ABSTRACTS
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DAIRY FOODS

1 Light-oxidized flavor and stability of Vitamins A, D, and C in value-added milk exposed to fluorescent light in various blow molded containers. K. W. Chapman*, L. C. Rosenberry, D. P. Brown, and K. J. Boor, *Cornell University, Ithaca, NY.*

The use of unpigmented blow molded plastic milk containers provides potential for photo-degradation of milk nutrients and development of light-oxidized flavors. Degradation of flavor and vitamins was compared in value-added lowfat milks fortified with vitamins A, D, and C, along with traditional 2% milk (fortified with vitamins A and D) in unpigmented blow molded plastic one gallon containers (standard) and in containers pigmented with 4% or 8% titanium dioxide (TD). Significantly less vitamin A degradation occurred in nonfat, 1% and 2% fat milk in both types of pigmented containers as compared to standard containers. After 24 h of fluorescent light exposure, vitamin A levels in milk held in pigmented containers were identical to those in unexposed milk. Vitamin C levels in 1% milk held for 42 h in 4% TD and in nonfat and 1% milk held for 24 h in 8% TD were comparable to those of samples held in the dark and were significantly higher than vitamin C levels of light-exposed milk in standard containers. Vitamin D levels were stable in nonfat and 1% milks in 8% TD containers at 24 h. At 8 h for 1% and 32 h for 2% milk, samples held in 4% TD had significantly less light-oxidized flavor than milk in standard containers. After 24 h, 1% milk held in 8% TD developed significantly less light-oxidized flavor than milk in standard containers. Light-oxidized flavor development did not differ among the nonfat milk samples, regardless of container. In summary, milk containers with TD significantly protected lowfat milk against development of light-oxidized flavor and vitamin degradation.

Key Words: Light-oxidation, Vitamins, Photodegradation

2 Detection and quantification of vitamin D in fortified milk by solid phase enzyme immunoassay. J. Jean*, C. Turcotte, R. E. Simard, and I. Fliss, *Universiti Laval, Canada.*

Vitamin D is one of the essential vitamins in the human diet for normal growth and function. In Canada and USA, fortified milk and milk products are the essential source of vitamin D. The recommended nutrient intake of vitamin D for Canadian adults is 200 to 400 I.U. per day. Additional amounts of vitamin D do not confer benefits and may even be toxic. In contrast, a deficiency of vitamin D leads to inadequate absorption of calcium and phosphorus and faulty mineralisation of bones and teeth. The actual methods for the quantification of vitamin D in milk are limited in terms of sensitivity, rapidity and simplicity. The development of a more accurate method is then urgently needed. The objective of this study was to develop a new strategy using an immunological approach for the quantification of vitamin D in fortified milk.
For this purpose, specific antibodies were raised in rabbits against vitamin D using cationized BSA as a carrier protein. No response was obtained with vitamin D alone. The IgG fraction was first purified by a Protein A/G column chromatography and then by CNBr-cBSA followed by CNBr-cBSA-vitamin D or directly by an Epoxy Activated-vitamin D columns chromatography. Purified antibodies were used to develop a direct solid phase enzyme immunoassay (DSP-EIA). Using this DSP-EIA, nanogram of vitamin D were detected within two hours. The signal obtained was proportional to the amount of vitamin D present in a given sample. The strategy developed seems to be very promising in terms of sensitivity, rapidity and simplicity. It offers a great potential for automation and use on a routine basis.

Key Words: Vitamin D, Polyclonal Antibodies, Milk and Milk Products

3 Gravity separation of raw bovine milk: Fat globule size distributions in milk fractions.

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Gravity separation is utilized by Italian cheesemakers to partially skim bovine milk. However, little is known about the resulting milk's globule size distributions. The purpose of this project was to employ laser light scattering to investigate this aspect. Raw, fresh, whole bovine milk (volume: 60 ml; height: 10.5 cm) was gravity separated at two temperatures: 4 or 15°C. Following incubation periods of 2, 6, 12 and 24 h, the bottom 5-ml (Fl) and top 5-ml (F2) fractions were drained from separation columns. At both temperatures, large fat globules moved to the top cream layer (F2) within ca. 2 h. Further incubation did not increase volume mean diameter significantly in F2. Higher temperature resulted in a faster rate of separation. Results of the experiment were as follows: for 24 h incubated milk, at the temperatures 4 and 15°C respectively, the fat content in Fl decreased from 3.13% to 0.61% and 0.26%, while that of F2 increased to 20.12% and 26.55%, thus achieving 53.6% and 70.1% creaming capacity. For the above same samples, concomitantly, the particle size distributions changed corresponding to changes of fat levels (see Table). Such gravity separation produced partially skimmed milk with a different particle size distribution than that of milk separated by mechanical means. As such, gravity separation possibly has unique applications to the cheese making industry, and its simplicity can make it an effective procedure for small scale dairy processors.

samples	volume mean diameter (μm)	specific surface area (m^2/g)	90 percentile diameter (μm)	10 percentile diameter (μm)
whole milk	3.13^b	5.39^c	5.97^b	0.45^b
4°C, F1	1.77^c	10.41^b	3.52^c	0.24^c
4°C, F2	3.60^a	4.20^d	6.77^a	0.80^a
15°C, F1	1.12^d	15.12^a	2.52^d	0.19^c
15°C, F2	3.66^a	4.06^d	6.80^a	0.84^a

Within the same column, different superscripts indicate significant differences ($p<0.05$).

Key Words: Bovine milk, Gravity separation, Globule size distribution

4 Determination of the free fatty acids content of cheese using ion exchange and gas liquid chromatography.

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Our objective was to compare the performance of the ion exchange binding method with two other methods. Samples of aged Ragusano cheese were analyzed in three different labs with different methods:Contarini G.et all.1989; C.De Jong,1990; and an acidic diethyl ether ether extraction plus an ion exchange resin were used to extract free fatty acids from cheese in our lab. The cheese is mixed with sodium sulfate and extracted with diethyl ether under acid conditions. Sodium sulfate and a free fatty acid internal standard (C13:0) were added to each flask. The neutral lipids and the free fatty acids are extracted by diethyl ether in two extraction steps.This extract is poured into a silicic acid chromatography column to bind phospholipids that will interfere with the binding of free fatty acids by the resin. All column effluent is collected in the bottle containing the resin (Amberlyst A-26). The column effluent is stirred with the resin overnight. The resin binds the free fatty acids but not neutral lipids. After this treatment the ether:methanol mixture is decanted and the resin and the container are rinsed five times with the ether:methanol mixture to remove all traces of neutral lipid. At this point the resin has bound all of the free fatty acids that were present in the original cheese sample plus the internal standard. The resin is transferred to a methylation flask containing a C17:0 methyl ester internal standard.The methylated free fatty acids are injected into a GLC to determine the qualitative and quantitative composition of free fatty acids per unit weight of cheese.There were no significant differences between estimates of total FFA C4:0, C12:0, C14:0, C16:0, C18:0,and C18:2, between the three methods, however the resin binding method gave significantly higher value ($p<0.005$)for C6:0, C8:0, C10:0, C14:1, C18:1. Further evaluation of the methods is being carried out to determine the source of these differences.

Key Words: Cheese, FFA, Method

5 Antioxidant activity and protection of SFME cell death by non-fat dry milk extract.

H. D. Jang*, A. Helmrich, and D. Barnes, *Department of Biochemistry and Biophysics, Oregon State University.*

The SFME cell line is a serum-free mouse embryo cell which can grow in serum-free medium indefinitely, maintains a normal karyotype and differentiates into neural cell types under appropriate conditions. The cells are cultured in medium in which serum is replaced with insulin, transferin, epidermal growth factor (EGF), high density lipoprotein (HDL), and selenium. Without HDL and selenium, the cells go into an apoptotic state, and die within 4 days. The ability of aqueous extract of non-fat dry milk (NFDM) to protect SFME cells from cell death associated with oxidative damage resulting from removal of protective agents (HDL, selenium) from the medium, was examined. The aqueous extract of NFDM was made by dissolving NFDM powder in deionized water and demineralization by chelex resin to remove metal ions, and the extractions divided into two fractions (filtrate, dialyzate) by dialysis(M.W. cut off 3,500) against deionized water. The antioxidant activity was measured in an in vitro model system using linoleic acid as model lipid and FeSO4 as a catalyst to cause the oxidation of linoleic acid. NFDM extract,demineralized NFDM extract and NFDM filtrate showed same level of antioxidant activity, and protected from SFME cell death.

Key Words: Antioxidant Activity, SFME Cell Death, Non-fat Dry Milk

6 Microstructure of whey protein-based, water-insoluble microcapsules.

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Effect of composition and microencapsulation process conditions on microstructure of whey protein-based, water-insoluble microcapsules (WIM) was investigated by SEM. Base emulsions (BE) consisting of anhydrous milkfat (AMF, 25–50%) in WPI solutions (15–25%, pH 4.5, 6, 7.2, 8) were prepared at 50°C and 50 MPa. BE were emulsified (50°C) into corn oil containing Span 65 to yield an O/W/O double emulsion (DE). Microcapsules were prepared by either thermal gelation (TG) of the protein matrix (90°C, 20 min) or by cross-linking (CL) the proteins with 1–10% gluteraldehyde (GA). Subsequently, capsules were harvested, washed with petroleum ether and ethanol, and dried. Outer topography and inner structure of gold-coated specimen were analyzed. WIM prepared by TG were spherical (20–60 μm), exhibited no surface dents, and had a limited number of surface pores (10–80 nm). In all cases, AMF droplets (0.1–0.6 μm) were evenly distributed throughout the protein matrix. WIM prepared by CL at GA conc. of 1–5% were spherical (10–50 μm) and dents-free. At GA conc. of 10% shape distortion, attributed to a too fast CL rate was evident. Inner structure of CL capsules was similar to that of TG ones; however, central voids were evident in some cases. Outer structure of WIM prepared by CL was significantly affected by the pH of BE. Capsules prepared at pH 4.5 were very porous and their surface features indicated aggregation of proteins rather than film formation. Extent of the latter was inversely related to WPI concentration. WIM prepared by CL from BE at pH 6.0 and 8.0 exhibited structural features similar to those observed at pH 7.2, however, capsules prepared from 15% WPI at pH 8.0 were elongated and formed large aggregates. AMF droplets (0.1–0.6 μm) were distributed throughout the protein matrix. Shape of embedded AMF droplet was affected by CL conditions, especially at WPI concentration of 15%. In all cases except at pH 4.5 results indicated that core phase was well isolated from the environment. Results indicated that structural features of WIM could be controlled by adjusting wall solids concentration and microencapsulation conditions.

Key Words: Whey, Microencapsulation, Proteins

7 Emulsifying properties of whey proteins in commercial whey samples. Z. U. Haque* and D. W. Olson, *Department of Food Science and Technology, Southeast Dairy Foods Research Center, MAFES, Mississippi State University, Box 9805, Mississippi State, MS 39762.*

Effect of processing stage in a commercial whey processing plant on emulsion activity index (EAI) and emulsion stability (ES) of whey proteins in whey samples was determined. Whey before ultrafiltration (5.3% total solids), whey before separation (6.3% total solids), liquid whey protein concentrate obtained from ultrafiltration (8.0% total solids), and condensed permeate (28.9% total solids) samples were obtained from a commercial whey processing plant. After freeze-drying 1.0, 0.5, 0.25, and 0.125% solutions in 10 millimolar imidazole-HCl pH 7.0 buffer were prepared for determining EAI and ES. Emulsions with a disperse phase fraction of 0.2 were formed by sonication. Emulsions for determining EAI were further diluted 1000 or 5000 fold in 10 millimolar imidazole-HCl pH 7.0 buffer containing 0.1% sodium dodecyl sulfate before measuring absorbance at 600 nm. EAI was calculated on a freeze-dried powder basis and expressed as surface area per gram. ES was determined by centrifugation of 1 ml of emulsion at 2000 × g for 30 min followed by removal of the aqueous phase and expressed by the remaining volume multiplied by 100%. ES of whey and whey protein concentrate stabilized emulsions at concentrations of 1 and 0.5% were normally between 22 and 35%. EAI ranged from 45 to 670 square meter per gram. The condensed permeate formed the most stable emulsions. Significant differences in EAI were present between different whey concentrations for each sampling location and between different sampling locations for each whey concentration. There appeared to be an inverse relationship between EAI and whey concentration for the condensed permeate. EAI and ES of whey proteins in commercial whey samples varied with both whey concentration and sampling location.

Key Words: Emulsion, Whey, Processing

8 Hydrolysis of whey proteins in solutions of whey protein concentrate by various commercial proteolytic enzymes. L.-B. Zhang* and N. Y. Farkye, *Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

The efficacy of six commercial proteolytic enzymes on whey protein hydrolysis in 20% solutions (16% protein) of commercial whey protein concentrate was studied. The enzymes used were: typsin (PTN 6.0), Alcalase™ (AL2.4), Neutrase™ (NEU 0.5), Flavourzyme™, Debitrase™ DD-6 and Debitrase™ HYW-20. Enzyme activities were standardized by the hemoglobin digestion method. Degree of hydrolysis (DH) of the whey proteins was measured by the TNBS method. Assay conditions were 50°C for 0.5 to 2 h at pH 6.4. Enzyme to substrate ratios (E/S) used were 1–4% for trypsin, Alcalase™ and Neutrase™; and 0.1 to 0.4% for Flavourzyme™, Debitrase™ DD6 and Debitrase™ HYW20. DH ranged from 2.74 to 38.40%. Results showed that under similar assay conditions, Debitrase™ DD6, Debitrase™ HYW20 and Flavourzyme™ were about 5–10 times more active than trypsin, Alcalase™ and Neutrase™.

Key Words: Hydrolysis, Proteolytic Enzymes, Whey Protein Concentration

9 Solubility of heat-treated and enzyme hydrolysed whey protein concentrate (WPC) at various pH. L.-B. Zhang* and N. Y. Farkye, *Dairy Products Technology Center, California Polytechnic State University, San Louis Obispo.*

The effect of heat treatment (85°C × 15 min) and degree of hydrolysis on solubility of whey protein concentration (WPC) solutions (20% solids, 16% protein) in the pH range 2.0 to 10.0 were studied. Commercial proteolytic enzymes, Debitrase® DD6 and Flavourzyme™, both containing mixtures of microbial proteases and peptidases were used for hydrolysis. Enzymes were used to hydrolyze WPC solution at E/S of 0.3 % for 0.5, 1.0 or 2.0 h at 50°C. Mean degree of hydrolysis (DH) of WPC using Debitrase® DD6 were 9.6, 13.8, and 18.4% at 0.5, 1.0, or 2.0 h, respectively. Mean DH of WPC using Flavourzyme™ were 13.8, 15.5, and 17.5%, respectively. Average solubilities of unheated WPC were 92.5, 89.4, 95.6, 99.0, and 98.9 at pH 2, 4, 6, 8 and 10, respectively. Corresponding mean solubilities of heated WPC were 66.5, 63.4, 67.0, 68.4, 70.8. Hydrolysis of WPC for 0.5, 1.0 and 2.0 h with Debitrase® gave DH of 9.6, 13.8, and 18.4%, with WPC solubilities of 80.5, 80.8, and 86.5%, respectively at pH 2.0 ; 79.3, 78.1, and 85.3% at pH 4.0; 82.9, 81.0, and 88.1% at pH 6.0; 81.2, 78.1 and 87.8% at pH 8.0; 85.0, 83.4 and 89.0% at pH 10.0. When Flavourzyme™ was used to give DH 13.8, 15.5, and 17.5%, solubilities were 82.6, 77.7, and 85.8% respectively at pH 2.0; 81.6, 77.5 and 85.0% at pH 4.0; 84.6, 80.1, and 87.5% at pH 6.0; 83.8, 80.4, and 88.3% at pH 8.0; 84.7, 81.6, and 88.5% at pH 10.0. Results suggested that heat treatment decreased solubility at all pH values. Solubility of hydrolysed WPC was higher than the heated WPC at all DH values.

Key Words: Solubility, Proteolytic Enzymes, Whey Protein Concentration

10 Chromatographic separation of various dephosphorylated forms of β-casein A^1-5P. F. Haidari*, E. D. Bastian, and L. S. Ward, *University of Minnesota, St. Paul.*

The objective of this research was to develop a chromatographic method to separate dephosphorylated forms of β-casein A^1-5P. β-Casein A^1-5P was isolated from a homozygous Holstein cow and showed one major peak using anion exchange chromatography. β-Casein A^1-5P was incubated at 37°C with bovine alkaline phosphatase. Samples were taken over time and analyzed using anion exchange chromatography. By adjusting the salt gradient, six different forms (β-casein A^1-5P, 4P, 3P, 2P, 1P, and 0P) were separated. The peak area for each form was integrated and plotted to show the concentration of the various forms with time. This method, developed for separating the different forms of dephosphorylated β-casein A^1-5P, permits subsequent collection of the dephosphorylated forms that will be used to study the functional and structual properties of phosphoserine residues in β-casein A^1-5P.

Key Words: β-Casein, Dephosphorylation, Chromatography

11 Simultaneous action of lysed cells and chymosin or plasmin under standardized conditions. K. A. Hassanein[1], A. Noomen[2], A. M. Darweesh[1], and S. I. Shalabi*[1], [1]*Minia University, Minia, Egypt.* [2]*Agricultural University, Wageningen, The Netherlands.*

Protein breakdowin by chymosin or plasmin in combination with lysed cells of lactococcus lactis subsp. Cremoris E8 was studied in analogues of cheese made from para caseinate calcium(PCC). Main parameter was the ratio of paracasein to water (P/W), but some experiments also aimed to study the effect of PH at preset P/W ratios. The analogues with chymosin and lysed lactococcal cells showed enhanced production of soluble nitrogen (SN), phosphotungestic acid soluble nitrogen (PTA-N) at the lowest ratio of P/W. In the analogues made from PCC containing indigenous plasmin (or this enzyme and added plasmin preparation) and lysed loctococcal cells (PH 5.3 or 5.6, P/W 1:3.5 kept at 13C), higher quantities of SN, PTA-N and total free amino acids were produced at PH 5.6 than PH 5.3 which indicate the enhanced action of plasmin at a higher PH. The results strongly support that SN substances produced by the concerted actions of chymosin and/or plasmin act as easily convertable substrates for peptidases of lysed cells, which speeds up the production of free amino acids and may affect cheese characteristics as well.

