, the *B. longum* strains decreased by 3 log₁₀ CFU/mL. Also, strain to strain variations were observed within the same species. For example reductions of 1.5, 1 and 3 log₁₀ CFU/mL in viable numbers of *B. lactis* strains RH-1, DSM 10140 and D 2908 was observed after 2h at pH 2.0, respectively. For all of the strains examined, conditions to induce an acid tolerance response (ATR) have been identified. Observed increases in survival during acid challenge after ATR induction ranged from 0.5 to 2 log₁₀ CFU/mL. Additionally, intrinsic resistance to NaCl has been examined with the *B. lactis* strains examined. No significant reductions in viability were observed after 20h in NaCl concentrations up to 12% (W/V). These results indicate the importance of both strain selection and culture treatments for enhancing the survival of bifidobacteria in foods and during gastric passage.

Key Words: *Bifidobacterium*, Acid Resistance, Salt Resistance

46 Growth substrates for nonstarter lactic acid bacteria. Biochemistry and transcriptional profile of *Lactobacillus casei* ATCC 334 in a Cheddar cheese model system. M. Budinich*¹, I. Diaz-Muniz¹, H. Cai¹, V. Smeianov¹, J. Broadbent², and J. Steele¹, ¹University of Wisconsin, Madison, ²Utah State University, Logan.

Cheese ripening is a dynamic, poorly understood process that is essential for development of cheese flavor and requires non-starter lactic acid bacteria (NSLAB). The energy sources utilized for growth by NSLAB are unknown. However, potential energy sources include simple carbohydrates, citrate, nucleic acids, glycopeptides and phosphopeptides. *Lactobacillus casei* is the typical dominant species of NSLAB present

in ripening cheddar cheese. Cheddar cheese extract (CCE) was used as a model system medium to examine the growth substrates present in Cheddar cheese at 2, 6 and 8 month of ripening. A decrease in both growth rate and maximum cell density of *Lb. casei* ATCC 334, a cheese isolate, indicate that substrates are being consumed and or changing during ripening process. Analysis of volatiles by SPME GC/MS indicated the appearance of several potential cheese flavor compounds. The completed genome of *Lb. casei* ATCC 334 permitted us to observe global gene expression patterns during fermentation of 2 month old CCE and identified 42 genes differentially expressed related to energy and metabolism. A putative pathway to derive energy from nucleic acid degradation was proposed and tested in chemically defined media. *Lb. casei* ATCC 334 was able to grow on milk derived glycopeptides and glycolipids and on *Lc. lactis* spp. *cremoris* SK11 derived nucleic acids but not on cheese derived phosphopeptides. Single carbohydrate studies showed that the ability to ferment carbohydrates also depends on the composition of the media and prior history of the bacterium

Key Words: Growth Substrates for NSLAB, Cheese Model System, Transcriptional Profile of *Lactobacillus casei* ATCC 334

47 Production of conjugated linoleic acid in cheese slurry by lactic acid bacteria. A. J. Pandit*, S. K. Anand, A. N. Hassan, and K. F. Kalscheur, *South Dakota State University, Brookings.*

Certain lactic acid bacteria (LAB) produce conjugated linoleic acid (CLA) from linoleic acid. Such CLA producing LAB, when used in cheese manufacture, can possibly increase CLA. Previous studies conducted in our laboratory have screened and identified CLA producing LAB. In the present investigation a slurry method was used to screen these CLA producing LAB for their suitability in cheese manufacturing with higher CLA levels. Cheese slurry (60% moisture, pH 6.6) from Cheddar curd was steamed, cooled to room temperature, inoculated in duplicates with respective cultures, and incubated for 5 day at 37°C. Treatments used were: 1) 4b cheese isolate (CI) (*Lactococcus* isolated from Cheddar cheese), 2) 4b + *Lb. helveticus* (ATCC 15807), 3) 4b + CI (*Lactobacillus* isolated from Cheddar cheese), 4) 850 + *Lb. helveticus*, 5) 850 + CI (*Lactobacillus*), and 6) CLA negative commercial starter culture (850, control). Cheese slurries were analyzed on day 0, 1, 3, and 5 for pH, Free fatty acids (FFA), bacterial counts on M17 and MRS, and CLA content. The CLA content of slurries before inoculation was 0.73 gm/100 gm fatty acids (FA). On day 1, CLA values of 0.94, 0.82, 0.78, 0.79, 0.73, and 0.74 gm/100 gm FA were observed in treatments 1, 2, 3, 4, 5, and 6, respectively. Maximum CLA content was observed in treatments 1 (0.99 gm/100 gm FA) and 2 (0.855 gm/100 gm FA) after 3 d of incubation. The CLA in treatment 1 (1.10 gm/100 gm FA) after 5 d of incubation was higher ($p < 0.05$) than that in the control (0.73 gm/100 gm FA). Counts on M17 agar declined from 10^{10} cfu/ml (d 1) to 10^7 cfu/ml (d 5), while counts on MRS agar increased from 10^7 cfu/ml (d 1) to 10^8 cfu/ml (d 5). None of the slurries were rancid after 5 d of incubation. This study provides useful information on the feasibility of some CLA-producing starter culture in cheese manufacture. It was also useful in anticipating the behavior of different CLA producing cultures during ripening.

