20 Temperature effect on structure-opacity relationships of nonfat Mozzarella cheese. A.J. Pastorino*[1], R.I. Dave[2], C.J. Oberg[3], and D.J. McMahon[1], [1]*Utah State University,* [2]*South Dakota State University,* [3]*Weber State University.*

Our objective was to determine the effect of heating on the structure of nonfat Mozzarella cheese, and then to relate changes in structure to changes in cheese opacity. Cheese was made according to a direct-acid, stirred-curd procedure. Cheese samples, at 4°C, were taken on d 1 and placed into glass bottles, which were sealed and heated. Once the cheese reached 10°C or 50°C, the bottles were placed on a scanner and color values measured. Samples of cheese at 10°C and 50°C were also taken on d 1 for structural analysis. Scanning and transmission electron micrographs of cheese were scanned and digitally analyzed. Applying heat increased cheese opacity, as measured by L-values (91.5 versus 78.0; $P < 0.001$), and induced changes in cheese structure. Larger protein aggregates (7.0 nm^2 versus 1.9 nm^2; $P = 0.074$) and increased protein concentration in the protein matrix were observed in cheese at 50°C (i.e., increased aggregate density, 157 versus 91; $P = 0.008$). Applied heat would favor hydrophobic interactions, and possibly, re-association of β-casein and calcium with the protein matrix, promoting protein-to-protein interactions. Thus, the protein matrix contracted, occupying less cheese matrix area (69 versus 99%; $P = 0.022$) and microphase separation occurred, causing serum pockets to grow in size (from a maximum diameter of 1.0 μm to 7.0 μm), and microstructural heterogeneity increased. It is proposed that the increased size of aggregates and heterogeneity of the cheese at 50°C promote light reflection, thus increasing cheese opacity. We concluded that applying heat alters protein interactions in the cheese matrix, which is manifest as changes in cheese structure. Such changes in structure help provide an understanding of changes in cheese opacity.

Key Words: Cheese, Structure-Opacity, Temperature

21 Rheological properties of rennet-induced gels made from coagulants of vegetable origin and chymosin. C. L. C. Esteves*[1,2], J. A. Lucey[1], and E. M. V. Pires[2], [1]*University of Wisconsin-Madison, Madison,* [2]*University of Coimbra, Coimbra, Portugal.*

The type of coagulant used in cheese-making plays an important role in the characteristics of the final product. In Portugal, the flowers of *Cynara* sp have been used for a long time in the production of vegetarian cheeses that are considered of high quality, because of their distinctive flavor and texture. However, little is known about the textural properties of rennet-induced gels made with vegetable coagulants. The rheological properties of rennet gels made from the vegetable coagulants *Cynara cardunculus* L. and *Cynara humilis* L. were compared with those of recombinant chymosin, using dynamic low amplitude oscillation. The large deformation properties of gels were studied by subjecting set gels to a constant shear rate (0.01 s^{-1}), up to yielding of the gel. Gelation experiments were performed at 32°C using NFDM reconstituted for 2 and 16 h, at 32°C. The general pattern of gelation curves was similar for the three coagulants. After 6 h of gelation, the storage modulus of chymosin and vegetable coagulants were 79±0.4 and 66±1.9 Pa, respectively for milk dissolved for 2 h and 74±0.2 and 57±2.4 Pa, respectively for milk dissolved for 16 h. The lower storage modulus in gels made from milk dissolved for 16 h was probably due to casein hydrolysis by plasmin. The effect of the time scale of the applied deformation on the rheological properties was determined by a frequency sweep (0.002 - 1 Hz). At low frequency (0.002 Hz), regardless of time allowed to dissolve the milk, loss tangent values for chymosin (0.54±0.01) were higher than those for *Cynara cardunculus* L. (0.49±0.01) and *Cynara humilis* L. (0.50±0.01). Higher loss tangent values indicate susceptibility to syneresis. The shear stress at yielding was higher for chymosin (53±2.2 Pa) than vegetable rennets (46±2.3 Pa), for milk dissolved for both periods. The shear strain at yielding was similar for all coagulants (0.98±0.11) except for chymosin (1.17±0.05) when milk was dissolved for 16 h. The rennet-induced gels made with *Cynara cardunculus* L. and *Cynara humilis* L. were alike, probably due to the similarity in their proteolytic enzymes.