Key Words: Lysed Cells, Chymosin or Plasmin, Protein

12 Use of polymerase chain reaction for bacterial identification in cheese. K. B. Houck[1]*, J. E. Christensen[2], M. E. Johnson[1], and J. B. Luchansky[3], [1]*Wisconsin Center for Dairy Research, Madison* [2]*Dept. of Bacteriology, University of Wisconsin-Madison* [3]*Food Research Institute, University of Wisconsin-Madison.*

Classical microbiological plating techniques have been used to differentiate or at least enumerate bacteria when the causative organism was the dominant bacteria in a cheese. However, if the culprit organism was not the dominant bacteria, or was not viable, the problem of isolation became much more difficult if not unlikely. Another method, PCR amplification, can be used to identify and verify bacteria not found by classical plating methods. In this study, DNA sequences for 16s rRNA were obtained for target species. The target species were a variety of bacteria commonly found to produce gas in cheese. Downloaded sequences were grouped by species and compiled to produce a consensus sequence showing perfectly conserved regions of the 16s rRNA for a given species. The resulting 16s rRNA consensus sequences were then compiled to determine regions of variability between species. Primers for PCR were developed from these variable regions and were used to search DNA sequence databases to determine the uniqueness of the proposed primers. Experimental determination of primer specificity was performed in duplicate with the targeted bacterial species. Chromosomal DNA was extracted from pure cultures and screened using PCR with all species specific primer sets. The DNA products of the PCR (if any) were electrophoresed, sized and quantitated. The results of these screens were tabulated and they indicated that the primers were specific for the targeted bacterial species. Though some cross-specificity of primers occurred, band patterns and size allowed unambiguous identification of each isolate screened. Extraction of DNA from cheese to identify potential gas formers is now being explored using the developed primer sets.

13 Effects of the abortive infection mechanism AbiK on the lactococcal phage p2. I. Boucher*, E. Emond, and S. Moineau, *Department of Biochemistry and Groupe de Recherche en Écologie Buccale (GREB), Université Laval, Québec, Canada.*

The natural plasmid pSRQ800 from *Lactococcus lactis* encodes for AbiK, a protein of 599 amino acids which aborts phage infection. It confers high resistance against phages belonging to the 936 and P335 species (EOP 10^{-6}). Previously, we reported that phage ul36 (P335 species) cannot replicate its DNA in cells expressing AbiK. Here, we show that phage p2 DNA (936 species) can be replicated within AbiK+ cells. However, only the replicative concatemeric form, but not the packaged form (linear genome), was present in those cells. These results indicate that AbiK acts at a later stage during the lytic cycle of 936 phages as opposed to an early phase for P335 phages. To our knowledge, this is the first time that an Abi protein has a clearly different impact on the development of phages from two species. A variant phage (p2K) derived from phage p2 but insensitive to AbiK was previously isolated. Electron micrographs and DNA restriction patterns were identical for both phages but minor differences were detected in the protein profiles of the two phages by SDS-PAGE analysis. The major structural protein in phage p2K occurred in lesser amount than the corresponding protein of phage p2. The N-terminal amino acids sequence of the major structural proteins of phages p2 and p2K were determined by Edman degradation. The sequence of the first 10 amino acids was MKLDYNSREI for both phages. Homology searches in databases revealed that this partial protein sequence was 100% identical to the N-terminal sequence of the major capsid protein (MCP) of phages sk1 and F4-1 (936 species). The MCP genes of phages p2 and p2K were sequenced as well as their flanking regions. The 1920-bp fragment sequenced, comprising the 906-bp mcp gene, 628-bp upstream and 386-bp downstream, were 100% identical. Other investigations are underway to elucidate the mode of action of AbiK and to identify the modifications that confers the resistance against AbiK to the phage p2K.

Key Words: *Lactococcus lactis*, Bacteriophage, Abortive Infection

14 Identification of 4 groups of plasmids in *Streptococcus thermophilus*. N. Turgeon* and S. Moineau, *Université Laval, Québec, Canada.*

Streptococcus thermophilus is widely used for the manufacture of various Italian cheeses. Twenty-one *S. thermophilus* strains were obtained from various collections and the species identification was confirmed by sugar fermentation profiles on API 50CHL. Thirteen strains are currently used in industrial applications whereas 8 strains were isolated from artisanal Italian cheeses. Strains were analyzed for their plasmid content. Twelve of them (57%), including 4 industrial strains and all of the wild-type strains, were found to contain plasmids. Two strains (SMQ-173 and SMQ-312) contained two plasmids whereas 10 strains had only one plasmid. Thus, a total of 14 *S. thermophilus* plasmids were found. They were relatively small and ranged in size from 3.5-kb to 16-kb. The aim of this project was to identify the number of different groups of *S. thermophilus* plasmids using DNA-DNA hybridization studies.
The first probe was made of two primers designed from a conserved region of the RepB gene of two previously sequenced *S. thermophilus* plasmids using the rolling circle mode of replication. Eight plasmids hybridized with the first probe and were classified within group I. A second probe consisted of *Xba*I fragments made from a plasmid that did not hybridized with the previous probe. Four plasmids hybridized with the second probe and were classified in the group II. Weak homologies were observed with plasmids from group I. The group III and IV contained only one plasmid as they hybridized only with themselves. Strain SMQ-312 carried one plasmid from group I and one from group II whereas SMQ-173 harbored one plasmid from group II and one from group IV. These results suggest that some plasmid families might be compatible. Studies are underway to elucidate the mode of replication of the plasmids from groups II to IV.

Key Words: Plasmid, *Streptococcus thermophilus*

15 Membrane Receptor for Prolate Phages Is Not Required for Infection of *Lactococcus lactis* by Small or Large Isometric Phages. J. Kraus and B. L. Geller*, *Oregon State University, Corvallis*.

We tested the hypothesis that the prolate phage receptor of *Lactococcus lactis* is required for infection by small or large isometric phages. *L. lactis* contains a chromosomal gene (*pip*) for a membrane protein that serves as a receptor for the prolate bacteriophage c2 and other phages of the c2 species. A mutated allele of this receptor gene was used to replace the wild-type allele in *L. lactis* strains MM210, NCK203 and C2. A restriction site marker in a PCR product from the mutated allele detected allele replacement. The mutated pip derivative of strain C2 was completely resistant to phages of the c2 species as expected but was fully sensitive to the small isometric phage sk1 of the 936 species. The mutated derivatives of MM210 and NCK203were fully sensitive to small isometric phages mm210b and 31 (p335 species), and large isometric phage 949 (949 species). These results show that pip is not required for infection by phages of species 936, p335, or 949. The resultant mutants grew as well as the parental strains in liquid media. The mutated derivatives of MM210 and C2 acidified and clotted milk as readily as the wild type strains. These results show that phage receptor replacement in a commercial strain (MM210) of L. lactis does not affect growth and acid production in milk.

Key Words: Lactococcus, Bacteriophage, Receptor

16 Enhanced Cheddar cheese flavor via starter culture uptake and hydrolysis of glutathione. D. A. Mikesell* and J. L. Steele, *University of Wisconsin-Madison*.

Previous research has indicated that hydrolysis of glutathione (γ glutamyl-cysteinyl-glycine), a natural component of milk, can significantly enhance the formation of volatile sulphur compounds in Cheddar cheese. Additionally, starter cultures having the ability to transport glutathione into the cell may result in an increased concentration of glutathione in the Cheddar cheese matrix. Furthermore, starter cultures able to hydrolyze the γ glutamyl-cysteinyl bond of glutathione may ultimately increase the development of volatile sulphur compounds in Cheddar cheese, thereby enhancing flavor production.
Gamma glutamyl transpeptidase (GGT), the enzyme responsible for the hydrolysis of the γ glutamyl-cysteinyl bond of glutathione, has been identified in lactic acid bacteria. Seven strains of lactococci were tested for GGT activity with four strains (*Lactococcus lactis* LM0230, *Lc. lactis* 1228, *Lc. lactis* 11007, *Lc. lactis* DL16) testing positively. Strains of *Leuconostoc, Pediococcus* and *Streptococcus* were also tested for GGT activity; out of six strains tested, *Leu. cremoris* ATCC 19254 and *St. thermophilus* ATCC 19987 tested positively.
To identify the gene(s) responsible for GGT activity, a genomic library has been constructed from *Lc. lactis* LM0230 (GGT positive). The lactococcal genomic library from *Lc. lactis* LM0230 will be cloned into *Escherichia coli* K-12 SH761 (GGT negative) followed by a positive selection for *E. coli* K-12 SH761 mutants expressing GGT activity. Identification of the gene(s) responsible for GGT activity in lactococcal strains will allow for starter culture selection or construction leading to enhanced hydrolysis of glutathione corresponding to enhanced flavor of Cheddar cheese.

Key Words: Cheese Flavor, Glutathione, Lactic Acid Bacteria

17 Characterization of the *Lactobacillus helveticus groESL* operon. J. R. Broadbent[1]*, L. Wei[1], D. J. McMahon[1], C. J. Oberg[2], [1]*Utah State University, Logan* [2]*Weber State University, Ogden, UT*.

Several species of *Lactobacillus*, including *L. helveticus*, are used as starter cultures for manufacture of cheese and fermented milks. Because dairy processing often subjects these bacteria to adverse environmental conditions, studies of adaptive physiology in these bacteria should facilitate worldwide efforts to improve the activity and function of lactobacilli and other starter lactic acid bacteria in fermented dairy foods. Our group previously investigated the heat shock response in *L. helveticus* LH212 and showed GroEL was the most tightly regulated (and heavily induced) heat shock protein in this organism. To learn more about GroEL regulation in *L. helveticus*, a series of inverse PCR reactions were used characterize a 2.7-kilobase region of the LH212 chromosome that included the *groES* and *groEL* genes and the 5' end of a third open reading frame (ORF). Protein homology searches showed that the truncated ORF downstream from *groEL* encoded a protein with good homology to the amino terminal end of the *Streptococcus pneumoniae* DNA mismatch repair enzyme, HexA. Nucleotide sequence analysis identified a putative transcriptional promoter upstream of *groES* that was comprised of -35 and -10 hexamers flanked, upstream and downstream, by copies of the conserved Gram-positive heat shock gene regulatory sequence, CIRCE. A large inverted repeat that may function as a rho-independent transcriptional terminator was located between *groEL* and the third ORF. Northern hybridization of a 1-kilobase LH212 *groEL* gene fragment to RNA isolated from cells that had been heat-shocked at 52°C for 0, 5, 10, or 15 min detected a 2.2-kilobase transcript in each of the cell preparations. Densitometry showed the concentration of this mRNA species was approximately 4-fold higher after heat shock for 5 or 10 min and 3-fold higher after 15 min of heat shock.

Key Words: Heat Shock, Lactic Acid Bacteria

18 Antimutagenic activity of *Lactobacillus* spp. isolated from European Kefir and Biogurt and non starter strains of *Lactobacillus* and *Bifidobacterium* using *Salmonella typhymurium* TA 98. Y. H. Yoon*, J. K. Cho, and Y. J. Baek, *Chung-Ang University, Korea*.

The antimutagenic activity of *Lactobacillus* spp. isolated from European Kefir and Biogurt and non starter strains of *Lactobacillus* and *Bifidobacterium* strains against 2-Nitrofluene have been determined by Ames test utilyzing *Salmonella typhimurium* TA 98 in order to get the basis of probiotic effect of the strains.
The optimal concentration of mutagen 2-Nitrofluene to give 3,000 revertant colonies per plate turn out to be 10 μg/plate. Thirty six strains out of forty strains of *Lactobacillus* spp. isolated from European kefir and biogurt revealed antimutagenic activity and *L. plantarum* CU 722 showed the strongest antimutagenic activity of 50.34%. The antimutagenicity of non starter *Lactobacillus* and *Bifidobacterium* strains showed average 15% and 20% of mutation inhibition rate respectively. The effect of S-9 mix addition on the inhibition rate of mutagenicity against 2-Nitrofluene was species and strain dependent.

Key Words: Antimutagenicity, *Lactobacillus*, *Bifidobacterium*

19 Manure management to control E. coli 0157:H7 and Salmonella. T. A. McCaskey* and N. K. Gurung, *Auburn University, AL*.

Foodborne pathogens such as E. coli 0157:H7 and Salmonella are the focus of much attention. Because animal manure management practices are likely to come under more scrutiny in the future relative to enteric diseases that can be contracted from food-producing animals, a study was conducted to determine the fate of E. coli 0157:H7 and Salmonella typhimurium in a liquid swine manure system. The manure system studied was a constructed wetland treating effluent from a two-stage lagoon system. Wastewater was collected from a primary lagoon (PL), secondary lagoon (SL), a detention pond (DP) which stores the water after treatment through the wetlands, and a recycle pond water (RP) which is the final treated water used to flush manure from the swine houses. The four sources of wastewater in flasks were inoculated with E. coli 0157:H7 and S. typhimurium to attain about 1 million bacteria per ml of wastewater. The inoculated samples were held at 32 C and viable bacterial counts were determined at 2-day intervals for 10 days. Populations of both pathogens declined more rapidly in the PL and SL water samples and reached lower populations than in the DP and RP samples. In the PL wastewater E. coli declined from 740,000 per ml to 62 per ml in 10 days with an average D-value (time for the count to decrease 90%) of 2.45 days. Each 2.45 days the count decreased 90% or 1 log value. An 8-log reduction requiring 19.6 days would reduce the E. coli count from 1 million per ml to 1 per 100 ml. Because lagoon wastewater is unlikely to have such a high initial count of E. coli 0157:H7, the 8-log reduction has a margin of safety. D-values for SL, DP and RP samples were 15.5, 18.2, and 19.5 days, respectively. Similarly, D-values to achieve an 8-log reduction of S. typhimurium were 14.8, 14.5, 15.5, and 18.0 days for PL, SL, DP, and RP samples, respectively. Results indicated that 20 days retention time would be required for treatment of swine lagoon wastewater to achieve an 8-log reduction for E. coli 0157:H7 and 18 days for S. typhimurium.

20 Influence of the oral administration of Lactic Acid Bacteria on IgA producing cells associated to bronchus. S. Alvarez, M. Medina, E. Vintiñi, E. Roux, and G. Perdigón, *Centro de Referencia para Lactobacilos (CERELA) Universidad Nacional de Tucumán (UNT) Chacabuco 145 - 4000 - Tucumán Argentina*.

Intestinal, respiratory and genitourinary mucosal surfaces are the most important routes of entry for microbial pathogens. Stimulating the mucosal immunity is not easy because the trigger keys for the activation do not follow the ones of the systemic immune response. In previous works we have demonstrated that some Lactic Acid Bacteria (LAB) orally administered can induce an enhance of the gut immune response. Taking into account the concept of common mucosal response, we studied the effect of *Lactobacillus casei, L. acidophilus, L. rhamnosus, L. delbrueckii ssp. bulgaricus, Streptococcus salivarius ssp. thermophilus and Lactococcus lactis* orally administered on the IgA secreting cells associated to bronchus. We demonstrated that oral immunostimulation with the LAB assayed induce an increase of the IgA+ cells by dose depending effect at intestinal level. However we observed that *L. bulgaricus* and *L. acidophilus* were not able to enhance IgA+ cells at bronchial level, but *L. casei, L. rhamnosus, S. thermophilus and Lac. Lactis* were, being this effect dose dependent. The enhancement made by some LAB in the number of IgA+ cells on the mucosa surface of the lower respiratory tract may be very important to prevent bronchus diseases.

Key Words: Lactic Acid Bacteria, Mucosal Immunity, IgA

21 Acid and bile resistance of *Bifidobacterium* spp., *Lactobacillus bulgaricus* and *Streptococcus salivarius* subspecies *thermophilus* and their β-galactosidase activities. S. A. Ibrahim* and D. J. O'Sullivan*, *University of Minnesota, St. Paul*.

Several investigations have indicated that consumption of dairy products containing live lactic acid bacteria with high β-galactosidase activities may improve lactose digestion and tolerance. Cultures selected for this purpose should exhibit resistance to conditions encountered in the digestive system such as the presence of bile salts and acid. In this study ten *Bifidobacterium* species encompassing *B. bifidum*, *B. breve*, *B. infantis*, and *B. longum* and a strain of *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus salivarius* subspecies *thermophilus* were evaluated for β-galactosidase activities, bile sensivity and acid resistance. The 10 Bifidobacteria strains tested showed levels of β-galactosidase ranging from 100 to 800 Miller units when induced (grown in the presence of lactose) and from 0 to 190 when it was not induced. The induced levels of β-galactosidase were 800 and 1100 Miller units for *L. bulgaricus* and *S. thermophilus*, respectively. No uninduced activity was observed. To determine their sensivity to bile salts strains were surface plated on their respective agar medium containing (0.0, 0.1, 0.5, 1.0, 1.5, 2.0, and 3.0%) bile salts. Two bifidobacteria were found to be fully bile resistant. The most sensitive strain decreased to 6.45 log from the initial count of 9.04 log. *L. bulgaricus* showed a 1 log decreased in the cell number and *S. thermophilus* showed a 2.3 log decrease. To test for acid resistance, strains were incubated in HCL acidified peptone water (pH=3.5) for 5 h. immediately after resuspension, cells were enumerated to determine the initial acid effect. Both *L. bulgaricus* and *S. thermophilus* showed a reduction in viable cell numbers by approximately 1 log after immediate exposure to HCl peptone water. Bifidobacteria strains showed reduction in viable cell number of 1.5 to 6 log. In all cases, no further cell number reductions were seen over the 5 h period. These data indicated that all strains tested can withstand pH 3.5 once the acid resistant phenotype is expressed. However, the expression of the acid resistant phenotype is much quicker in some strains of bifidobacteria than others.

Key Words: Lactic Acid Bacteria, Bifidobacteria, Acid Resistance

22 Translocation of microorganisms in mice fed fermented and non fermented milk containing *Lactobacillus acidophilus*. C. L. Ferreira*, N. Brunoro, E. Fernandes, L. Borba, and A. Benatti, *Universidade Federal de Vicosa, Brazil*.