Key Words: Cheese Slurry, Conjugated Linoleic Acid, Lactic Acid Bacteria

48 Profiling flavor related biochemical changes in cheddar cheese during ripening using infrared spectroscopy. A. Subramanian*, J. Harper, and L. Rodriguez-Saona, *The Ohio State University, Columbus.*

Composition and flavor quality of Cheddar cheese, which influence the consumer acceptance, price and food processing application, develop during the ripening process. However, ripening is not well understood due to the heterogeneous nature of cheese. Profiling cheese ripening by means of few selected variables can save time and money for the cheesemaker. Mid infrared (MIR) spectroscopy is an attractive technology for rapid, sensitive, and high-throughput analysis of food components. Preliminary results have shown that MIR could classify Cheddar cheese based on flavor quality. The objective of this research was to use MIR to further the understanding on biochemical changes during ripening and their influence on flavor.

Twelve different Cheddar cheese samples were ripened for a period of 73 days. Samples were collected on days 7, 15, 30, 45 and 73 during ripening and analyzed for organic acid and amino acid content using liquid and gas chromatography, respectively. For MIR analysis the samples were treated using organic solvents and the extracts were scanned between 4000-700 wavenumbers. The spectra of samples were matched with the composition and quality data to develop multivariate statistical regression and classification models. The spectra correlated well (r -value > 0.95) with the flavor quality as well as the changes in organic and amino acid levels. Furthermore, age of the cheese could also be predicted within a standard error of 1.6 days. Some of the prominent infrared marker bands that were responsible for the correlation were 1411 (amino acids), 1710-1450 (fatty acids), and 1200-1050 cm^{-1} (organic acids). Interestingly, greatest change in composition of cheeses was observed between the days 15 and 30. This could be valuable information for prediction of cheese flavor quality early in the ripening process. Early identification of flavor quality will assist in controlling ripening parameters, and deciding the marketability and application of cheese. FT-IR spectroscopy shows great promise as a rapid, simple and cost-effective analytical and quality control tool for monitoring and understanding cheese ripening.

Key Words: Cheddar Cheese, Flavor, Infrared Spectroscopy

49 Sensory evaluation of reduced fat cheddar cheese fortified with omega-3 fatty acids for oxidized, rancid and fishy flavor attributes.

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Omega-3 fatty acids reduce inflammation and help prevent risk factors associated with certain diseases. However, because the body cannot synthesize them they must come from diet. Therefore, interest in fortifying cheddar cheese with omega-3 fatty acids exists, but there is concern that doing so may have detrimental effects on the flavor of the final cheese product.

Fifty percent reduced fat cheddar cheese was manufactured to contain 16, 32 (typical fortification level), and 64 mg docosahexaenoic/eicosapentaenoic acids (DHA/EPA) per 28-g serving (in triplicate). This was accomplished by comminuting to particles about 2 mm in diameter, adding an encapsulated fish oil powder, pressing to form a cheese block overnight, and storing at 8 °C. Control cheese contained no fish oil. Samples were rated on a five-point categorical scale (No, Slight,

Moderate, Strong, and Extremely Strong Flavor) by 11 trained panelists for oxidized, rancid, and fishy flavor attributes at 1, 7, 30 and 90 d. For analysis, numerical values of 0 to 4 were assigned respectively. Omega-3 fatty acid fortification caused no change in oxidized ($p = 0.08$) or rancid flavor ($p = 0.35$). Mean oxidized flavor scores were from 0.2 to 0.4 throughout 90 d for all cheeses. Rancid flavor increased during storage ($p < 0.0001$) with mean scores of 0.4 at d 1 and 0.9 at d 90 (score of 1 = "Slight Flavor"). Fishy flavor increased with level of DHA/EPA fortification ($p < 0.0001$), but decreased during storage ($p < 0.0001$). At d 1, average fishy flavor scores for control, and the fortified cheeses

were 0.2, 0.3, 0.8, and 1.2 respectively. By 30 d these had decreased to 0.1, 0.1, 0.2, 1.0, and by 90 d to 0.0, 0.0, 0.2, 0.5 leaving the 64 mg DHA/EPA sample with fishy flavor significantly different from control. Thus, cheddar cheese can be fortified with up to 32 mg DHA/EPA per serving using an encapsulated fish oil without causing an increase in oxidized, rancid or fishy flavors provided the cheese is stored for 30 d because initial fishy flavor diminishes during storage.

Key Words: Omega-3 Fatty Acids, Cheese, Flavor