Key Words: Vegetable coagulants, Rennet-induced gels, Rheology

22 Evaluation of Quality Properties of Butter and Ice Cream with a High Content of Linoleic and Oleic Acid. S Gonzalez*, S.S. Duncan, S.S. Sumner, S.F. O'Keefe, and J. Herbein, *Virginia Tech, Blacksburg, VA/USA.*

Milk fat composition determines specific rheological, sensory and physicochemical properties of dairy products such as texture, melting point, flavor, color, oxidation rates, and viscosity. Previous studies have shown that milkfat containing higher levels of long chain polyunsaturated fatty acids have lower melting point and decreased solid fat content which leads to softer textured products. An increased risk of higher oxidation rates can be a disadvantage of high levels of polyunsaturated fatty acids. Three different milkfat compositions were obtained through dietary manipulation of cows: high oleic content, high linoleic content and standard milkfat. Ice cream and butter were processed from the treated and control milk. The samples were then analyzed to measure differences in texture (firmness), oxidation rate and sensory perception. Textures of butter and ice cream were performed by doing compression measurements. No significant difference was found between control and treated ice cream samples. However untreated butters were firmer (10.54 J) than butters containing higher amounts of unsaturated fatty acids (6.09-6.95 J) in a temperature range of 4.3-5.5 C. Peroxide values in ice cream were measured before freezing and after sensory testing to monitor oxidation behavior of the three treatments. Initial peroxide values were similar between the treatments, however after 3 months of storage the values increased at a higher rate for the linoleic treatment. Sensory analyses included a scooping test on the three treated ice creams at -18C to detect differences in texture. An overall difference test was conducted to determine if consumers could taste a difference between the three ice cream treatments. The overall difference test showed significant difference between the control and the oleic treatment as well as the control and linoleic treatment at a p= 0.05. No significant difference was found in the scooping test.

Key Words: Ice cream , Texture, Linoleic and Oleic Acid

23 Effect of high-pressure on two strains of *Lactococcus lactis* subsp. *cremoris* in a phosphate buffered saline (PBS) cell suspension. A. S. Malone*, T.H. Shellhammer, and P. D. Courtney, *Food Science and Technology, Ohio State University.*

Cellular lysis or membrane permeability of cheese starter cultures is thought to play an important role in cheese ripening due to release of intracellular enzymes. Thus, researching a method, such as high-pressure processing, to induce or prohibit cell lysis or membrane permeability may provide a path to accelerate or arrest cheese maturation. Two strains of *Lactococcus lactis* subsp. *cremoris*, MG1363 and SK11, were grown to early stationary phase at 30°C in GM17 and LM17 broth, respectively. Cells were washed and resuspended in sterile phosphate buffered saline (PBS) at pH 7.0 to concentrations of approximately 6 X 10^8 to 1 X 10^9 cfu/ml. Cell suspensions were examined for their response to five minute, high-pressure treatments (100 to 800 MPa) at 25±4°C. Cell viability was assessed as cfu/ml immediately after pressure treatment. Both strains were completely inactivated at pressures of 400 to 800 MPa. There was no significant effect on cell viability at pressures of 100 and 200 MPa. At 300 MPa, the MG1363 population decreased by 7.3 log cycles, whereas the SK11 population decreased by 2.5 log cycles. Cell lysis was monitored by recording suspension absorbance at 600 nm (A_{600}) after pressure treatment for time intervals up to 24 hours. Pressure treated MG1363 cell suspensions decreased in A_{600} over time when compared with non-pressure treated controls. Twenty-four hours after pressure treatment, the A_{600} decreased by 0.15 to 0.30. The A_{600} of pressure-treated SK11 suspensions did not differ significantly from non-pressure treated controls. These data indicate that high pressure can induce subsequent cell lysis and that lysis is strain dependent. Further studies are aimed at the extent of membrane permeability and autolysin activity in each strain after pressure treatment.