Lactobacillus acidophilus (*L. acidophilus*) is a well studied microorganism, and one common component of probiotics. Although the dietary adjuncts containing this type of microorganism have been well studied, there is still a lack of information about translocation of this microorganism to other organs in the body. To evaluate this, a 28 day period experiment was devised with three groups of 10 Albine mice each. Group one (control) received daily, by mouth, 0,1mL of Skimmilk without the test microorganism. Group two, and three received each 0,1mL of the unfermented or fermented milk containing an average of 1,0 x 10^9 UFC/mL of *L. acidophilus* NCFM. The three groups, aside the test diet, received a basic mice feed. After the 28 day period, all the mice were killed. The organs were weighed and examined by plating in MRS (De Man, Rogosa, Sharpe) agar after appropriate dilutions were made. Incubation were followed for 48–72h at 37°C under anaerobiosis. There were no growth in any dilution in none of the kidneys, livers and hearts of all the mice from the control group.Translocation was observed in the organs from the animals which received the sweet acidophilus and the fermented milk. Organs originated from the sweet acidophilus milk indicated viable counts of Lactobacilli in the levels of (log of the average followed by standard deviation) 2,97 (3,30), 2,96 (3,16) and 2,27 (2,42) for kidneys, livers and hearts, respectively. In the same order were found, for the group receiving fermented milk, levels of 2,57 (2,65), 3,28 (3,79), 2,26 (1,55). The results demonstrated that translocation occurs when mice are fed milk containing fermented or non fermented milk with one billion viable cells of *L. acidophilus* NFCM. Although the numbers found in the different organs are considered low, they suggest that there is a need to compromise when developing a probiotic. The frequency of the dosage, the cell number concentration and the species, should be evaluated and probably a screening should be made for the strains that translocate less once it is not known how this translocation will affect the host. Studies of clearance of the translocated organism is in process.

Key Words: Translocation, Lactobacillus, Mice

23 Incidence of spore-former bacteria through the milk powder processing and correlation of its presence in milk with its presence in the air. R. Rodriguez*, B. C. Hampson, R. Jimenez-Flores, *California Polytechnic State University, San Louis Obispo*.

Aerobic endospore forming bacteria are commonly found in raw milk and survive milk powder manufacture. The objectives of this work was to find the main source of endospore-formers during milk powder processing, to characterize them, and to correlate their presence in milk with the plant environment. To pursue these objectives a survey was made in 3 different plants in the San Joaquin Valley, in California, during Summer 1997. Raw, pasteurized, condensed, and powdered milk samples were taken at the beginning, middle and end of each run which varied from 24 to 36 hours. Air samples were taken every 12 hours at specific points in the processing areas with a portable air sampler (Burkard Manufacturing Company Limited, England). Both, air and milk samples, were tested for mesophilic and thermophilic endospore-formers. Mesophilic and thermophilic spore-formers were found in all stages of the milk powder manufacturing and all of the air samples. However, thermophiles predominated over mesophiles. Although mesophiles were found in the pasteurized, condensed and powder milk, their numbers were lower than the raw milk. This suggests a reduction through processing, and its presence in the samples obeys to long term runs or deficiencies during sanitation of the equipment. On the other hand, the number of thermophiles increased through all stages of processing. This indicates that thermophiles are more resistant to temperature changes during processing and they are able to stay and multiply. The spore-former species found in both type of samples, air and milk, corresponded to the genera *Bacillus*.

Key Words: Incidence, Spore-formers Bacteria, Stages for Milk Powder Processing

24 Characterization of *Lactococcus cremoris* strains for improved Cheddar cheese starters. N. Thivierge*, R.E. Simard, and I. Fliss, *Université Laval, Canada*.

Lactic acid bacteria used as starter in Cheddar cheese production are major contributors to the development of its desired texture and flavor, due mainly to the proteolytic and autolytic activities of the bacterial strains. The proteolytic activity consists of an enzymatic degradation of milk proteins by proteinases and peptidases into low molecular weight peptides and free amino acids. Considering the intracellular location of the peptidases (aminopeptidases), autolysis of the cells is a desired event in cheese ripening, because a good correlation was shown between autolysis of cell starters and development of cheese flavor. The aim of this research was to develop new tools for evaluating cheese starters based on proteolytic and autolytic activities. In this project, we studied six lactococci mesophilic starters (obtained from Agropur) from wich individual strains were recovered and evaluated. The best strains autolysed rapidly (26% in less than 3.5h) while only 10% autolysis was obtained with strains yielding lower quality cheeses. Also, highly proteolytic strains, as evidence by SDS-PAGE, degrade milk as well as whey proteins. Proteolytic activity was evaluated on fermented milk using OPA (o-phthaldialdehyde). Low or high proteolytic activity strains give values varying from 1 to 10 mM and up to 65 mM (expressed as NH_3 bonds). Low proteolytic strains give better cheese, because lactococci proteinases tend to produce bitter peptides. Strains were grown in 500 ml chemostat, in 6% reconstituted skim milk at 30°C with pH fixed at 5.8 with gentle agitation for 12-20 hours (end of exponential growth phase). Cells were recovered, washed and used for autolytic and aminopeptidasic activities (glutamic acid and leucine aminopeptidases). Results from the different activity tests led us to develop a predictive model for the development of new starters having Cheddar cheese aptitudes.

Key Words: Proteolysis, Autolysis, Cheddar cheese

25 Whiteness change during heating and cooling of Mozzarella cheese. L. E. Metzger[1]*, D. M Barbano[1], M. A. Rudan[1], P. S. Kindstedt[2], and M. R. Guo[2], *Northeast Dairy Foods Research Center, [1]Cornell University, Ithaca, NY and [2]University of Vermont*.

Mozzarella cheese undergoes a dramatic change in temperature and whiteness during baking on Pizza. Our objective was to determine why whiteness changes during baking. The color of four samples of low fat (6% fat) and four samples of LMPS (20% fat) Mozzarella cheese was evaluated during heating to 60°C and cooling back to 7°C after 15, 30, and 60 days of 4°C storage. The Hunter L value of low fat Mozzarella increased ($p < .05$) from 76 at 7°C to 87 at 60°C for 15 day old cheese. Upon cooling back to 7°C the L value decreased ($p < .05$) to 84. Similar trends occurred after 30, and 60 days of storage, except as the cheese aged the L value before heating and the L value after heating and cooling were lower ($p < .05$) than for younger cheese. The L value of LMPS Mozzarella was higher than the low fat before heating and lower than the low fat after heating and cooling. The serum phase of two of the low fat and two of the LMPS Mozzarella cheeses was removed at 3 days of refrigerated storage by centrifugation at 12,500 × g for 75 min at 25°C. The serum was heated from 7°C to 49°C and the L value was determined at each temperature. As was the case in the cheese, the L value of the serum increased dramatically during heating. The L value of the serum increased from 35 to 66 and from 27 to 55 for the LMPS and low fat Mozzarella cheese respectively when heated from 7 to 49°C. As the serum was heated from 49 to 71°C a white precipitate formed. SDS PAGE and urea PAGE were performed on the precipitate and supernatant to determine which proteins from the serum phase are involved in this temperature induced interaction. The casein and peptides derived from casein in the serum phase interact and produce the white precipitate which increases the L value during heating of Mozzarella cheese.

Key Words: Whiteness, Mozzarella Cheese

26 Construction of bacteriophage resistant strains of *Streptococcus thermophilus* by pGh9::ISS*1* insertional mutagenesis. J. M. Sturino[1]* and J. L. Steele[2], *Departments of [1]Bacteriology and [2]Food Science, University of Wisconsin-Madison, USA*.

As with other lactic acid bacteria, prolonged use of *Streptococcus thermophilus* as starter cultures has resulted in the emergence of lytic bacteriophages. Bacteriophages are the leading cause of slowed or failed dairy fermentations and translate to inferior products and significant financial losses to the industry. Bacteriophage insensitive mutants (BIM) were constructed using pGh9::ISS*1*-mediated insertional mutagenesis. A pool of *S. thermophilus* JLS130 (pGh9::ISS*1*) integration-derivatives was challenged with phage jud29 at a multiplicity of infection (MOI) ranging from 1–0.001, plated onto Belliker Agar + erythromycin (1 μg/ml) and incubated at 42°C overnight. Seventeen BIM from the MOI 1–0.01 dilutions and were chosen for future study. After mild alkaline lysis, parent and BIM genomic DNAs were isolated using the Quiagen QIAamp Tissue Kit and subjected to Southern hybridization (30% formamide and 42°C) against a ISS*1* probe. A total of nine banding classes were found in the BIM strains. The major banding class (8/17) was found to absorb jud29 like the JLS130 parent but jud29 plaques are detected with an efficiency of plaquing (EOP) of less than 10^{-6}. The remaining classes exhibited a reduction in EOP ranging from 10^{-1} to 10^{-2} and varying degrees of jud29 absorption. The objective of this ongoing study is to characterize the native loci involved in lytic bacteriophage proliferation in *S. thermophilus* and to construct bacteriophage resistant stains useful in industry.

Key Words: Bacteriophage, *Streptococcus thermophilus*

27 Identification, gene cloning, sequencing and expression of pyruvate carboxylase in fast milk-coagulating *Lactococcus lactis* subsp. *lactis* C2. H. Wang*, D. J. O'Sullivan, and L. L. McKay, *University of Minnesota, St. Paul*.

Fast milk coagulation (Fmc^+) by lactococci is an essential property for fast acid-producing starter cultures and is critical for successful cheesemaking. We previously reported the isolation of an $Fmc^-(Lac^+Prt^+Opp^+)$ mutant of *Lactococcus lactis* subsp. *lactis* C2, designated *L. lactis* KB4. KB4 required supplementation with aspartate or asparagine for growth and was found to lack pyruvate carboxylase activity (Pyc^-). The objective of this study was to clone the *pyc* gene and determine the role of pyruvate carboxylase on the Fmc^+ phenotype. To locate the *pyc* gene in C2, a *pyc* specific gene probe was synthesized by polymerase chain reaction (PCR) using degenerate primers which targeted conserved regions within pyruvate carboxylase. The probe was used to isolate a *pyc*-containing DNA fragment from a lambda phage-C2 genomic library. A *Sau*3AI fragment of about 20kb containing *pyc* was isolated and subcloned. A region of the fragment which included the *pyc* gene was sequenced. This sequence also revealed the presence of a gene cluster coding for open reading frames with homology to citrate synthase, aconitase, and a truncated isocitrate dehydrogenase. A PCR fragment containing only the functional C2 *pyc* was synthesized and cloned into pCI372. The resulting plasmid was electroporated into both C2 and KB4. The acid production rate of the transformants from both strains was found to be comparable when propagated in milk, indicating that complementation of the mutant *pyc* gene had occurred. PCR results showed that KB4 carried the complete *pyc* gene. However, data from immunoblotting with Pyc specific antibody indicated that the amount of the protein in KB4 was much less than in either C2 or the complemented transformant. The results suggested that Pyc activity is required for the Fmc^+ phenotype. The significance of the gene cluster encoding three putative tricarboxylic acid cycle enzymes in the metabolism of lactococci is currently under investigation.

28 Mutational analysis defining ropy exopolysaccharide expression in Lactococcus. E. P. Knoshaug[1]*, M. M. Skinner[1], A. S. Bishop[1], J. A. Ahlgren[2], and J. E. Trempy[1], [1]*Oregon State University, Corvallis, OR* [2] *Biopolymers Research Unit, USDA-NCAUR, Peoria, IL*.

Fermented milk products have been produced using starter cultures composed of lactic acid bacteria for centuries. These bacteria are capable of producing many types of polysaccharides. The polysaccharide of interest is a ropy exopolysaccharide (R-EPS). Sensory studies from this lab revealed that the R-EPS expressed by lactococcal strain Ropy 352 provided a slightly sweet character to fermented milk. Two studies are being pursued in addition to sugar compositional analysis of Ropy 352 R-EPS: 1) To characterize R-EPS genes, chemical and transposon mutagenesis were used along with a whey-ruthenium red screening assay. Ropy 352 mutants have been generated that show a loss of the desirable R-EPS with a concomitant loss of the desirable sensory characteristics in fermented milk products. This suggests that the R-EPS genes have been identified. Molecular analysis indicates that the location of the R-EPS genes appears to be chromosomal. 2) To develop stable R-EPS producing strains, we are searching for negative genetic regulators such as the Lon protease of the colanic acid capsular polysaccharide system. Certain bacterial species mutant for the highly conserved Lon activity overexpress capsular polysaccharide and are sensitive to DNA damaging agents (UV) which result in the formation of long filaments. Transposon mutagenesis was used to generate lactococcal mutants that were screened for UV sensitivity and the long filament phenotype. A lactococcal mutant has been identified which is sensitive to UV and forms long filaments. The phenotype of this lactococcal mutant suggest it is mutant for Lon activity, thus indicating that the lactococcal lon gene has been located by transposon insertion.

Key Words: Ropy exopolysaccharide, Lactococcus lactis sp. cremoris, Lon protease

29 Cloning and sequencing of a lactoccocal branched-chain amino acid aminotransferase. M. W. Atiles*, E. G. Dudley, and J. L. Steele, *University of Wisconsin-Madison*.

Enzymatic catabolism of amino acids is believed to be involved in the formation of flavor compounds and precursors in ripening cheese. Transamination is potentially the first step in the catabolism of amino acids. A branched-chain aminotransferase gene (*ilv*E) from *Lactoccocus lactis* LM0230 was cloned on a 9kb chromosomal insert in *Escherichia coli* DL39 ($ilvE^-$,$aspC^-$, $tyrB^-$). Complementation analysis and couple enzyme assays specific for leucine were used to confirm that a leucine aminotransferase activity was encoded by the insert. Subcloning experiments failed to isolate *ilv*E in a smaller chromosomal fragment. Sequencing of different subclones identified *ilv*E based upon identity to other sequences found in Genbank. A total of 2041 bp were sequenced. A 1023 bp open reading frame encoding a 340 amino acid polypeptide was found. The deduced amino acid sequence has 62% identity to *ilv*E of *Haemophilus influenza* and high similarity to the *ilv*E of a variety of organisms found in the GenBank. A transcript size of 1200 bp determined by Northern blot analysis suggests that *ilv*E is transcribed monocistronically. A promoter region and a potential RBS sequence were identified. In addition , a *rho*-independent terminator was identified downstream of the *ilv*E open reading frame using the terminator program of GCG. Hydrophobicity plot analysis of the deduced amino acid sequence and the lack of a signal peptide sequence suggests *ilv*E is a cytosolic protein. To study the role of this enzyme in the catabolism of amino acids by lactococci will require the construction of an isogenic strain lacking IlvE activity.

Key Words: *Lactoccocus lactis*, Branched-chain Aminotransferase

30 Integration of milkfat nutrition research with product development: A nutrition researcher's perspective. M. Lefevre*, *Pennington Biomedical Research Center, Baton Rouge, LA*.

In recent years, our understanding of the processes contributing to cardiovascular disease has grown along with our knowledge of what factors (risk factors) contribute to the development of the disease process. With dietary recommendations currently focused on reducing risk derived from elevated LDL cholesterol, it has been important to examine how these same recommendations affect the more recently identified risk factors. Data from a number of well-controlled studies have shown that many of these risk factors are actually adversely affected by current low-fat diet recommendations. Many of these adverse changes in risk factors can be specifically linked to recommendations to reduce total fat intake. With increasing recognition of the potential problems with low-fat diets, we are beginning to see a move away from generalized low-fat diet recommendations. For dairy foods, it is likely that future nutritional concerns will be focused less on the fat content and more on the fatty acid profile of the products. Of particular concern will be the content of palmitic, myristic and lauric acids. The challenge will be to develop products which have a more desirable fatty acid profile while maintaining desirable functional qualities and consumer acceptability.

Key Words: Dairy Fat, Heart Disease

31 Unresolved issues in designing diets to meet dairy research needs. C. M. Champagne*, *Pennington Biomedical Research Center, Baton Rouge, LA*.

Menus for dietary treatments used in metabolic feeding studies are often designed and analyzed using computerized nutrient software analysis systems. The nutrient values for foods in these software systems result primarily from the USDA nutrient databases for standard reference. Laboratory analyses generate the nutrient values which are a reflection of the particular product used in the analysis and is therefore dependent on the market availability of foods at that point in time. Experiences in two multi-center feeding trials brought attention to the fact that a menu generated from foods in the computerized nutrient database might not necessarily match those items that could be procured easily in the marketplace. Chemical analyses of composited menus were conducted to validate the calculated menus. Two problematic nutrients were fat and sodium. Issues directly involving dairy products were the exact fat percentage of milk, yogurt, and other commercially available milk-based foods, as well as the availability of altered sodium dairy products, such as reduced-sodium full-fat cheeses. The challenge to multi-center trials is to locate appropriate sources to acquire these items from a central location to decrease variability. Additionally, to be able to accurately match the available products to database descriptors or to obtain extensive nutritional data required by database developers would facilitate the development of usable menus and confidence in database calculations of nutrient content.

Key Words: Diet, Nutrition, Nutrient Database

32 Funding—The DMI perspective. D. B. DiRienzo*, *Dairy Management, Inc., Rosemont, IL*.

Current and future nutrition funding initiatives and priorities will be reviewed. Find out the important characteristics of a DMI funded nutrition research project and get an up-to-date summary on the hot research areas that will be targeted for future funding dollars.

Key Words: Nutrition Research, Research Funding

33 The role of nutrition research in food product development: An industry perspective. R. S. Rambo*, *Dean Foods Company, Rockford, IL*.

Is nutrition a part of food product development today? The answer is yes if Sales and Marketing, through good consumer research, determines it is. However, Sales and Marketing requirements, either on claims made or product attributes, must be compatible with product development and manufacturing capabilities. While some progress is being made in these areas, there remains much work to be done, particularly for nutrient/ingredient compatibility in dairy foods. Also, shelf life is an issue. Question is who will do the research? Probably not the food companies. It will fall on universities, pharmaceutical companies and trade associations. Dairy/Food companies will do the applications research perhaps via joint ventures.

Key Words: Food Product Development, Shelf-life, Nutrient Compatibility

34 Merging dairy foods processing expertise with food nutrition expertise—A case study and discussion. J. U. McGregor*, *Dairy Science Department, LAES, LSU Agricultural Center, Baton Rouge*.

got milk?—not milk!, milk does a body good—milk does a body in. Conspiracy? Consumers are constantly bombarded with conflicting information on the nutritional benefits of consuming dairy products. What effect is this conflict having on consumer views of dairy products and what effect will this information have on the future consumption patterns of dairy products? Current information being given to consumers and how this information is being delivered will be reviewed. Understanding this trend in consumerism is vital to the development of successful strategies for the creation of new dairy products that have high consumer acceptance. One key component for success is the merging of dairy foods processing expertise with food nutrition expertise in collaborative research and marketing efforts. During this presentation the audience will be invited to participate in a discussion on how to make nutrition research work for the dairy industry.

Key Words: Consumer, Nutrition Information, Dairy Product Consumption

35 Hydrolysis of casein derived peptides by peptidase-deficient *Lactobacillus helveticus* CNRZ32 derivatives. J. E. Christensen[1] and J. L. Steele[2]*, *Departments of [1]Bacteriology and [2]Food Science, University of Wisconsin-Madison, USA*.