Key Words: *Lactococcus lactis*, high-pressure, lysis

24 Alpha-galactosidase as a novel molecular tool for the genetic modification of *Lactococcus lactis*. I. Boucher*[1], M. Parrot[1], C. Vadeboncoeur[1], and S. Moineau[1], [1]*Universite Laval, Quebec, Quebec, Canada*.

Atypical sugar fermentation phenotypes represent an attractive alternative to antibiotic resistance as a selection marker on plasmid vectors used for the genetic modification of lactic acid bacteria (LAB). For instance, melibiose is an alpha-galactoside rarely fermented by LAB. This disaccharide is hydrolyzed into galactose and glucose by alpha-galactosidase. The resulting monosaccharides are subsequently degraded through metabolic pathways that are commonly found in LAB. Using a degenerated oligonucleotide probe, the alpha-galactosidase gene (*aga*) and a gene coding for a transcriptional regulator from the LacI/GalR family (*gal*R) of *Lactococcus raffinolactis* ATCC43920 were located on a 4 kbp DNA restriction fragment. When transferred into various *Lactococcus lactis* strains using the plasmid vector pNZ123, *aga* conferred the ability to ferment melibiose. A *Pediococcus acidilactici* strain also became melibiose-positive when transformed with the *L. raffinolactis aga* gene. Sequences coding for homologues of *L. raffinolactis aga* and *gal*R products were also obtained from a raffinose-fermenting *L. lactis* subsp. *lactis* strain. The genes of both organisms displayed a similar organization on the chromosome and their nucleotide sequences were over 90% identical. *L. raffinolactis aga* was finally associated to the minimal region essential for the maintenance of a *L. lactis* natural plasmid to create a functional cloning device. Detection of melibiose-positive *L. lactis* transformants on a solid medium was made possible by first culturing transformed cells into an enrichment medium containing melibiose as the unique energy source. Constituted entirely of lactococcal DNA and exempt of antibiotic resistance determinants, this plasmid construction should be appropriate for food-grade applications. Lactococcal alpha-galactosidases represent a valuable new molecular tool suitable for the genetic modification of lactococci and other LAB.

Key Words: *Lactococcus raffinolactis*, alpha-galactosidase, selection marker

25 Influence of proteolytic enzymes from thermophillic lactic acid bacteria on the functional properties of Mozzarella cheese. B. S. Oommen*[1], D. J. McMahon[1], J. R. Broadbent[1], and C. J. Oberg[2], [1]*Utah State University,* [2]*Weber State Univeisty*.

Part-skim Mozzarella cheeses were manufactured from 2%-fat milk using *Streptococcus thermophillus* as the starter and three different strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* as adjunct starter culture to make three treatments and one without the addition of adjunct starter culture as the control. Three strains of the bacteria differed in their proteolytic specificity on α_{s1}-CN (f1-23) and their extent of proteolysis in milk. Based on their specificity, they were grouped as R2I, R6III, and R13V (this also represents the decreasing order of their extent of proteolysis) and the cheeses with those cultures were designated CR2I, CR6III, and CR13V respectively. Four replicates of these cheeses were aged and tested for functional attributes at 7, 14, and 21 d. Moisture, fat, and protein content in cheeses ranged from 45.2 to 48.4%, 19.9 to 20.5%, and 26.80 to 28.55% respectively. Salt content were not different ($P > 0.05$) among treatments. Cheese meltability increased ($P < 0.05$) from 58.05% in control to 81.86% in CR13V (CR2I, 73.20%; CR6III, 74.19%). TPA-hardness was 168.20 N for control and similar ($P > 0.05$) for CR2I (88.77), CR6III (101.45), and CR2I, CR13V (80.34 N). Melt strength, a measure of strand forming ability was highest ($P < 0.05$) for control (3.17 N) and lowest ($P < 0.05$) for CR13V (1.42 N). The ability of cheese strands to remain as a cohesive mass while being pulled called stretch quality was highest ($P < 0.05$) in control (0.6438 N) and lowest ($P < 0.05$) in CR13V (0.2113 N). Stretch quality and melt strength were similar ($P > 0.05$) for CR2I and CR6III. Meltability and TPA-cohesiveness increased ($P < 0.05$) with age while melt strength and stretch quality decreased ($P < 0.05$) with age. Water soluble peptides in cheese as measured by the total area under the peaks on HPLC correlated highly with meltability and stretch characteristics. Proteolytic specificity of lactic acid bacteria may be used as an indicator of the functional properties of the resultant cheese.