The purpose of this project is to identify and characterize enzymes from *Lactobacillus helveticus* CNRZ32 which hydrolyze bitter peptides that accumulate in Cheddar cheese. A peptide that is known to accumulate to high levels in some Cheddar is β-casein (f193-209), a chymosin-derived peptide which has been associated with bitterness. Previously, PepC$^-$, PepE$^-$, PepN$^-$, PepO$^-$ and PepX$^-$ deletion derivatives of *Lb. helveticus* CNRZ32 were constructed by a two-step gene replacement procedure. The potential of *Lb. helveticus* CNRZ32 and the peptidase deletion derivatives to hydrolyze β-casein (f193-209) *in vivo* was investigated. β-casein (f193-209) was used to supplement defined media lacking all essential amino acids that are potentially obtained through complete hydrolysis and transport of the peptide. *Lb. helveticus* CNRZ32 and the peptidase deletion derivatives were all capable of growth with β-casein (f193-209) as the sole source of the amino acids Arg, Ile, Leu, Phe, Pro, Tyr and Val. Growth of the peptidase deficient strains indicates that complete hydrolysis of β-casein (f193-209) occurred, though the rate of hydrolysis is not known. The contribution of individual enzymes in hydrolysis of β-casein (f193-209) is being examined using cell-free extracts prepared from *Lb. helveticus* CNRZ32 and the peptidase deletion derivatives. The CFE were incubated with the peptide at 37°C at pH 6.50 or pH 5.20/4% NaCl (the pH and NaCl content of Cheddar) and the resulting peptides were separated via RP-HPLC and monitored with a diode-array detector. Individual peptides were identified through a combination of DAD spectral data and mass spectrometry. The results indicate PepN contributes significantly toward the hydrolysis of β-casein (f193-209) by CFE at pH 6.50 and pH 5.20/4% NaCl. Further characterization of the β-casein (f193-209) derived peptides will provide information about the specificity of *Lb. helveticus* CNRZ32 peptidases. Similar experiments are currently in progress to characterize the hydrolysis of α_{S1}-casein (f1-9).

Key Words: *Lactobacillus*, Peptidase, Casein

36 Characterization of proline-specific peptidase-deficient derivatives of *Lactobacillus helveticus* CNRZ32. G. U. Yuksel and J. L. Steele*, *University of Wisconsin-Madison*.

The contribution of four proline-specific peptidases to the ability of *Lactobacillus helveticus* CNRZ32 to obtain essential and/or growth stimulating amino acids from milk proteins, caseins, was examined. The proline-specific peptidases examined included X-prolyl dipeptidyl aminopeptidase(PepX), prolinase(PepR), prolidase(PepQ), and proline iminopeptidase (PepI). The growth and acidification rates of CNRZ32 and ten proline-specific peptidase-deficient CNRZ32 derivatives in skim milk were evaluated. Three of these derivatives (PepX$^-$, PepR$^-$, and PepQ$^-$) have been reported previously. *Lb. helveticus* CNRZ32 PepI$^-$, PepX$^-$PepQ$^-$, PepX$^-$PepR$^-$, PepX$^-$PepI$^-$, PepQ$^-$PepR$^-$, PepR$^-$PepI$^-$, and PepX$^-$PepR$^-$PepI$^-$ derivatives were constructed in this study. When grown in milk, the specific growth rates for CNRZ32 and its proline-specific peptidase mutants were separated into two groups. The PepR$^-$, PepQ$^-$, PepI$^-$, PepQ$^-$PepR$^-$, and PepR$^-$PepI$^-$ mutants grew like CNRZ32 (μ_{max}=0.54−0.61). The PepX$^-$PepQ$^-$, PepX$^-$PepR$^-$, PepX$^-$PepI$^-$, and PepX$^-$PepR$^-$PepI$^-$ mutants grew like the PepX$^-$ mutant (μ_{max}=0.24−0.29). The results suggest that PepX is the only proline-specific peptidase which has an essential role in CNRZ32's ability to obtain essential amino acids efficiently from caseins.

Key Words: *Lactobacillus*, Peptidase

37 Methionine Catabolism in Lactococci, a ^{13}C Nuclear Magnetic Resonance Study. S. Gao*[1], E. S. Mooberry[2], and J. L. Steele[1], *Department of *[1]Food Science and *[2]Biochemistry, University of Wisconsin-Madison*.

The catabolism of methionine is widely believed to play a major role in development of Cheddar cheese flavor. However, the catabolism of methionine in cheese related microorganisms has not been well characterized. Two enzymatic pathways may exist in lactococci: 1) elimination and 2) transamination. This study was designed to investigate Met catabolic pathways in lactococci. ^{13}C NMR using the uniformly enriched ^{13}C Met was utilized to differentiate between the two pathways, as well as, the possible distribution between the two pathways. The catabolism of methionine in whole cells and with cell-free extracts of four starter lactococcal strains was examined. The intermediate and products were determined to be 4-methylthio-2-oxobutyric acid and 2-hydroxyl-4-methylthiobutyric acid. Transamination pathway was found responsible for the catabolism of methionine in strains examined. Additionally, this pathway was shown to be active under cheese ripening like conditions.

Key Words: Methionine, Catabolism, ^{13}C NMR

38 Strategies to improve the viability of *Lactobacillus acidophilus* in probiotic yogurts: responses to heat and acid stress. S. McKechnie*, M. L. Britz, and N. P. Shah, *Victoria University of Technology, Australia*.

The main factor for poor survival of *Lactobacillus acidophilus* in yogurt has been attributed to a decrease in the pH of the medium resulting from accumulation of lactic acid during growth. *Lactococcus lactis* has been found to adapt to normally lethal acidic conditions after being exposed initially to moderate acid shock. The aims of this study were to characterise the response of *L. acidophilus* to temperature and pH stresses and to determine whether the response improved the survival of *L. acidophilus* during yogurt manufacture and storage. *L. acidophilus* strains were grown at optimal conditions to mid-log phase before being exposed to moderate heat or acid stress. Samples were taken before and after stress and whole cell protein extracts were run on SDS-PAGE gels. The proteins obtained after PAGE were transferred by electroblot to a nitrocellulose membrane and Western immunoblotting was performed using a variety of specific antibodies for the detection of stress proteins including GroEL and DnaK. Viable counts of stressed *L. acidophilus* cells were monitored after transfer to MRS broth with extreme acid or temperature challenge, and after manufacturing of yogurt with stressed cells. Results showed a specific increase in polypeptides during acid and temperature stresses. During heat stress, there was a significant increase in a 60KDa protein, which was homologous to the GroEL heat stress protein produced by *Eschericia coli*. Exposing *L. acidophilus* cells to moderate acid or heat stress prior to inoculation into extreme acid and temperature conditions improved survival of this organism by 100-fold as compared to the unstressed control. Viable counts of *L. acidophilus* in yogurt improved to some extent over the 7 week storage period compared to unstressed cells. These results show a potential for improvement of *L. acidophilus* in probiotic yogurts and increased health benefits to the consumer.

Key Words: *Lactobacillus acidophilus*, SDS-PAGE, Viability

39 Characterization of lactic acid bacteria isolated from Cheddar cheese with slit defect. M. Wiedmann*[1], C. Golnazarian[2], D. Weilmeier[1], T. Arvik[1], S. S. Dineen[1], K. J. Boor[1], and C. Donnelly[2], *Northeast Dairy Foods Research Center, [1]Cornell University, Ithaca, NY, [2]University of Vermont, Burlington.*

Slit defect in long-hold Cheddar cheese is a major economic problem for the dairy industry. This study was designed to further characterize the bacterial flora associated with cheeses developing this defect. Lactic acid bacteria were isolated from samples of Cheddar cheese showing the slit defect (approx. 90 isolates) and non-defect cheese (approx. 20 isolates). Using the API CH50L system, isolates from slit cheese were identified as *Lactobacillus curvatus* (n=26), *L. paracasei* subsp. *paracasei* 1 (n=6), *L. paracasei* subsp. *paracasei* (n=27). A total of 33 isolates could not be identified using the API CH50L database. A subset of 23 isolates was further characterized by automated ribotyping (a DNA "fingerprinting" method) using the restriction enzyme EcoRI, revealing 11 different ribotypes ("DNA fingerprints"). These data establish baseline information on the lactic acid bacteria present in Cheddar cheese with the slit defect and allow us to further investigate our hypothesis that gas-producing lactic acid bacteria play an important role in the development of this defect. We show that the API CH50L can only characterize to the species level <70% of the lactic acid bacteria isolated from Cheddar cheese. Application of the BioLog system for biochemical characterization of these isolates showed that aerobic incubation of the BioLog substrate plates often results in less than 5 wells (out of 95 wells) with positive reactions for a given isolate. Ribotyping showed promising results as a highly discriminatory tool for fingerprinting of our lactic acid bacteria isolates, indicating that this technique could be used for tracking the sources and spread of lactic acid bacteria during cheese manufacturing.

Key Words: Slit Defect, Lactic Acid Bacteria, Cheddar Cheese

40 Microbiological and chemical characteristics of surface mold ripended cheeses. J. N. Nanua* and J. U. McGregor, *LAES, LSU Agricultural Center, Baton Rouge.*

Camembert and Brie cheeses were collected from retail outlets and analyzed for coliforms, psychrotrophic bacteria and chemical characteristics. Surface and interior sections were sampled for analysis. Some of the cheeses were highly contaminated with coliforms, especially Brie, although no fecal coliforms were found. The coliform counts were higher on the surface than the interior of the cheese. Psychrotrophic bacteria counts were significantly higher on the surface for all cheese samples except canned Camembert, which showed no growth. Mold growth was mainly confined to the cheese surface. The mean composition of Brie was 17.3% protein, 34.7 % fat and 43.2 % moisture, while Camembert had 20.3 % protein, 26.8 % fat and 47.7 % moisture. There was a pH gradient from the surface to the center with a mean surface pH of 6.65 and center pH of 6.16. Acid degree value (ADV) decreased sharply from the surface to the interior of the cheese. The high pH and ADV are attributed mainly to proteolytic and lipolytic activity of the molds respectively. There was no significant difference between the surface and interior moisture content. The high pH and moisture content are likely to encourage growth of contaminants, some of which could be pathogenic. Coliform distribution in the cheese suggests that contamination occurred during handling. Brie with herbs had a high coliform count in the interior sections suggesting that contamination occurred during incorporation of the herbs into the cheese. Higher coliform count in Brie than Camembert could be due to contamination of Brie during cutting into wedges which is not done in Camembert processing. Canned Camembert is heat treated after ripening.

Key Words: Surface Ripened Cheese, Brie, Camembert

41 Effect of carbon source on bacterial capsules size and production. A. N. Hassan[1], J. F. Frank[2], and S. I. Shalabi*[1], [1]*Minia Universiy, Minia, Egypt* [2]*The University of Georgia, Athens.*

The effect of different sugars on capsule production was examined. Cultures (11 strains of lactic acid bacteria) were grown in milk with different total solids contents (8%, 11% or 14%) or in Ellikers broth media which contained lactose, sucrose, glucose or galactose as a sole source of carbon. Sterilized milk was inoculated with the grown culture and observed using confocal scanning laser microscopy in its reflactance mode. The obtained data revealed that sugar required for capsule production was strain dependent. Non ropy strains produced their maximum capsule size when grown in milk. Increasing lactose content of Ellikers broth from 0.5 to 5% produced larger capsules. Ropy strains seemed to be less affected by grown medium. Neither carbon/nitrogen ratio nor nitrogen source affected capsule size. The difference between the osmosity of milk compared to other medium might reduce the capsule size of some strains and completely inhibit the production of the others.
It seems that sugar content is not the only key factor influncing capsule production.

Key Words: Carbon, Bacterial, Capsules Production

42 Texture analysis of mozzarella cheese. N. P Shah* and R. K. Bhaskaracharya, *Victoria University of Technology, Australia.*

Mozzarella and pizza cheeses have a typical texture which is affected with reduction in the fat content. A low fat mozzarella cheese is inferior in quality to the full fat cheese and there is a need for making low fat mozzarella cheese which has similar characteristics as the full fat mozzarella. The recent trend for low fat and cholesterol free cheeses in the healthy diet has led to studies to overcome the problems in cheeses with reduced fat contents. The major textural characteristics of mozzarella cheese constitutes of primary parameters of hardness, cohesiveness, adhesiveness and springiness. Gumminess and chewiness are the important secondary characteristics based upon the primary characteristics. In this study, mozzarella cheeses were made using milk with various fat contents. Milk with 4, 3.5, 2.5, 1.5 percent fat and with skim milk were used to make mozzarella cheeses. *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus*, were used at the rate of 0.5 percent each as starter culture. Cheeses were analysed for their textural characteristics using an Instron Universal Testing Machine (Model 5564) with Merlin software. The cheeses were cut into uniform cylindrical specimens measuring 25 mm diameter x 20 mm height and compressed to 50 percent and 70 percent of their original height. The compression was achieved using a 500 N load cell with a flat plunger and cross head movement was adjusted to 50 mm per minute. Cheese with higher fat content showed lower values of hardness, cohesiveness, springiness, gumminess and chewiness. Adhesiveness values were higher for cheeses with higher fat content. Cheese made with skim milk showed similar characteristics to that made with 1.5 percent fat milk after 2 weeks of storage at 4°C. These observations were similar at both levels of compressions.

Key Words: Texture Analysis, Mozzarella Cheese, Low Fat

43 Ultrastructure of a full fat mozzarella cheese made with exopolysaccharide producing starter culture. R. K. Bhaskaracharya[1], N. P. Shah[1], A. Friedhuber[2], [1]*Victoria University of Technology,Melbourne, Victoria, Australia* [2]*Melbourne University, Parkville, Victoria, Australia.*

Several approaches have been taken for making low fat mozzarella cheese with the desired characteristics of a typical full fat mozzarella cheese. Use of exopolysaccharide (EPS) producing starter or an adjunct culture is becoming popular as one of the methods for making low fat high moisture mozzarella cheese. Cheeses made with EPS starter culture show better melt and stretch properties in comparison to low fat mozzarella cheese made with non EPS producing starter culture. In this study mozzarella cheese was prepared with full fat milk using *L. delbruckii ssp bulgaricus* and EPS producing *S. thermophilus* and the product was stored at 4°C. Its microstructure was examined after 28 days of manufacture. Cheese samples were cut to approximately 2 × 2 × 10 mm size and fixed using gluteraldehyde and osmium tetroxide. The specimens were rinsed with ethanol and acetone and fractured at room temperature after drying in a critical point apparatus. The specimens were mounted on aluminium stubs with the fractured surfaces facing up and sputter coated with gold in Edwards Sputter Coater S150B. Philips SEM 515 scanning electron microscope was used at 20 KV to study the ultrastructure of cheese specimens at about 10,000-13,000 times magnification. The ultrastructure of specimens showed microorganisms to be mostly located in cavities, i.e. at the junction of serum and protein. Some microorganisms were observed on the surface of the cheese strands which may be due to extraction during preparation of the specimen. A few chains of *S. thermophilus* were attached to the cheese network structure by filaments which appeared to have been produced by these microorganisms. Exopolysaccharide produced by the microorganisms such as *S. thermophilus* helps to retain moisture in cheese which enhances its melt and stretch properties.

Key Words: Low fat mozzarella cheese, Exopolysaccharide

44 Milk pre-acidification with citric acid: Impact on characteristics of low fat Mozzarella cheese. L. E. Metzger[1], D. B Barbano[1], M. A. Rudan[1]*, and P. S. Kindstedt[2], *Northeast Dairy Foods Research Center,* [1]*Cornell University, Ithaca, NY and* [2]*University of Vermont.*

Low fat Mozzarella cheese (6% fat) was manufactured using a 3×3 randomized complete block design. The three treatments included a control, acidification to pH 6.0 and pH 5.8 with citric acid prior to cheese making. A shorter ripening time and less starter culture were used for the pre-acidified milks. Milk pre-acidification did not affect ($p > 0.05$) cheese moisture, fat, protein or salt content. The pH of control cheese at 3 days was significantly lower than those made from pre-acidified milk (5.09, 5.36, and 5.39 for the control, pH 6.0, and pH 5.8 treatments, respectively). Acidification to pH 6.0 decreased ($p < 0.05$) calcium content by 20% and acidification to pH 5.8 decreased ($p < 0.05$) calcium content by 37%. During storage at 4° C, the cheese pH for the pH 6.0 and 5.8 milk treatments decreased while the control cheese pH remained stable. The pre-acidification did not influence the level of pH 4.6 soluble nitrogen, but the 12% TCA soluble nitrogen was lower, probably due to the lower amount of starter. Pre-acidification to pH 5.8 caused the unmelted cheese to be less hard and the apparent viscosity of the melted cheese to be lower at day 3, but by day 50 all cheeses had similar hardness and apparent viscosity. Post-melt chewiness determined by sensory testing and an objective chewiness test was lower ($p < 0.05$) in the pH 5.8 citric treatment than in the control or pH 6.0 citric treatment at 50 days of storage. The reduction in total calcium content caused by pre-acidification to pH 5.8 with citric acid made the cheese softer than the control cheese immediately after cheese making and also caused the post baking chewiness to be lower ($p < 0.05$) after 50 days of refrigerated storage.

Key Words: Pre-acidification, Low fat Mozzarella

45 Effect of homogenization on the serum phase of reduced fat Mozzarella cheese. M. R. Guo[1], P. S. Kindstedt[1]*, M. Rudan[2], D. M. Barbano[2], [1]*University of Vermont, Burlington* [2]*Cornell University, Ithaca, NY.*

Reduced fat (ca. 9% wet basis) Mozzarella cheeses were made from milk that was not homogenized, milk that was homogenized (13.8, 3.45 mPa), and milk in which only the cream portion was homogenized (13.8, 3.45 mPa). Expressible serum (ES) was obtained from cheeses after storage (4°C) for 2, 4, 6, 8 and 10 d after manufacture. Expressible serum was analyzed for crude protein and pH 4.6 soluble protein, Ca, Mg, Zn, Na, P, and K, and by urea-PAGE. Cheese manufacture was repeated on three different days using a randomized complete block design. Data were analyzed as a split-plot ANOVA. Amounts of ES decreased significantly from d2 (13.86 g/100g cheese) to d 10 (4.0 g/100g cheese), but did not differ among homogenization treatments. Crude protein and pH 4.6 soluble protein increased significantly with storage time but were not affected by homogenization. Protein that was insoluble at pH 4.6 (a measure of intact caseins) increased significantly from d2 (.65%) to d8 (1.37%); however, increases were much smaller than those observed in full fat Mozzarella cheeses from other studies. Low levels of α_s- and β-CN that increased with storage time were evident in ES samples by urea-PAGE. Calcium, Mg, and Zn increased significantly from d 2 (4093, 331, 2.8 mg/kg) to d 8 (4833, 409, 6.3 mg/kg), but did not differ among treatments. In summary: 1) water-holding capacity of reduced fat Mozzarella cheese increased during storage, as has been observed for full fat Mozzarella; 2) casein-associated minerals (Ca, Mg, Zn) and intact caseins increased in the serum phase during aging, as occurs in full fat Mozzarella; 3) lower levels of intact caseins in the serum phase of reduced fat Mozzarella cheese may indicate that the interaction between protein matrix and the serum phase is different from that in full fat Mozzarella, which may have implications for structure and functional properties; 4.) Homogenization had no significant effect on the parameters of the serum phase measured in this study.