Key Words: Mozzarella, proteolysis, functionality

26 Fluorescence microscopy and recrystallization rate of model ice cream solutions as influenced by stabilizer type. A. Regand* and H.D. Goff, *University of Guelph, Guelph, Ontario, Canada*..

Stabilizers are known to retard ice recrystallization in frozen systems during storage at fluctuating temperatures, however the mechanism is not clear. Stabilizers were labelled with rhodamine isothiocyanate (RITC) and incorporated into solutions of sucrose (24%) and sucrose (16%) with skim milk powder (SMP) (14.7%). Solutions contained either no stabilizer or 0.3% of carrageenan, carboxymethyl cellulose (CMC), xanthan gum, sodium alginate, locust bean gum (LBG) or gelatine. The solutions were quench frozen to -50°C, precycled to get similar ice crystal size at t=0 ($p \leq 0.05$), and cycled between -3.5°C and -6°C, 5 times. Different precycling treatments were required for samples with or without SMP due to lower initial values of ice crystal size in the presence of SMP. Digital brightfield and fluorescent images were acquired at t=0 and -3.5°C on each cycle. Recrystallization rate was calculated as the slope of the linear regression of the ice crystal median diameters obtained by the brightfield data. Location of the stabilizer was observed using fluorescence microscopy. Of the sucrose solutions, only the sample containing LBG showed a gel-like structure after cycling. Nevertheless, its recrystallization rate was not different from the control ($p \leq 0.05$). Lowest rates of recrystallization were detected with xanthan or alginate in sucrose solutions ($p \leq 0.05$). Cryo-gel formation and/or phase separation of proteins were identified by fluorescence microscopy after cycling in all solutions containing SMP. In the presence of proteins, all stabilizers retarded recrystallization compared to the control ($p \leq 0.05$). The most effective stabilizers in this case were alginate, CMC and carrageenan ($p \leq 0.05$), but they did not show any distinguishing structural features. These results suggest that molecular interactions between polysaccharides and proteins play an important role in retarding ice recrystallization. In the absence of proteins, cryo-gel formation is not essential to slow recrystallization.

Key Words: Ice Cream, Stabilizers, Fluorescence Microscopy

27 Monoclonal antibodies raised against native structural proteins of *Streptococcus thermophilus* bacteriophage DT1. C. Bart*[1], A. Darveau[1], C. Vadeboncoeur[1], and S. Moineau[1], [1]*Universite Laval, Quebec, Quebec, Canada*.

Streptococcus thermophilus is a lactic acid bacterium widely used for the production of yogurt and speciality cheeses. The manufacture of these dairy goods has risen sharply over the past years. A known fact about increased productivity within existing facilities is that milk fermentation processes may be delayed due to lytic bacteriophages. *S. thermophilus* phages are currently classified into two groups based on the number of major structural proteins (MSP) and their mode of DNA packaging. Phages with two MSP and cohesive genome extremities (cos-type) appear to occur more frequently. To better understand phage-host interactions, our laboratory recently sequenced the first genome of a cos-type lytic phage of *S. thermophilus*. The sequence analysis of DT1's genome revealed several open reading frames (ORF) coding for putative structural proteins. To confirm the presence of these proteins on the phage structure, monoclonal antibodies were produced using bacteriophage DT1 as the antigen. Furthermore, two different approaches were utilized, the classical method using mice spleen hybridoma cells and the phage-display technology. One antibody raised against the major capsid protein of DT1 was obtained by the first strategy and its specificity was confirmed by immunoelectron microscopy. An ELISA assay showed that this antibody has affinity for two out of the three 2MSP phages tested (DT1, MD4) and none for the two 3MSP phages tested. On the other hand, the phage-display technology allowed us to create a library of antibody fragments (ScFv) expressed as fusion proteins on the filamentous *E. coli* phage M13. Following two rounds of panning using complete DT1 phages as the antigen, four ScFv were chosen for further characterization. The phage-display library can be a useful tool in understanding the proteomic of phage DT1 and other phages of *S. thermophilus* since members of the same phage group (2MSP) share a high level of similarity in structural proteins.

Key Words: Monoclonal antibodies, Bacteriophages, *Streptococcus thermophilus*

28 Effect of linoleic and conjugated linoleic acids on *Lactobacillus* species in broth and milk. J. K. Jenkins* and P. D. Courtney, *The Ohio State University, Columbus, Ohio.*

Conjugated linoleic acid (CLA) is formed by the enzymatic or chemical isomerization of linoleic acid (LA). CLA is reported to reduce cancer cell growth, improve immune response and reduce body fat composition. Both CLA and LA are found in dietary triacylglycerols which are hydrolyzed by lipases in the human gastrointestinal tract. CLA content is highest among fatty dairy foods due to microbial activity in the rumen. Several bacteria have the ability to enzymatically isomerize linoleic acid to CLA. It has been hypothesized that bacteria may counteract the toxic effects of linoleic acid by converting it to a less toxic form, CLA. Studies directly comparing bacterial inhibition by both fatty acids are lacking. Five *Lactobacillus* strains from mammalian intestines or fermented dairy products were grown in MRS broth or UHT milk containing various concentrations of linoleic acid or CLA. All strains were inhibited to a greater extent by the fatty acids in broth than in milk. In MRS broth, fatty acid concentrations were 0, 250, 500, 750, or 1000 μg/ml linoleic acid or CLA, whereas in UHT milk higher concentrations were used (0, 500, 1000, 3000 and 5000 μg/ml). Both fatty acids were more inhibitory at higher concentrations. CLA was consistently less inhibitory or less lethal than linoleic acid. The population of the most linoleic acid-sensitive strain was reduced by 3 log cycles after 10 hours in MRS broth containing 750 μg/ml LA, whereas a slight increase (0.5 log) in population was observed with the same concentration of CLA. Free fatty acids can be present in foods and in the ileum, cecum and colon of humans, thus these results have implications regarding survival of *lactobacilli* in foods and human intestinal tract.

Key Words: *Lactobacillus*, conjugated linoleic acid

29 Development of two analytical methods to quantify the concentrations of insoluble and soluble Calcium in Cheddar cheese. A. V. Hassan* and J. A. Lucey, *University of Wisconsin-Madison.*

Two methods were developed, which could be routinely used to quantify the proportions of soluble and insoluble Ca in cheese. The concentration and type of residual Ca greatly influences cheese texture and functionality. The first method ("titration"), involved the use of a novel computer controlled, acid-base titration system to quantify buffering properties of milk and cheese. During acidification of milk, there was a well-defined buffering peak with a maximum at pH 5.1, due to the solubilization of colloidal calcium phosphate (CCP). When cheese was acidified there was a strong buffering peak at pH 4.8 which was due to residual CCP of milk that was not solubilized during cheese making. The area of this buffering peak in cheese was expressed as a percent of the original area of this peak in milk and was used to estimate the concentration of residual CCP (insoluble Ca) in cheese. Cheddar cheese homogenates were prepared for titration by mixing 8 g of cheese with 40 g of distilled water at 50°Cin a homogenizer for 3 min. Cheese homogenates and milk samples were titrated from initial pH of cheese and milk to pH 3.0 with 0.5 M HCl and then back titrated to pH 9.0 with 0.5 M NaOH at 25°Con a Mettler DL50 Autotitrator. Total area under this buffering peak was analyzed using a program developed with MatLab software. Proportion of soluble Ca in cheese increased from 36% to 44% of total Ca during the first four wk of ripening. The second method was based on extracting the aqueous phase ("juice") of cheese under high pressure (Morris et al., 1988) and determining the concentration of soluble Ca in juice using atomic absorption spectroscopy. 800 g of freshly grated cheese was thoroughly mixed with 1000 g of sand and placed in a specially designed stainless steel mould, and subjected to pressures up to 8 MPa, over a period of 3 h. To remove fat, the juice was centrifuged at 1650 *g* for 10 min at 4°C. By this "juice" method the proportion of soluble Ca in cheese increased from 33 to 38% of total Ca during the first four wk of ripening. Both methods gave similar results for the proportions of soluble and insoluble Ca in cheese. These methods will be very useful tools in monitoring changes in the detailed mineral composition of cheese during ripening.