Key Words: Mozzarella Cheese, Expressible Serum

46 Milk pre-acidification with acetic and citric acid: Calcium content and post-melt chewiness. L. E. Metzger[1]*, D. B Barbano[1], M. A. Rudan[1], and P. S. Kindstedt[2], *Northeast Dairy Foods Research Center,* [1]*Cornell University, Ithaca, NY and* [2]*University of Vermont.*

Low fat Mozzarella cheese (6% fat) was manufactured using a 4×4 randomized complete block design. The four treatments included a control, acidification to pH 6.0 and pH 5.8 with acetic acid and to pH 5.8 with citric acid prior to cheese making. Acidification with both acid types had no effect ($p > .05$) on fat on a dry basis, moisture content or initial cheese pH. The pH 4.6 and 12% TCA soluble nitrogen of the acidified treatments was higher than the control after 15 days of storage. Initially the TPA hardness and apparent viscosity of the pH 5.8 citric cheese was less than the other treatments, but values for all treatments decreased and converged over time. The calcium contents of the cheeses were .981, .875, .754 and .577% and the water-soluble calciums at day 2 were .291, .254, .218, and .197% respectively for the control, pH 6.0 acetic, pH 5.8 acetic, and pH 5.8 citric treatments. In the acidified treatments the water-soluble calcium increased over time, whereas in the control it remained stable. A similar but opposite trend occurred in the pH of the cheese during storage. A plot of water-soluble calcium as a function of cheese pH indicates a significant ($p < .01$) negative linear relationship exists. Acidification to pH 5.8 with citric acid decreased ($p < .05$) post-melt chewiness at 30 and 60 days of refrigerated storage, whereas acidification to pH 5.8 with acetic acid had no effect ($p > .05$) at 30 days, but decreased ($p < .05$) post-melt chewiness at 60 days. Acidification to pH 6.0 with acetic acid had no effect ($p > .05$). The decrease in post-melt chewiness resulted from the reduction in calcium and increase in water-soluble calcium.

Key Words: Pre-acidification, Low fat Mozzarella, Chewiness

47 Microstructure and ultrastructure of nonfat Mozzarella cheese made using direct acidification. D. J. McMahon*[1], B. M. Paulson[1], and C. J. Oberg[2], *Western Dairy Center, [1]Dept. Nutrition and Food Sciences, Utah State University, Logan, and [2]Dept. Microbiology, Weber State University, Ogden, UT.*

Scanning and transmission electron microscopy were used to study the structure of nonfat Mozzarella cheese. Eight directly-acidified cheeses of differing moisture and calcium contents, and pH were examined using a 2×2×2 randomized design in duplicate. Targets for moisture, calcium and pH were 66% and 70% moisture, 0.6% and 0.3% Ca and pH 5.8 and 5.3. Samples of cheese were taken from each replicate and moicrographs prepared from two or more different fields. At pH 5.8 and calcium levels of 0.56 to 0.67% the cheese predominantly showed poor fusion of the protein network. In some cheeses, the protein network appeared compressed together with individual proteins strands (ca. 1 to 2 mm diameter) still being evident. Large serum pockets (ca. 1 to 10 mm) were also present throughout the protein matrix. At pH 5.3, a similar openness, and lack of protein fusion, was observed in cheese that had the higher calcium levels (0.56 to 0.67% Ca). When calcium content was lowered to ca. 0.3%, there were no individual strands of protein evident; rather the protein matrix appeared as a homogeneous mass. Transmission microscopy of thin sections of cheese showed the same trend with poor fusion of protein strands at high calcium levels and a more homogeneous structure with low calcium. As well as the large scale folding of protein strands, strands of protein ca. 200 nm in width were observed along with numerous small serum pockets of 50 to 400 nm throughout the protein matrix. Differences at the ultrastructure level were not as apparent, although it appeared that the proteins in the high calcium cheeses (especially at pH 5.8) were aggregated into larger strands (ca. 25 to 60 nm width) than the lower calcium cheeses in which the protein chains appeared to be only (ca. 20 to 25 nm).

Key Words: Mozzarella, Microstructure, Calcium

48 Temperature-dependency of the opacity of nonfat Mozzarella cheese. D. J. McMahon*[1] and C. J. Oberg[2], *Western Dairy Center, [1]Dept. Nutrition and Food Sciences, Utah State University, Logan, and [2]Dept. Microbiology, Weber State University, Ogden, UT.*

When cheeses are made at reduced fat levels, a loss of opacity occurs. We had previously observed that this translucency of nonfat cheese was temperature dependent. During cheesemaking, the curd remained opaque until the cheese was salted. Then as salt was absorbed the curd became translucent. When the curd was heated during stretching it returned to being opaque. Upon cooling the cheese blocks again became translucent. This shift between translucency and opacity was also observed when the cheese was cooked on a pizza and the cheese became opaque and returned to being translucent when the pizza was cooled. To test this, we made nonfat Mozzarella cheese using direct acidification at fat levels between 0.1% to 3% fat. Whiteness, measuured by L* values, was observed to increase from 80.0 to 83.6 as the fat level increased. This influence of fat on opacity was expected as the fat globules in cheese act as light scattering centers and provide most of the light scattering. In contrast, the proteins aggregates are usually do not scatter light. When the cheese was heated, an increase in L* values was observed for all the cheeses. There were no changes in L* values between 40 and 50°C, then between 50 and 60°C a large change was observed with the lowest and highest fat content cheeses increasing to L* values of 87.0 and 88.1 respectively. This was followed by moderate changes from 60 to 90°C at which L* values between 89.0 and 90.2 were recorded. The greatest increases were observed in the cheese with the least amount of fat which implies that the increase in opacity is a result of changes in the light scattering properties of the proteins. It was concluded that the proteins within the protein matrix are becoming more aggregated as the cheese is heated. The temperature-dependence of this phenomenon suggests that the aggregation between the proteins is controlled by hydrophobic interactions.

Key Words: Mozzarella, Color, Temperature

49 Influence of pH, calcium, and moisture on physical properties of nonfat Mozzarella cheese. B. M. Paulson[1], D. J. McMahon[1], and C. J. Oberg*[2], *Western Dairy Center, [1]Dept. Nutrition and Food Sciences, Utah State University, Logan, and [2]Dept. Microbiology, Weber State University, Ogden, UT.*

Nonfat Mozzarella cheese was made using direct acidification to test the influence of pH, calcium, and moisture on physical properties. Ten kilogram batches of skim milk were acidified to either pH 5.8 or 5.3 (using either acetic acid or acetic/citric acid combination) and then coagulated at 35°C with rennet. Modifications to the manufacture procedure were used to produce cheese with either high (ca. 70%) or lower (ca. 66%) moisture by adjusting make time in the range 50 to 90 min depending on the pH and calcium targets. After draining, the cheese curd was salted with 40 g NaCl and stretched by hand in 5% brine at 82°C, molded and cooled in cold water. Melt was measured using a tube test in an oil bath at 90°C for 16 min. Calcium content of the low pH cheese was increased by adding calcium chloride to the milk before coagulation so that a calcium content typical of cheese made by starter cultures (ca. 0.6% Ca) could be obtained as well as the low calcium content (0.3% Ca) typical of directly-acidified cheeses. Attempts to lower the calcium content of the high pH cheeses by adding citric acid to the milk before coagulation and EDTA to the whey before draining the curd were not as successful. Cheese meltability was not influenced by moisture content or pH but was increased >300% when Ca content was reduced from 0.6% to 0.3% in the pH 5.3 cheese. Cheese hardness decreased as moisture content was increased at all pH and Ca combinations. Hardness also decreased as Ca content or pH was lowered. Adhesiveness was highest in the pH 5.3 cheese containing 72.7% moisture and 0.30% Ca. Cheese gumminess decreased as moisture content increased in a similar manner to cheese hardness. From this data, it appears that within the moisture range tested, calcium content is the predominant factor influencing the meltability of nonfat Mozzarella cheese.

Key Words: Mozzarella Cheese, Calcium, Functionality

50 Milk pre-acidification for low fat Mozzarella: impact on cheese yield. L. E. Metzger[1], D. M. Barbano[1]*, M. A. Rudan[1], and P. S. Kindstedt[2], *Northeast Dairy Foods Research Center, [1]Cornell University, Ithaca, NY and [2]University of Vermont.*

Two low fat (6% fat) Mozzarella cheese making trials were conducted: trial 1 was a 3 × 3 with a control and two levels (pH 6.0 and 5.8) of pre-acidification with citric acid and trial 2 was a 4 × 4 with a control, two levels of pre-acidification with acetic acid (pH 6.0 and 5.8), and one level of citric acid (pH 5.8). In trial 1, fat and protein recovery in the cheese was significantly lower than the control for the pH 5.8 pre-acidification. The moisture (55%) and salt (1.5%) adjusted cheese yields were 7.33, 7.15, and 6.95 kg/100 kg of milk for the control, pH 6.0 and pH 5.8, respectively, while the cheese yield efficiencies using the modified Van Slyke formula for Mozzarella cheese were 103.3, 100.8 and 97.7%, respectively. These were all significantly different ($p < 0.05$). Based on the experience in trial 1, the cheese making procedure was modified slightly with respect to starter culture usage and stretching temperature for use in trial 2. In trial 2, the moisture and salt adjusted cheese yields were 7.35, 7.17, 7.10, and 6.99 kg/100 kg of milk and the cheese yield efficiencies were 105.0, 102.4, 101.5, and 99.8% for control, pH 6.0 acetic, pH 5.8 acetic, and pH 5.8 citric respectively. The composition adjusted cheese yields and the cheese yield efficiencies were significantly different for all treatments in trial 2. Pre-acidification of milk prior to rennet addition shifts insoluble calcium from the casein micelles into the serum phase and this will cause a decrease in cheese yield. At a pre-acidification to pH 5.8, yield decrease will depend on the type of acid used, but we observed a 3.5 to 5.5% lower yield than would be expected without pre-acidification.

Key Words: Pre-acidification, Low fat Mozzarella, Yield

51 Test for measuring stretch characteristics of Mozzarella cheese. R. L. Fife[1]*, D. J. McMahon[1], and C. J. Oberg[2], [1]*Dept. Nutrition and Food Sciences, Utah State University, Logan, UT* [2]*Dept. Microbiology, Weber State University, Ogden, UT.*

The purpose of this research was to develop an objective test for measuring the stretchability of melted Mozzarella cheese. Three non-fat (NF) and four low-moisture part-skim (LMPS) Mozzarella cheeses were obtained from commercial sources or manufactured at Utah State University. Each cheese was analyzed for fat, protein, moisture, Ca, and Na. Functional characteristics of melt and stretch were measured by a modified tube test, helical viscometry, and a fork test as used by manufacturers of pizza cheese. Values obtained from these tests were compared with the new stretch test using a texture profile analyzer. To evaluate the new stretch test, cheese was placed into a stainless steel cup and tempered (30 min) in a water bath at 60, 70, 80, or 90°C until melted. The cup was then placed in a water- jacketed holder mounted on the texture profile analyzer. A three-prong probe was lowered into the melted cheese and then pulled vertically until all of the strands broke or the beam stroke maximum was reached. Stretch strength, the maximum load (g) obtained during the tensile test, stretch quality, the average load (g) as the cheese fibers stretched and elongated, and stretch length were computed. Stretch strength correlated with apparent viscosity and stretch length with the fork test. There was a significant difference in stretch quality between NF and LMPS cheeses, and within each cheese category. This elongation stretch test, along with more traditional compression/deformation tests, can provide useful information about the functional properties of Mozzarella cheese.

Key Words: Stretch test, Mozzarella, Melt

52 Effect of freezing, storage time and heating method on free oil formation in Mozzarella cheese. C. K. Yeung*, J. D. Braa, P. S. Yeung, and P. S. Tong, *Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

The effects of initial physical state, storage time and heating method on free oil formation in low-fat and part-skim-low-moisture retail Mozzarella cheeses were studied. Cheese samples were tested in duplicate immediately after purchase and after 35 days storage at 4.4°C. The free oil of cheese samples was determined by using a modified Babcock fat test method and the results were expressed as percent of total cheese fat. Heating methods were: a boiling water bath for 4–5 minutes, a conventional oven at 191°C for 3.5–5 minutes and a microwave oven (1.5kW) at high power for 25–35 seconds. Specific heating conditions were selected to give a similar bulk cheese temperature of approximately 74°C at the end of heating. Proteolysis in cheese was characterized by polyacrylamide gel electrophoresis. All analyses were done in duplicate. All Mozzarellas showed significant differences in free oil due to the effect of heating method ($p < 0.01$). Further analysis indicated that the microwave heating caused the greatest free oil formation, followed by conventional oven heating, and then water bath heating in all cheese samples. The mean and standard deviation of free oil percentages were 19.9±2.3%, 22.3±2.4%, 24.3±2.0% for water bath, conventional oven and microwave heating, respectively, of part-skim-low-moisture Mozzarella. The effect of initial physical state contributed significant difference to low-fat Mozzarella only, while the storage time produced no significant effect among all Mozzarellas. The free oil percentage was lower (<12.2%) in low-fat cheese.

Key Words: Mozzarella, Free Oil

53 Monitor and control of steam seals and barriers on an aseptic pasteurization system. J. Schlesser*[1], W. Wilson[2], A. Cinar[3], F. Kosebalaban[3], and J. DeCicco[3], [1] *Food and Drug Administration, National Center for Food Safety and Technology, Summit-Argo, IL* [2] *Anderson Instruments, Fultonvile, NY* [3] *Illinois Institute of Technology, Chicago, IL.*

An aseptic valve was assembled with a defect in the steam seal gasket to evaluate the effects that the size of defect had on the temperature and pressure in the steam seal area. Defects of 1.5, 5, and 10 mm in the steam seal gasket were evaluated. The aseptic valve was located on the pasteurized product line at the end of the cooling section and equipped with temperature and pressure sensors. The processing conditions were monitored with data acquisition software. Initial experiments evaluated a range of low steam seal pressures from 0 to 6 psig and a range of low pressures in the pasteurized product line from 0 to 12 psig. The measurements were taken for 2, 4, and 6 psig initial steam pressure. The steam seal temperature and pressure were measured as the pressure in the pasteurized product line was increased from 0 to 12 psig and then decreased from 12 to 0 psig. Later experiments evaluated a range of medium steam seal pressures from 10 to 20 psig and a range of medium pressures in the pasteurized product line from 0 to 60 psig. The measurements were taken for 10, 15, and 20 psig initial steam pressure. The steam seal temperature and pressure were measured as the pressure in the pasteurized product line was increased from 0 to 60 psig and then decreased from 60 to 0 psig. The on-line temperature and pressure sensors were able to monitor changes caused by defects in a steam seal gasket of an aseptic pasteurization system as small as 1.5 mm. These results provide evidence that the temperature and pressure sensor and the monitoring techniques can be effectively used to monitor temperature and pressure changes caused by steam seal gaskets defects that could cause process deviations.

Key Words: Aseptic, Pasteurization, Steam Seals

54 Evaluation of multiwall bags for dried milk. N. Farkye[1], D. Twede[2], S. J. Lee[2], [1]*Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo, CA* [2]*School of Packaging, Michigan State University, East Lansing, MI.*

This research evaluates the durability of multiwall paper bags for dried milk. Ten bag constructions were compared; they were filled and sealed by the dairy which uses each bag. The bags were evaluated in butt and side impact tests. Although all ten constructions had similar paper plies (3 plies of 60 lb natural kraft), the performance varied widely. The bags with a separate inner liner (Stone container's patented "Cap-sac" bag) performed best. There was found to be some relationship between the thickness of the liner and performance in the butt drop test. However, the side drop test did not show the same relationship since the liner seal depends more on the quality of the sealing operation. The side drop tests revealed that the top end sealed by the dairy is usually weaker than the bottom end which is sealed by the paper bag manufacturer.

Key Words: Dried milk, Bags, Packaging

55 Manufacture of Mozzarella cheese from highly-concentrated microfiltration retentate. R. L. Brandsma* and S. S. H. Rizvi, *Cornell University, Ithaca, NY.*

Most semi-hard and hard cheeses made from concentrated ultrafiltration (UF) retentates have not been commercially viable due to their increased content of whey proteins and calcium-phosphate salts, which can be detrimental to cheese texture and functionality. The objective of this research was to develop a Mozzarella cheese manufacturing process using concentrated, skim milk microfiltration (MF) retentate. Skim milk MF (0.2μm pore size ceramic membrane, 0.4m^2 surface area) was done at 3 pH levels to determine optimal processing conditions. This work was used to produce concentrated, pH 6.0 retentate containing 47.4% and 65.9% of the whey proteins and calcium, respectively, originally in the skim milk feed. Retentate and butteroil were homogenized together at 4.1MPa/52°C, resulting in cheesemilk composition of 38.2% TS, 15.7% protein (1.8% whey protein), 17.0% fat, 1.7% ash, 0.56% calcium, and 3.8% lactose. Glucono-delta-lactone powder (2% by weight of retentate) was added immediately after homogenization to achieve desired curd acidity without localized coagulation. The mixture was cooled to 34°C and renneted with 120μl rennet/kg cheesemilk. Set curd was cut into 2cm cubes and heated at 46°C until adequate moisture levels were attained. Whey was drained and curd hand-stretched using 70°C water, after which the cheese was brine salted and stored at 4°C. Finished cheese composition was 52.2% TS, 22.5% protein (2.06% whey protein), 22.3% fat, 3.3% ash, 0.7% calcium, 2.0% salt, and 2.1% lactose, with pH ranging from 5.1 to 5.3. Cheese meltability, as measured by a modified Schreiber test, ranged between 80 to 90% (3 to 50 days age) of the meltability of same age control low-moisture, part-skim (LMPS) Mozzarella, likely due to lesser amounts of rennet used, absence of starter culture, and presence of whey proteins. Stress relaxation and lubricated squeezing flow tests were used to quantify the rheological differences between control LMPS Mozzarella and experimental cheeses at 3, 9, 18, 29, and 50 days of age using an Instron Universal Testing machine. A promising cheesemaking process using MF has been developed that can be made continuous and generates sterile, fat-free permeate containing native-state whey proteins with enhanced functional properties.

Key Words: Mozzarella Cheese, Microfiltration

56 Use of surface modified polysulfone ultrafiltration membranes in whey processing. S. Somayajula[1]*, T. K. Singh[1], E. D. Bastian[1], and H. Nomura[2], [1]*University of Minnesota, St. Paul MN USA,* [2]*Neomecs, Inc. St. Louis Park MN USA.*

Ultrafiltration (UF) is a widely used membrane separation technique in the dairy industry. However, this technology suffers from problem of fouling, a decline in permeate flux over operating time. Fouling occurs due to chemical interactions between the membrane and feed constituents, in particular proteins. Any modifications to enhance inertness of the membrane may lead to improved performance, longer life of membranes and decreased energy requirements of UF operations. In this study we tested the performance of surface modified polysulfone UF membranes compared to untreated polysulfone membrane. Surface modification invloved coating membranes with low temperature plasma. Four plasma treatments were used. The membranes were tested with Cheddar cheese whey and permeate flux was measured at regular intervals. Three plasma treatments showed higher permeate flux during whey processing. We think that the improvement can be attributed to reduced fouling and increased physical resilience of the membranes due to plasma modification. Surface modification by plasma polymerization offers a promising technology for users of membrane separation techniques.

Key Words: Whey, Ultrafiltration, Plasma Polymerization

57 Novel application of whey proteins to stabilize micro-cellular structure of starch-based foods by supercritical fluid extrusion. B. Gogoi, M. Khan, S. H. Alavi, and S. S. H. Rizvi*, *Cornell University, Ithaca, NY.*

Supercritical fluid extrusion(SCFX) has the potential of producing homogenous microstructure in a starch-protein matrix. Nucleation and growth of microscopic cells in the starch-protein melt is achieved by supercritical carbon dioxide(SC-CO_2). Low-shear and high-moisture processing conditions permit utilization of heat sensitive ingredients and minimize degradation of starches. However, high moisture tends to collapse the extrudate and the expanding carbon dioxide can also rupture the weaker cells. Thermosetting ingredients such as whey proteins can enhance the strength of the cell walls and the structure can be set by post extrusion drying. The objective of this study was to evaluate the effect of added whey protein and drying temperatures on stabilization of the expanded cellular structure, cell size and distribution, density and expansion ratio of extrudates containing pre-gelatinized corn and potato starches after SCFX processing. A Wenger TX-52 co-rotating twin-screw extruder, with L/D ratio of 27, was used. The processing conditions were screw speed 100 rpm, throughput 35.58 kg/h, moisture content 35% (wet basis), and SC-CO_2 flow rate 4.55 x 10^{-3} kg/min. Extrudates were dried at 22, 70, 85 and 100°C. Scanning electron micrographs showed that sample cell size was below 100 μm and cell density was over 10^8 cells per cm^3. Drying at 100°C caused rupture of the cells and non-uniform cell size distribution. Temperatures below 85°C resulted in a more homogenous cell structure. Bulk density and expansion ratio were more affected by temperature than whey protein content. Expansion ratio increased up to 65% with temperature increase from 22 to 100°C, while increasing whey protein content from 0 to 10% increased expansion up to 25%. Whey protein at 4% concentration best stabilized the extrudate with the highest overall expansion and most homogenous cell structure in the temperature range 22 to 85°C. This study shows that whey protein is a good thermosetting ingredient for stabilizing starch based extrudates.

Key Words: Micro-structure, Whey Protein, Supercritical Fluid Extrusion

58 Effect of phosphates/citrates, methyl cellulose, hydroxypropyl methylcellulose, and microcrystalline cellulose with cellulose gum on shrinkage-expansion of nonfat and full fat ice cream. U. K. Dubey, C. H. White, and J. C. Wilson*, *Mississippi State University.*

The objective of this study was to control the phenomenon of shrinkage-expansion which occurs when ice cream is shipped to or from high altitude. The study was completed in three phases: phase one consisted of evaluating different phosphates and citrates (dipotassium phosphate, tetrasodium pyrophosphate, disodium phosphate, trisodium citrate); phase two evaluated methyl cellulose at 0.05 or 0.10% with or without normal ice cream stabilizers/emulsifiers; and phase three studied low, medium, and high viscosity hydroxypropyl methylcellulose (HPMC) with normal stabilizers/emulsifiers, and low and high viscosity microcrystalline cellulose with cellulose gum and normal stabilizers/emulsifiers. Three replicates were made at 64 m altitude with samples being divided into two sets. The first set was shipped to an altitude of 1676 m above sea level at -29 ± 5°C. The second set of samples was subjected to a vacuum of 200 mm of Hg at -29 ± 5°C to simulate the atmospheric pressure. Results indicate that phosphates/citrates did not influence ($P > 0.05$) shrinkage-expansion. Methyl cellulose at 0.05% was the most effective treatment in the second phase. In phase three, both low and medium viscosity HPMC were the most effective treatments in controlling shrinkage-expansion in both nonfat and full fat ice creams. The shrinkage-expansion effect was noted more in the nonfat ice creams than in the full fat ice creams.

Key Words: Ice Cream Shrinkage-expansion, Phosphates/Citrates, Altitude

59 Effect of the implementation of a premium payment system in a northern Mexican cooperative. Payment of milk on the basis of compositional and hygienic quality. J. M. Elizundia* and P. Salazar, *Grupo Industrial LALA*.

The effect of the payment of milk by compositional and hygienic quality was studied on the main quality indexes and statistical variables of fat, protein, SCC, and SPC in a mexican dairy cooperative with a production of approximately 2,000,000 l/day. The four parameters were improved in quality as we had a statistical significance $P < 0.05$ in the beyond specification and Cpk index. The % observed beyond specification for protein, SCC, and SPC; and the Cpk for SCC, and SPC were increased at $P < 0.01$.
For fat most of the statistical variables were changed with a statistical significance of $P < 0.05$ and the SD, and CV were different at $P < 0.01$. For protein only the Cpk, and the median were affected at $P < 0.05$, and the % observed beyond specification was at $P< 0.01$. The mode, SD, CV, stdandard skewness, and standard kurtosis were not affected $P > 0.1$.
In SCC all the variables were modified $P < 0.05$ and the % observed beyond specification, Cpk, SD, and CV were greatly changed $P < 0.01$. Most of the variables for SPC shifted $P < 0.1$ but not the standard skewness and standard kurtosis. The % observed beyond specification, Cpk, mean, median, and mode were changed at $P < 0.01$.
The conclusion is that the implementation of a pricing policy based on quality helped to improve the quality, but it does not mean that this change was accomplished without certain resistance to it.
Abbreviation key: CV = coefficient of variation, Cpk = process capability index; NAFTA = North America Free Trade Agreement, SD = standard deviation; SCC = somatic cell count; SPC = standard plate count.

Key Words: NAFTA, Milk Payment System, Quality

60 WisLINK™ - Development of an electronic network for dairy producers. M. J. Joyce[1]* and J. M. Mattison[2], [1]*Wisconsin Milk Marketing Board, Madison, WI,* [2]*ReQuest Ltd., Madison, WI*.

Wisconsin LINK (WisLINK^{TM}) Electronic Network was designed and built as an interactive decision support tool for Wisconsin dairy producers. WisLINK^{TM} is now being used by extension agents, ag consultants, veterinarians and other agri-support personnel as the front door to dairy information resources located all over the world. This interactive information site was BETA tested with 40 dairy producers located throughout Wisconsin prior to its launch on the World Wide Web in June 1996. The content of the WisLINK^{TM} web site is totally user-driven based on requests, suggestions and feedback received from producers and their agri-support companies, organizations and personnel. Information categories on the WisLINK^{TM} site include: dairy news and events, weather, markets, business and farm management, searchable ag databases, state and national dairy resources, on-line companies and organizations, ag/dairy media, computer resources, and dairy discussion groups. Since its launch and through December 1997, the WisLINK^{TM} site has received over 3,000,000 information requests and averages more than 10,000 information requests per week. It is being accessed 24 hours-a-day, 7 days-a-week by more than 3,500 regular users.

Key Words: Internet, Dairy Producer, Decision Support Tool

61 Examination of cheddar cheese ripening using FTIR. E. Chen[1], J. Irudayaraj[2], D. McMahon[1], *Utah State University, Logan* [2] *Penn State, University Park*.

Fourier transform infrared spectroscopy coupled with microtome sampling technique was used to analyze characteristics of ripened Cheddar cheese. Absorbance of bands arising from fat- and protein-related functional groups varied during cheese ripening. Change in absorbance of the bands at 1744 and 2850-2930 cm-1 arising from ester and C-H bond, and 1650 and 1540 cm-1 from protein amide I and II was greater than other functional group. Bands at 1116 and 1240 cm-1 arising from C-O, C-N, C-C stretch, changed slightly during cheese ripening. A correlation coefficient of 0.97 was obtained for absorbance at bands arising from fat ester and C-O bond (fat A and C band) and 0.93 for bands arising from protein amide I and II. A ripening index model developed by correlating ripening time with the change in key reactive groups in full-fat and reduced-fat cheddar cheese (FFCC and RFCC) had an R-square of 0.83 and 0.59 respectively. The index could be used as an indicator of ripening.

Key Words: FTIR spectroscopy, cheddar cheese, ripening index

62 Enhancing the intrinsic value of milk through dairy herd management practices. J. H. Herbein, *Virginia Polytechnic Institute and State University, Blacksburg*.

Desirable modifications in the composition of milk fat presently can be obtained by supplementing contemporary dairy cattle diets with high-oleic oils, but further improvement in milk fatty acid content is limited by the extensive hiohydrogenation of dietary unsaturated fatty acids (UFA) in the rumen. Apparent biohydrogenation of UFA to stearic acid ranges from 50 to 75% for oleic acid and 70 to 95% for linoleic acid and linolenic acid. Feeding supplemental UFA in a chemical or physical form that limited biohydrogenation, however, resulted in a very favorable increase in the ratio of unsaturated to saturated fatty acids in milk (fat-modified). Compared with consumption of conventional dairy products, consumption of fat-modified milk and dairy products lowered plasma total and LDL cholesterol of men and women. Another desirable modification of milk fat may be enhancement of conjugated linoleic acid (CLA) content. The primary source of CLA, an anticarcinogenic agent, in the human diet is bovine milk fat. The CLA in bovine blood is a byproduct of ruminal biohydrogenation of dietary linoleic acid. Although CLA content of milk can be increased up to 5-fold when CLA availability for absorption and transport to the mammary gland is enhanced, undesirable effects on milk fat yield and composition may result. Studies using bovine mammary cell cultures indicated elevated CLA in the medium inhibited fatty acid synthesis, desaturation, and elongation. When abomasal infusion of CLA was used to increase CLA availability in vivo, the inhibitory effects indicated by cell cultures were confirmed. Mammary cell cultures will be used for further evaluation of metabolic regulation of lipid metabolism in the mammary gland, focusing on methods to enhance the gland's capacity for stearic acid desaturation to oleic acid. Continued improvement in pre-harvest technology will allow production of fat-modified milk and dairy products with enhanced nutritional value and consumer acceptance.

Key Words: Fat-Modified Milk, Conjugated Linoleic Acid, Ruminal Biohydrogenation

63 Isolating the value in milk through separation technologies. L. M. Huffman[1] and W. J. Harper[2], [1]*New Zealand Milk Products (North America)* [2]*The Ohio State University*.

It is all too easy to take milk for granted, to dismiss it as simply a pleasant beverage for children and adolescents. Milk is much more than something to pour on the morning cereal. It is the source of a wide range of proteins that deliver nutrition and health to today's most promising new food products. Isolated milk proteins are natural, trusted ingredients with excellent functionality.
The major milk proteins, casein protein and whey protein, can be isolated by manipulating their chemical and physical properties and then using various separation technologies to recover the proteins. Additionally, they can be treated in various ways to create a wide range of ingredients with diverse functional characteristics. These ingredients include MPC (milk protein concentrate), MPI (milk protein isolate), casein, caseinate, WPC (whey protein concentrate), WPI (whey protein isolate) and hydrolysates. Within each of these ingredient categories there is further differentiation according to the functional and nutritional requirements of the finished product.
Technology often precedes market demand. As milk is further fractionated into component proteins, it becomes possible to study the potential benefits of those ingredients. In the future, as clinical evidence develops, the opportunity for adding value to dairy products as functional foods with continue.
Adding value to the milk by expanding from consumer products to ingredients often requires different technologies, marketing structure and distribution channels. The amount of technical support required for an ingredient business is also different. The worldwide market for both consumer products and ingredients from milk continues to grow. The research and development of today will be the basis of those value-added milk products for tomorrow.

Key Words: Protein Ingredients, Separation Technologies, Value-added

64 Enhancing market milk value of milk by adding cultures. R. Chandan, *Global Technologies, Inc., New Brighton, MN*.

Fluid milk and several dairy products are an excellent medium to generate an array of products that fit into the current consumer demand for health-driven foods. Several technologies associated with culture addition and/or fermentation for effecting innovation of an assortment of flavors and textures in milk products are available. It appears that by accentuating the positive attributes of inherent milk constituents, incorporating health-promoting cultures, and offering a variety of flavors and textures to the consumer is likely to enhance milk consumption.
Recent advances in probiotic research shows much promise in new product development of functional foods. Several scientifically sound clinical studies have verified some of the anecdotal reports of the past. Among the reported beneficial effects of consuming certain strains of cultures and/or their metabolites are: immune modulation, balancing of colonic microbiota, vaccine adjuvant effect, reduction of fecal enzymes implicated in cancer initiation, treatment of diarrhea associated with travel, antibiotic therapy, rotavirus and *Clostridium difficile*, and control of ulcer related *Heliobacter pylori*, reduction of serum cholesterol, antagonism against food borne pathogens and tooth decay organisms, and amelioration of lactose malabsorptions symptoms. The mode of action in most cases seems to involve modulation of ecosystem of the gastrointestinal tract of the host.
Probiotic strains safe of human consumption should be carefully selected to provide the health benefits. It is desirable to use a strain of human origin with proven resistance to stomach acid and intestinal bile secretion. Furthermore, the strain should exhibit colonization in human intestinal tract, and produce anticarcinogenic as well as antipathogenic activity in vivo. Several strains belonging to Lactobacillus and Bifidobacterium genera showing desirable clinical benefits are now available. They are being incorporated in yogurt type fermented dairy snacks, breakfast foods, drinks, refrigerated desserts, cheeses, spreads, frozen desserts as well as in unfermented milk drinks. These functional foods are designed to deliver unique flavor, desirable texture, excellent nutritive/health value and a widely accepted natural image.

Key Words: Cultured Dairy Products, Value-added, Probiotic

65 New product image: Improving the acceptability of milk and milk components. L. Dunning*, *Germantown International Limited, Germantown (U.S.A.) Company*.

Enhancing the value of milk and milk components as an ingredient of new product image is a challenge that faces today's dairy industry. Due to the presence of butterfat and cholesterol, milk has received negative publicity that has affected the consumption of dairy and dairy ingredients. Changes in the composition of milk, utilization of nutritional enhancers, development of optimum formulations and ingredient choices, and utilization of newer processing techniques are methods available to improve the image of dairy based products.
Removal of fat and cholesterol and the use of fractionated fats are methods available for changing the amount of fat and cholesterol and the degree of saturation in dairy products. Further enhancement is available through the addition of vitamin and mineral fortification, as well as the utilization of probiotics and prebiotics. The success of nutritional enhancement is evident in the sales of calcium fortified skim milks.
As consumers demand lower fat products, manufacturers are forced to optimize formulations so that they are similar to their higher fat counterparts. The use of new ingredients and processing techniques create dairy based products that have a higher acceptance among consumers. The choice of ingredients affects the textural and flavor characteristics and, in some cases, may provide nutritional benefits. Lactitol and Inulin are commercially available products that are just beginning to take hold in the market while hydrocolloids such as PrimaCel have just been introduced to the market. Dairy ingredients, such as why protein concentrates, are also finding their functional niche in food products.
Formula optimization combined with the use of processing techniques such as high pressure homogenization and pre-whipping further enhances the eating characteristics and shelf life of some foods.
It is through a balance of compositional changes, nutritional enhancement, ingredient choices, and new processing techniques that will allow food manufacturers to better utilize dairy ingredients and promote the image and consumption of dairy products.

Key Words: Formulation, Ingredients, Dairy Products

66 Wrapping it all up ... the value of packaging. D. M. Gorski, *Dairy Foods magazine, Des Plaines, IL*.

Of the 12,398 branded foods introduced to retail markets in 1997, 862 were dairy foods. On average, about 25% of all new products are truly unique. Most are simply line extensions, which includes new flavors and new package sizes. Of that number, about 33% of the truly new products fail, and 78% of the line extensions fail. With that many new foods introduced in a single year, and with such poor odds for success, dairy food manufacturers must think beyond the product and recognize the value of packaging.
Market research indicates that more than 50% of all decisions are made at point-of-purchase, with a package having about one-seventh of a second to attract a buyer's attention. Furthermore, new product life cycles are getting shorter. There are two ways that dairy food manufacturers can overcome these issues. The first is to make over the packages of old favorites in order to keep consumers interested. Second, for truly new foods, bold colors and graphics are necessary to generate interest among consumers, to grab their attention and get them to put a product from the shelf into the cart and then home.
Package design, color and graphics are extremely important with influencing the success of a product. For example, yellow makes a package look larger; black implies luxury, and often price; brown projects a natural, earthy feel; and green says healthy and fresh.

Key Words: Packaging, New Products, Graphics

67 Detection of cholera-toxin-binding activity of glycomacropeptide from bovine κ-casein and optimization of its production by use of response surface methodology. S. Oh*[1], R. Worobo[1], B. C. Kim[2], S. Kim[2], and S. Rheem[2], [1]*New York State Agricultural Experiment Station, Cornell University, Geneva;* [2]*Korea University, Seoul.*

Cholera toxin binding activity of purified glycomacropeptides (GMP) from bovine κ-casein was detected. In addition, a statistical model was developed to optimize the production of GMP by chymosin. GMP was obtained from the isolated κ-casein by chymosin hydrolysis followed by 3% trichloroacetic acid treatment. GMP was further fractionated on ion exchange column using FPLC. One peak eluted at .18M NaCl revealed the cholera toxin binding activity determined by ELISA. This fraction was a 8.9 kDa peptide without tyrosine and arginine residues. The cholera toxin binding activity of the fraction was decreased rapidly by carbohydrase treatment. The condition for the GMP production by chymosin was optimized by using response surface methodology (RSM). A central composite design was used to assign treatment combinations in this experiment. Because of the significant lack of fit of the full second-order polynomial model which was at first fitted to the data, cubic and quadratic terms were incorporated into the model through variable selection procedures. Thus, a subset fourth-order polynomial model without a significant lack of fit was built up, which was superior to the full second-order polynomial model in the coefficient of determination, in the coefficient of variation, and in significances of regression coefficients. The optimum levels of the factors were estimated as follows: reaction temperature = 38.5°C, pH = 6.44, and time = 35.9 min. A validation experiment in which the GMP production were compared among different conditions ascertained that the chosen optimum condition gave better production of GMP than the other conditions.