Key Words: Calcium, Buffering, Cheese texture

30 The effects of NaCl, $CaCl_2$, lactose and pH on the interfacial behavior of β-lactoglobulin. J P Davis* and E A Foegeding, *North Carolina State University, Raleigh NC/USA.*

Changes in the dynamic surface tension of aqueous β-lactoglogbulin (10 μM) solutions, with varying concentrations (0-0.4 M) of either NaCl, $CaCl_2$ or lactose, were investigated using a pendant drop technique. Surface tension was measured over a time scale of 0 to 1200 s with a 2 s resolution. For all treatments, two distinct regions of protein adsorption were observed: 1) an initial rapid and nonlinear decrease in surface tension (0 to 250 s) followed by a 2) more gradual, linear decrease in surface tension (250 to 1200 s). The rate of decline for the second region was very similar for all treatments and no treatment had reached equilibrium by 1200 s. Therefore, the final values of surface tension for any treatment depended mainly on the processes occurring during the first 250 s. At pH 5.0, which is near the isoelectric point of 5.2 for β-lactoglogbulin, the initial rate of adsorption was greatest for all treatments due to the reduction of an electrostatic energy barrier at the air/aqueous interface. At pH levels above (pH 7.0) and below (pH 3.0) the isoelectric point of β-lactoglobulin, the initial adsorption was significantly slower. The addition of either NaCl or $CaCl_2$ at levels up to 0.1 M increased the initial rate of adsorption due to a reduction of the electrostatic barrier to protein adsorption. The effect of lactose concentration was minimal.

The success of any protein-based food foam ultimately depends on the initial rapid adsorption of the protein and its subsequent behavior, i.e. unfolding and protein-protein interactions. Our results suggest that pH, NaCl and $CaCl_2$ mainly alter the rate and extent of surface tension decrease. Once β-lactoglogbulin is adsorbed, the gradual unfolding and lowering of interfacial tension is the same regardless of pH or co-solute.

31 Isolation and Analysis of Glycomacropeptide from Goat Sweet Whey. Eryck Silva*, Takuo Nakano, and Lech Ozimek, *University of Alberta, Edmonton, Alberta, Canada.*

Glycomacropeptide (GMP) found in cheese whey (or sweet whey) is the hydrophilic C-terminal peptide released from k-casein by the action of chymosin during cheese making. GMP has been the subject of growing interest in recent years. Because of its various biological activities, and unique amino acid composition having no phenylalanine, GMP is thought to be a potential ingredient for dietetic foods and pharmaceuticals. Bovine GMP has been studied in many laboratories. There is, however, very limited information available on goat GMP. This study was, therefore, undertaken to purify GMP from goat sweet whey, and to determine its chemical composition. A sample of sweet whey was prepared from pasteurized goat milk by chymosin treatment. After dialysis, the whey sample was applied to an anion-exchange column of DEAE-Sephacel (Pharmacia Biotech Inc.) equilibrated with water adjusted to pH 3.0. Most GMP, accounting for 97 % of total recovered sialic acid (a carbohydrate moiety of GMP), was adsorbed on DEAE-Sephacel and eluted from the column by applying 1 M NaCl. The GMP fraction obtained after DEAE-Sephacel chromatography was further purified using hydrophobic interaction chromatography on phenyl-agarose (Sigma Chemical Co.) equilibrated with 0.01 M sodium phosphate, pH 6.8 containing 5 M NaCl. The final preparation of GMP, corresponding to 0.06 % (w/v) of sweet whey, was of considerably high purity with its amino acid composition showing a trace (0.4 mol % or < 1 residue/peptide) of phenylalanine. The overall amino acid composition in goat GMP is, in general, comparable to that in bovine GMP. The content of sialic acid was, however, approximately three times lower in goat (25 mg/mg dry weight) than in bovine (80 mg/mg dry weight) GMP. It was concluded that goat sweet whey is a potential source to prepare GMP to partially substitute amino acids used for the dietary treatment of phenylketonuria.

Key Words: Glycomacropeptide, Goat whey, Sialic acid