Key Words: Glycomacropeptide, Cholera-toxin-binding Activity, Response Surface Methodology

68 Microencapsulation of lactase for improvement of milk digestion. H. S. Kwak*, M. R. Lim, and J. J. Ahn, *Sejong University, Korea.*

The present study carried out to find an appropriate coating material and the conditions for lactase microencapsulation. As a coating material, MCT (medium chain triglyceride) and PGMS (poly-glyceride monosterate) were chosen. Three different percentages of microcapsules (2,4 and 6%) were added, and subdivided into 3 groups by washing time (0,1 and 2). The highest micro-encapsulation efficiency was found in the ratio of 15:1 as coating to core material with both MCT and PGMS. Those were 94.9 and 72.8% with MCT and PGMS, respectively. Subsequently, storage study was achieved during 12 days at 5°C. When 2% of two-time washed microcapsule was added into milk, lactose content was not significantly changed upto 12 day with MCT and upto 8 day with PGMS ($p > 0.05$). The sensory data also indicated that the sweetness and off-flavor in the milk added was not significantly increased during storage, compared with those of a commercial market milk. The present study provides an evidence that emulsifiers could be used as an effective coating material for lactase and the addition of 2% of microcapsules may not result in a negative effect on milk quality during 8 days. In addition, this study showed the possibility for a sufficient milk consumption of people suffering from lactose intolerance.

Key Words: Microencapsulation, Lactase, Milk

69 Cholesterol reduction in cream using β-cyclodextrin by response surface methodology. H. S. Kwak* and J. J. Ahn, *Sejong University, Korea.*

A process of cholesterol reduction in cream by using β-cyclodextrin (β-CD) was optimized by response surface methodology. For determining the effects of five factors (β-CD concentration, mixing time, temperature and speed, and centrifugation speed), a five level rotatable central composite design was used. Among those, three independent variables such as β-CD concentration, mixing time and speed appeared to influence on cholesterol reduction. When β-CD was added as 5%, the cholesterol reduction was 80.17%. With 10% β-CD addition, the effect of mixing speed was more profound than that of mixing time. The highest reduction (94.42%) was obtained in the condition of 30 min mixing and 1330 rpm of speed at 30°C. With 15% β-CD addition, 93.85% of cholesterol was reduced. When mixing time was fixed as 10 and 20 min at 30°C, 15% β-CD addition resulted in the highest reduction as 94.81 and 97.99%, respectively. The present study indicated that although percentage of cholesterol reduction varied with different factors and conditions, above 94% of the cholesterol in cream could be removed and β-CD concentration may play the most important role in cholesterol reduction process.

Key Words: Cholesterol Reduction, β-cyclodextrin, Cream

70 Cholesterol removal in homogenized milk with β-cyclodextrin. H. S. Kwak*, D. K. Lee, and J. J. Ahn, *Sejong University, Korea.*

The present study was designed to develop the optimum conditions for cholesterol removal in 3.6% homogenized milk by response surface methodology. The effects of five different factors such as β-cyclodextrin (β-CD) concentration, stirring time, temperature and speed, and centrifugation speed were determined by using a five level rotatable central composite design. Cholesterol content was measured by saponification following hexane extraction and gas chromatography. The important factors influencing on cholesterol removal were β-CD and stirring temperature. The optimum conditions for the cholesterol removal in 3.6% homogenized milk were the addition of 1.15% β-CD, 17.5°C of stirring temperature, 10 min of stirring time, 800 rpm of stirring speed and 1500 rpm of centrifugation speed. Based on above conditions, about 98.4% of cholesterol in milk could be removed, and β-CD may be an effective compound on cholesterol removal process.

Key Words: β-cyclodextrin, Cholesterol Reduction, Milk

71 Binding of retinyl and palmitate moieties of retinyl palmitate at separate sites on β-lactoglobulin. Q. Wang*, J. C. Allen, and H. E. Swaisgood, *North Carolina State University, Raleigh*.

β-Lactoglobulin (β-LG), the major protein in whey, is related to a group of hydrophobic molecule transporters that includes retinol-binding and fatty acid-binding proteins. All these proteins share a common three-dimensional structure consisting of an eight- or ten-stranded anti-parallel β-barrel constituting a hydrophobic pocket. β-Lactoglobulin can bind tightly one retinal molecule in the calyx and also interact with long chain fatty acids. Recently, we found that native β-LG isolated by bioselective adsorption had a higher capacity for retinoids than the β-LG in whey and also that it strongly bound vitamins D, E, K and conjugated linoleic acid. Furthermore, binding of fatty acids to β-LG strongly depended on protein concentration and followed a two-site binding model. The two-site model did not fit the binding of retinal to β-LG, suggesting that retinal and palmitic acid bound at different sites. A competitive binding study confirmed this observation. Because production of low-fat or non-fat foods results in removal of the fat-soluble vitamins A and D, these vitamins are usually added to restore the nutritional value. For example, retinyl palmitate dissolved in corn oil is often used because of its stability. However, β-LG isolated by bioselective adsorption may be a good carrier of fat-soluble vitamins A and D because of its strong binding properties, and thus could replace the use of corn oil. The fluorescence changes that occurred when retinyl palmitate bound to β-LG could be separated into the respective contributions of the retinyl and the palmitate moieties, each binding at a different site, thus allowing analysis of the binding of each moiety. The results showed that the β-LG binds the retinyl group at one binding site with Kd=0.12 μM and n=0.99 and the palmitate group at another site with Kd=0.039 μM and n=1.17. Thus, two molecules of retinyl palmitate are tightly bound to one β-LG molecule.

Key Words: β-lactoglobulin, Retinyl Palmitate, Binding

72 Molecular design and characterization of a trypsin-streptavidin fusion protein. D. A. Clare*, V. W. Valentine, and H. E. Swaisgood, *North Carolina State University, Raleigh*.

Herein, we describe the design and characterize the expression of a trypsin-streptavidin (TRYP-SA) fusion protein. The streptavidin gene was initially cloned into pET26b. The pET-streptavidin gene was modified by PCR to incorporate a 5'BamHI site and a 3'SacI site (pETSA7). The gene encoding trypsin was cloned upstream into pETSA7. The trypsin gene was PCR modified to incorporate a 5'MscI site and a 3'BamHI site generating a co-linear fusion of trypsin/streptavidin genes. The cloning procedure for each construct was verified by restriction enzyme digestion and sequencing. pTRYP-SA was transformed into the cell line, BL21, for protein expression. Cells were grown in LB broth containing 1 mM IPTG for 24 hr at 37°. Standard protein preparations were made. All samples were dialyzed against 50 mM Tris, pH 8.1, containing 10 mM calcium chloride, and assayed for trypsin activity. Enzyme activity was measured in the periplasmic and inclusion body fractions. Immunoblotting was accomplished using anti-streptavidin antiserum. Western blotting of a cell-free extract evidenced a single protein band exhibiting a molecular weight of 38,000 daltons. A biotin affinity matrix was prepared by immobilizing NHS-LC-biotin on controlled-pore glass beads. Selective adsorption on biotinylated glass resulted in a *one step* purification and immobilization of TRYP-SA from crude cell lysate. Enzymatic modification of whey proteins affords the opportunity to change gelling properties and improve foaming characteristics and emulsifying properties. This trypsin bioreactor will serve as an excellent prototype for design of functionality of milk proteins.

Key Words: Proteolysis, Bioreactor, Cloning

73 Yogurt quality and milk protein degradation as detected by capillary electrophoresis. L. S. Mendiola* and R. Jiménez-Flores, *California Polytechnic State University, San Luis Obispo*.

Yogurt quality greatly depends on the starting milk's protein integrity. Requiring minimal sample preparation, Capillary Electrophoresis (CE) rapidly detects milk peptides. The objective of this work was to determine whether a relationship existed between CE electropherograms of milk and physical properties of the resulting yogurt. Trypsin was added to skim milk at a 1:50 dilution and allowed to digest for 4 hours at 37 °C. To increase the solids content to 12%, both the proteolyzed and fresh skim milks were fortified with non-fat dry milk. After the two milks were heat treated at 90 °C for 30 minutes, mixtures were made in fresh to trypsin-digested ratios of 1:0, 3:1, 1:1, 1:3, and 0:1. Samples of each were taken for CE and SDS-PAGE analysis. The CE system used a pH gradient stacking method with a citrate-based running buffer and a Tris-based sample buffer. With a 20 second pressure injection, samples were run through a 40 cm capillary, 50 μm in diameter, at 18 kV. The capillary temperature was 20 °C. Meanwhile at 43.3 °C, the five milk mixtures were inoculated and incubated until a pH ∼ 4.4 was reached. The yogurts were cooled, packed, and refrigerated overnight. Physical properties compared among the five yogurts included syneresis, firmness, viscosity, and stress yield. Syneresis was determined by a drainage test, firmness by a Texture Analyzer TXT.A-2, viscosity by a Brookfield viscometer LVTD, and stress yield analyses by a Scientific Rheometric rheometer SR-5000. A complex relationship was observed between the CE electropherograms of the various milks and their corresponding yogurts, when B-casein degradation reached detectable levels by CE. As more peptides appeared on the electropherograms, syneresis increased while firmness, viscosity, and stress yield decreased. This study indicates that CE has the potential to predict yogurt quality by assessing the starting milk's protein integrity.

Key Words: Capillary Electrophoresis, Protein Hydrolysis, Yogurt

74 Interactions of microfibrous cellulose with milk proteins & its use in solid and semi-solid milk products. S. Moran * and R. Jimenez-Flores, *Dairy Products Technology Center, Cal Poly State University, San Luis Obispo, CA*.

Microfibrous cellulose (MC) offers promise as an ingredient allowing the development of new low-fat and fat-free dairy products. An initial qualitative assessment of the effect of this ingredient on systems that mimic solid and semi-solid dairy products is presented, while rheological and thermal analyses quantify important interactions between MC and milk proteins. Dispersion and activation (via a shearing process similar to homogenization) of 0.5 % MC in milk protein solutions or skim milk leads to a large increase in viscosity (by more than a factor of 40 and by a factor of 175, respectively), giving the mixture the consistency of yogurt. Comparison of mixtures made from skim milk and milk protein concentrates in either buffer or milk permeate shows that small ionic and/or polar components in the milk serum increase ease of dispersion of MC, but milk salts and/or lactose lower the final viscosity of an activated milk protein-MC mixture. Milk gels approximating soft-curd cheese are produced enzymatically (via chymosin treatment) from these mixtures. Gels generated with activated MC have better water retention (extremely low syneresis) than those lacking MC or including unactivated MC. Gel strength measurements obtained with a texture analyzer show that activated MC softens the gel by a factor of 5. However, slurries made by breaking up the curd and mixing to homogeneity give samples with a yogurt-like consistency that have a much higher yield stress than samples without MC or with unactivated MC. These results appear to indicate that activation of the microfibrous cellulose forms an additional internal matrix that improves the hydration and textural properties of milk protein gels.

Key Words: Milk Proteins, Cellulose, Texture

75 Some physical properties of acid gels made using added whey protein concentrate. L. Materon, G. Castro*, and R. L. Richter, *Texas A&M University, USA*.

The properties of acid milk gels are important in many dairy products. Whey protein concentrates are now available which can be incorporated into these gels to change the physical properties of these gels. The objective of this study was to determine the effect of whey protein concentrate on the viscosity and syneresis of an acid milk gel. Nonfat dry milk, a whey protein concentrate with 80% protein, and cream were combined to prepare milks with 4.5% protein and 0% milk fat or 4.5% protein with 1.5% milk fat. Samples containing milk fat were homogenized at 30MPa. The ratio of nonfat dry milk to whey protein for each series of samples was 100:0, 80:20, 60:40, and 40:60. The mixtures were heated at 90-95°C for 10 minutes and then cooled to 4.5°C. Sufficient glucono-delta-lactone was added to acidify the samples to a pH of approximately 4.1. The initial viscosity and the rate of viscosity decrease were determined at constant shear rate using a Brookfield viscometer. Syneresis was determined using a centrifugation method. Syneresis remained constant or decreased slightly as the ratio of whey protein in the nonfat protein caused an increase in syneresis in the nonfat samples. Addition of whey protein to samples that contained milk fat caused a decreased syneresis in all samples. The initial viscosity was greater for samples containing milk fat than for the nonfat samples and it decreased as the percentage of whey protein increased in both groups of samples. The rate at which the viscosity decreased was greater in the samples which contained milk fat. The rate at which viscosity decreased decreased in both groups of samples as the percentage of whey protein in the samples increased.

Key Words: Glucono-delta-lactone, Whey protein concentrate, Acid milk gel

76 Physicochemical properties of retort sterilized dairy beverages during storage. M. E. Cano-Ruiz, C. A. Lin*, and R. L. Richter, *Texas A&M University, College Station, TX*.

The effect of composition (nonfat dry milk, milk fat, carrageenan, and sodium tripolyphosphate), storage time, and storage temperature on the physicochemical properties of a retort sterilized dairy beverage were investigated. Drinks with eight formulations were stored at 4, 25, and 37°C for six months and analyzed monthly for pH, apparent viscosity, homogenization index, and soluble calcium. The changes in the beverages increased with storage time and temperature. The degree of change was affected by composition of the product. Sodium tripolyphosphate was implicated in promoting age gelation of samples with 11% nonfat milk solids, whereas sedimentation was observed in the absence of sodium tripolyphosphte and carrageenan. Apparent viscosity of samples was associated with the rate of age gelation and sedimentation which increased with decreased viscosity. Interactions between milk fat, carrageenan, and milk solids nonfat were important in detemining the apparent viscosity of the beverages and the rate of change observed during storage.

Key Words: Dairy beverage, Retort sterilization, Age gelation

77 The inhibition of the Maillard reaction in whey protein concentrates. S. A. Chen, *AMPC, Inc, Ames, IA*.

Whey protein concentrates containing 80 percent protein are widely utilized as nutritional and functional ingredients in various food products. The Maillard reaction, nonenzymic browning due to the interaction of proteins and reducing sugars, could give undesired off-flavor, dark color and nutrition loss in whey protein concentrates. Chemical inhibition, metal ion chelating, moisture control, oxygen scavenger, removal or oxidation of reducing sugars, or pH control could inhibit the Maillard reaction. The objective of this study was to determine the effect of different treatments on shelf life of whey protein concentrates by controlling the Maillard reaction. Addition of sulfites or vacuum packing was applied to six different whey protein products with pH of 6.5, 6.0, 5.5, 5.0, 4.5 and 4.0 at each whey products. The treated whey protein concentrates were stored at 35, 40 and 45 degree C in high and low moisture incubators for 25 days. The color change and sugar content of whey protein concentrates were monitored daily by Hunter Lab colorimeter (L, a, b color values) and HPLC during the accelerated temperature incubation. The reducing sugars were decreased and the colors were darkened with time due to the Maillard reaction. The reaction rate constants were calculated from zero-order Arrhenius plots and used to predict shelf life of whey protein concentrates at 21degree C. The results showed the lower pH and addition of sulfites significantly inhibited the Maillard reaction in whey protein concentrates.

78 Properties of emulsions consisting of whey protein-coated milkfat droplets and non-fat milk solids. M. H. Wang* and M. Rosenberg, *University of California, Davis, CA* .

Whey proteins (WP) adsorbed at O/W interfaces may enhance the oxidative stability of milkfat included in food products. For systems containing caseins (CN), minimizing displacement of WP by CN is thus critically important. The objective of our research was to develop emulsions of anhydrous milkfat (AMF) in milk solid solutions in which AMF is coated mainly by WP. Base emulsions (BE) consisting of AMF (40-50%) in WPI solution (0.5-5%) were prepared (50°C, 50 MPa). Final emulsions (FE, 20% solids non-fat) were prepared by adding dry milk powder (NFDM) or skim concentrate (CS) to BE at 50°C or after heating BE for 30 min at 90°C(HTBE). Effect of composition and heat treatment on mean particle diameter (MD), surface excess (SE) and the ratio WP/CN at the O/W interface were investigated. MD of BE was inversely related to WP concentration and ranged from 0.36 to 0.59 μm and from 0.48 to 0.78 μm at 40 and 50% AMF, respectively. SE of BE was affected by WPI and AMF concentration and ranged from 0.79 to 3.3 mg/m^2 and from 0.84 to 1.49 for 40 and 50% AMF, respectively. MD of FE prepared from BE with more than 0.5% WPI did not differ from that of the original BE. Adsorption of CN was inversely related to WPI content of the BE. The ratio WP/CN was significantly affected by composition of BE, type of milk solids, and heat treatment. The ratio WP/CN ranged from 0.182 to 0.831; from 0.171 to 0.878; from 0.132 to 0.740 and from 0.118 to 0.731 in FE prepared, with NFDM or CS, from BE with 40 and 50% AMF, respectively. WP/CN ratio ranging between 0.276 and 1.248 and between 0.200 and 1.182 were obtained for FE prepared with NFDM from HTBE at 40 and 50% AMF, respectively. Results indicated that emulsions in which proportions of WP adsorbed at the O/W interface are larger than those of CN can be prepared and the composition of the interfacially adsorbed films can be adjusted.

Key Words: Emulsions, Whey proteins, Casein

79 Microstructure of whey protein-based edible films containing candelilla wax and butter fat. S-J. Kim*, Z. Ustunol, *Michigan State University, E. Lansing*.

Like other protein-based edible films, whey protein-based films are poor moisture barriers. However, it has been demonstrated that the incorporation of lipids into the film forming solution increased hydrophobic characteristics of protein films, thus, improves their moisture barrier properties. The purpose of our study was to investigate the microstructure of whey protein-based films with and without candelilla wax (CW) or butter fat (BF) incorporated, to gain an insight to the differences in their barrier and mechanical properties. Whey protein isolate (WPI) or whey protein concentrate (WPC) (5%, w/w) and sorbitol were mixed in distilled water. The pH of the solutions were adjusted to 8 using 2 N NaOH. The mixtures were heated at 90 degree C for 15 min while being stirred continuously. CW (0.2, 0.4 or 0.8%, w/w) or BF (0.1 or 0.2%, w/w) was added during the heat treatment and allowed to melt into the protein solutions. The solutions were homogenized and vacuum was applied. The film forming solutions were cast on a teflon surface and dried at room temperature (approximately 23 degrees C) overnight at a constant relative humidity. Films produced were transparent and flexible. Microstructure of the films was determined using scanning electron microscope (SEM). Cross-sections and surfaces of the films were examined. The cross-section was obtained by crosscutting the film using a razor blade. Film pieces were mounted on aluminum stubs using double-sided cellophane tape then gold coated with gold-palladium alloy to form a 10nm thick layer. Two different magnification of 1,500x and 400x were used to examine surfaces and cross-sections of the films, respectively. SEM was conducted with accelerating voltage of 15 kV. A thicker lipid phase was observed on the surface microstructure upon increasing CW or BF content in the films. The CW phase appeared compact, while BF incorporated films showed void spaces in the lipid layer. Cross-sections of the films showed dense protein matrix of the WPI films but a rather porous structure of WPC films. Microstructure of the films accounts for the differences in their barrier and mechanical properties.

Key Words: Edible Films, Whey Protein, Microstructure

80 Tindalization of goats' milk in glass bottles. J. A. M. Mesquita, M. A. Teixeira, and S. C. C. Brandao*, *Universidade Federal de Vicosa, Brazil*.

Whole goats' milk in 500 ml glass bottles sealed with metallic caps was submitted to three tindalization conditions. In condition A bottles were immersed in boiling water (97.7°C) during 30 minutes, cooled with tap water, kept at room temperature until the next day (20 hours), then submitted to the same treatment for two more times. In condition B bottles were immersed in boiling water for 45 minutes, cooled, and then submitted to the same treatment once more in the next day. Condition C was similar to condition B, with the exception of the immersion time, which was 60 minutes. The treatments were done with and without the addition of 20.000 *Bacillus subtilis* spores per ml. Microbial, physical-chemical, acceptance, and color analysis were performed during storage of the treated bottles for up to 90 days at room temperature. Raw goats' milk presented averages of 10^6 CFU/ml for mesophilic and 10^4 CFU/ml for psicrotrophic bacteria, 100 CFU/ml for mesophilic and 3 CFU/ml for thermophilic spores. The bottles submitted to conditions A, B, and C, without the addition of *B. subtilis* spores, presented 4%, 1%, and 0 (zero)%, respectively, cumulative microbial defects during storage at room temperature for up to 90 days. The bottles with the addition of *B. subtilis* spores presented 59% and 46% cumulative defects for conditions A and B, respectively. Condition C presented no defect, with the exception of one bottle with multiple contamination including yeast, suggesting air leakage of the cap. All samples presented a strong characteristic heat treated flavor. Acceptance tests made by hedonic scale showed that milk submitted to condition C presented lower scores than conditions A and B. Storage for up to 90 days presented a waved acceptance score sequence. Treatment C also presented stronger color modification. Storage for up to 90 days increased browning of all samples.

Key Words: Goat, Milk, Tindalization

81 The influence of non-starter lactobacilli on proteolysis in Cheddar slurries and cheeses during ripening. M. R. Muehlenkamp and J. J. Warthesen*, *University of Minnesota, St. Paul*.

Non-starter microorganisms are known to dominate the microflora of Cheddar cheese during ripening The proteolytic enzymes of these organisms may play an important role in desirable or undesirable flavor development in cheese, thus contributing to quality. The objective of this research was to evaluate non-starter lactobacilli influence on peptide and amino acid development in cheese slurries ripened for 12 days and cheese ripened for 6 months. Eleven non-starter lactobacilli were screened for peptides and amino acids produced in cheese slurries made from fresh cheese curds manufactured in a 20 L vat within a laminar air-flow hood. All curds contained the starter culture SK11, with the control slurry only containing the SK11. All slurries were produced twice and incubated for twelve days at 30 C with samples taken at time 0, 3, 6, 9 and 12 days. Analyses included monitoring non-starter and starter counts and extraction of water solubles from which peptide profiles and amino acids were determined by HPLC. Based on the slurry results, three strains were chosen to be added to cheese and ripened for six months at 9 C. All cheeses were manufactured in triplicate in the laminar air-flow hood with SK11 starter. Analyses of the cheeses were the same as those for the slurries, but evaluated at times 0, 2 weeks, 1, 2, 3, 4 and 6 months. Results indicate that a cheese slurry model may be a useful tool in screening a large number of organisms for amino acid production in a cheese model system. However, differences in proteolysis development between cheese and slurries are likely because of higher moistures and temperatures in slurries that will influence enzyme activities.

Key Words: Non-starters

82 Cold solvent extraction of Cheddar cheese aroma compounds. L. M. Colchin and S. A. Rankin*, *University of Maryland, College Park*.

Our objective was to characterize the performance of a cold solvent extraction technique to recover flavor volatiles present in Cheddar cheeses. Equal parts of finely shredded cheese sample and Celite 545-AW were combined and packed into a 22 × 400 mm glass separation column. The column was eluted with double distilled diethyl ether at 4°C. The eluent was held at 4°C, allowing a majority of the neutral lipid fraction to crystallize out. The liquid fraction was decanted and the crystalline portion washed with additional diethyl ether. Solvent fractions were combined, dried over anhydrous sodium sulfate and condensed with a Kuderna-Danish concentrator to 200 μl. Chromatography was conducted on a Hewlett-Packard 5890 GC with FID and MS detectors using a polyethylene glycol column. Compounds were identified based on correlation of retention indices and mass spectra to those of known standards. The extract possessed an aroma characteristic of whole Cheddar cheese. Major compounds identified included diacetyl, acetic acid, acetoin, butanoic acid, 2,3-butanediol, hexanoic acid, BHT, octanoic acid, 2,4-dimethylheptane, benzoic acid, dodecanoic acid, and 3-methyl-3-hexanol. This method represents a relatively simple method for the recovery of compounds of both high and intermediate volatility considered as important in Cheddar aroma.

Key Words: Cheddar, Aroma, Chromatography

83 Study of the mineral distribution in liquid concentrated dairy-based infant formulas. L. P. DesMarchais*, M. Pouliot, and Y. Pouliot, *STELA Dairy Research Centre, Université Laval, Québec, Canada.*

Minerals are among the most important elements in the liquid concentrated dairy-based infant formulas. It has been suggested that mineral balance plays an important role in the physico-chemical stability of this product. The objective of our work was to study the state of salt distribution and its effect on physico-chemical properties of these formulas (pH, viscosity, protein load of the emulsion) as affected by added salts or alkali. Formulas were prepared following a conventional industrial process. The salts used were Na_2SO_4 and K_2SO_4 and the alkali were NaOH and KOH. The additions corresponded at 0, 2 and 4mM of cations. The conditions were applied according to a complete factorial design repeated 3 times. The concentrated dairy-based soluble fractions were obtained by centrifugation on 500kD ultra-filtration membranes (Centricon®, Amicon Canada). The permeates were analyzed by capillary electrophoresis in order to quantify the soluble content of potassium, sodium, calcium and magnesium. Atomic absorption (flame photometry) was used to determine the total minerals in the dairy-based formulas. The results were expressed as percentage of soluble minerals on total content. It was found that 85% of the potassium was in the soluble phase. Calcium was soluble at 17%, magnesium near 65% and sodium a little higher than 95%. The addition of the sodium ion (salt or alkali form) in the dairy-based formulas involved a decrease of the other soluble minerals. The pH increase was significant ($p<0.0001$) and linear when NaOH and KOH were added. The pH increase was not significant when K_2SO_4 or Na_2SO_4 were added. The ion (Na or K) did not show significant effect on the properties of dairy-based formula but the quantity added (0mM, 2mM or 4mM) combined with the kind of adding (salt or alkali) had a significant effect ($p<0.0001$). The viscosity was not influenced by the nature of the cation (Na or K). The results from this study revealed slight but consistent changes in mineral distribution, which in turn, had an apparent impact of product's physico-chemical properties.

Key Words: Infant Formula, Minerals, Properties

84 Proteolytic activity in yogurt cultures producing different size exopolysaccharides during fermentation and storage. P. Thangpong and K.A. Schmidt*, *Kansas State University, Manhattan.*

In the dairy industry, EPS-producing starter cultures have been advocated because of their effects on physical properties of yogurt-primarily increasing the viscosity. Little is known about the different enzymatic processes of these starter cultures. The objective of this research was to investigate the proteolytic behavior of yogurt cultures with varying sizes of EPS (0–4μ). Sterilized skim milk was inoculated with 2% of a single strain culture and incubated at 42°C until a pH of 4.5 was achieved. After reaching pH 4.5, samples were refrigerated (4°C) and stored. During fermentation, results showed both species were proteolytic, with the lactobacilli being more proteolytic than the streptococci strains. Within a species, as EPS size increased, proteolytic activity was higher for both the *Streptococcus thermophilus* and *Lactobacillus bulgaricus* organisms. As expected, microbial counts and titratable acidity increased during fermentation and during 7 days of refrigerated storage; whereas the pH decreased during this time frame. Implications of this research suggest that these microbes differ not only in the presence of EPS character, but perhaps also in metabolic needs.

Key Words: EPS-producing Culture, Proteolysis

85 A new method for measuring Mozzarella cheese stretchability. J-H. Kim*, K. A. Schmidt, and I. J. Jeon, *Kansas State University, Manhattan, Kansas.*

Melting and stretching characteristics cheese are very important in Mozzarella cheese because it is mostly consumed in a melted state on pizza and other foods. However, helical viscometry, uniaxial horizontal extension, vertical extension, squeezing flow method, or simple fork test are time-consuming, require complicated preparation and are not always reproducible. A new method for measuring Mozzarella cheese stretchability monitoring its direct stretching characteristics was developed using a Texture Analyzer TA.XT2 and a new test cell. Young Mozzarella cheese samples were purchased from three different manufacturers and stored at 7°C. Comparisons of the new stretchability method, helical viscosity, firmness, free oil content, and electrophoresis testing were performed during 10 wk of storage. Data showed that elongation length varied by sample and storage time and as such were inconclusive in monitoring the stretching quality. However, the initial force to lift and toughness decreased over storage for all cheese samples. Initially, cheeses with higher fat contents had longer elongation lengths which decreased over time. These results can be explained by the protein:fat ratio and protein degradation. The viscosity of the melted cheese and firmness of the samples at room and refrigerated temperatures decreased over time for all cheese samples. Free oil content varied among the samples. The new stretchability test could distinguish properties of each product as affected by its initial composition.

Key Words: Mozzarella Cheese, Stretching, Electrophoresis

86 Viscoelastic properties of ice cream mixes made with various fat contents. S. Adapa and K. A. Schmidt*, *Kansas State University, Manhattan.*

Ice cream mixes with fat levels of 12%, 8%, 6%, 6% + a protein based fat replacer, and 6% + a carbohydrate based fat replacer were tested for viscoelastic properties by dynamic testing involving sinusoidal oscillatory tests at various frequencies. The energy stored (storage modulus, G'), the energy dissipated (loss modulus, G''), and tangent of the loss angle (tan δ, G''/G') were calculated in all the treatments of ice cream mixes to determine both elastic and viscous properties. In all the treatments, G' and G'' exhibited a strong frequency dependence. The G'' was higher than G' throughout the frequency range (1–8 Hz) examined, without any crossover. The tan δ values were constant throughout the frequency range within each treatment. When comparing data of ice cream mixes at 4 Hz, elastic properties of the ice cream mixes decreased as fat content decreased. The presence of fat replacers did not enhance the elastic properties of the ice cream when comparing to ice cream mix at the equivalent fat content.

Key Words: Viscoelastic Properties, Ice Cream

87 The environmental scanning electron microscope (ESEM): a new tool for dairy product research. R. I. Thompson*[1] and J. U. McGregor[2], [1] *California Milk Producers,* [2] *LAES, LSU Agricultural Center, Baton Rouge.*

Various dairy products were examined using an environmental scanning electron microscope. Samples were mounted on aluminum stubs with double stick tape; no other preparation was necessary. The samples were examined at 250x magnification at 5 torr, 20 kV using an Electroscan Environmental SEM. Milk powder samples performed very well, although magnification above 500x caused some burning of the samples. When compared to samples run on an SEM at the same magnification the images from the ESEM are as clear and sharp as those from the SEM. However, the real advantage of the ESEM is the capability to look at samples under dynamic conditions. Milk powder was looked at after a drop of water was applied (either in the chamber or outside the chamber) and after the milk powder had been subjected to a high humidity environment. These treatments were not possible on the SEM. Process cheese and yogurt were mounted on aluminum stubs and examined on the ESEM with no preparation. The images for these two products showed differences in microstructure. Butter and Cheddar cheese were also examined but images were not clear or there was no image at all. ESEM has the potential for use as a quick method to examine the microstructure of some dairy products under dynamic conditions. Also, with the use of high humidity environments in the chamber it is possible to view the hydration of milk powders.

Key Words: Milk Powder, Environmental Scanning Electron Microscope, Microstructure

88 The effect of dairy ingredient formulations on the quality of fresh blended iced espresso beverages. C. A. Boeneke* and J. U. McGregor, *Dairy Science Department, LAES, LSU Agricultural Center, Baton Rouge.*

A study was performed to determine the role of dairy product formulations on the quality of fresh blended iced espresso beverages. Three soft serve ice cream mixes varying in sweetener type were manufactured using 100% sucrose, 50:50 blend of corn sweetener and sucrose, and 100% corn sweetener. The three mixes were used as ingredients in the manufacture of dairy based espresso beverages. Soft serve mixes were combined with fresh espresso plus ice and blended. Soft serve mixes and blended beverages were analyzed for color, separation, composition, pH, and viscosity. Sensory analysis was carried out by trained and consumer panels. The coffee beverages made using soft serve ice cream mix sweetened solely with sucrose were significantly sweeter and preferred by consumers. Fresh blended iced espresso beverages manufactured using soft serve ice cream mixes sweetened with sucrose resulted in less foaming and lower viscosity.

Key Words: Sweetener, Espresso, Coffee

89 Effect of fat content and whey protein fortification on milk frothing properties. M. I. Levy* and J. U. McGregor, *LAES, LSU Agricultural Center, Baton Rouge.*

Skim (.39%), lowfat (1.69%), and whole (3.20%) milk with and without added WPC (1% fortification) were tested for frothing properties. For each sample, 200 grams of milk were frothed with a Faema Expresso machine (model c85/1) using a 7.5-cm diameter cylinder for 25 seconds. Froth characteristics were observed and initial height of froth, height of froth after 4 minutes of dissipation, froth stability, and air cell size were recorded. Skim milk had a mean froth height of 3.23 cm. Skim milk with 1% WPC had a mean froth height of 3.15 cm. Lowfat milk had a mean froth height of 2.40 cm. Lowfat milk with 1% WPC had a mean froth height of 3.27 cm. Whole milk had a mean froth height of 2.72 cm. Whole milk with 1% WPC had a mean froth height of 3.20 cm. The froth of milk fortified with 1% WPC remained more stable over time, and gave a "creamier / heavier" froth at all fat levels.

Key Words: Cappuccino, Whey Protein, Milk Froth

90 The water vapor barrier properties of whey protein-fatty acid emulsion films. C. P. Sherwin, D. E. Smith, and R. G. Fulcher, *University of Minnesota.*

An edible film is a thin layer of material which can serve as a barrier to moisture, oxygen, aromas, and other physical transfers when applied as an external coating or as a continuous layer between food components. Previous research on edible films did not focus on microstructure to explain the functional properties. Whey protein (WP) edible films have been found to be poor moisture barriers, but can be improved with the incorporation of lipid. Experiments were designed to test the hypothesis that the water vapor barrier properties of WP edible films can be enhanced by the incorporation of lipid using microfluidization technology and are affected by fatty acid type and environmental conditions.
In preliminary experiments effects of formulation, homogenization, casting surface, and drying conditions were determined in the preparation of a viable film of WP and fatty acids (FA). To study the effect of FA chain length six formulations were prepared that contained myristic, palmitic, stearic, arachidic, behenic, or no FA.
Water vapor permeability (WVP) was measured by the ASTM E96-80 method. Glass cups containing a saturated salt solution and sealed with the test film were placed in a controlled humidity chamber. Permeant flux was measured as the weight gain of the saturated salt solution over time, and was used to calculate WVP. Films were tested at 53:11, 83:57, and 94:83% relative humidity (RH) gradients. WVP increased as chain length increased at 53:11, but no statistical differences were found at the higher gradients. Additionally, the films were poor barriers at the higher gradients. WP edible films had not previously been examined at these real-world model gradients. Dispersed phase particle sizes of the films were measured by polarized light microscopy and digital image analysis. Particle size increased as chain length increased. Quantitative microscopy is an improved technique for particle size determination that has not previously been employed on an edible film system. Mathematical models were developed to describe the mode of inhibition that dispersed lipid particles impart on the hydrophilic continuous phase matrix. Unique combinations of permeant path extenuation and phase interactions were found to govern the inhibition for each formulation.

91 Comparative study of the effect of storage on some properties of commercially produced milk powders. C. M. Kim* and N. Y. Farkye, *Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo*.

Functional properties of whole milk or skim milk powders are important determinants of their use as food ingredients. Powders are frequently stored for several months under non-ideal conditions before being used. When the powder is exported to foreign countries, storage at high temperatures and high relative humidity are commonplace. Conditions as such induce property changes (oxidation, pH, solubility, water activity, wettability, viscosity, flowability, lactose crystallization, etc.) which are critical factors controlling the functionality and value of the powders. The purpose of this study is to identify changes that occur in milk powders subjected to harsh conditions for an extended period of time with the focus on the effect of liner type on the change in properties. Several lots of bags of powders (three whole milk powder and eight skim milk powder) were obtained from various manufacturers with each lot consisting of fifteen 25-kg bags. Two to three bags were taken from each lot and sub-sampled into 5-kg portions and sealed under inert condition in either three or four millimeter thick polyethylene liners (cap-sac method). Same number of samples were also prepared with 3 mm liners and stored in tin cans. Samples were stored under "tropical condition" (37°C at 90% relative humidity). Also, comparisons are made between powders stored in "tropical" conditions with those stored in ambient environment. Initial results showed percent moisture level ranged from 1.6 to 3.4% and water activity levels were 0.17 to 0.25. Results also showed that storage and package material influenced milk powder stability.

Key Words: Milk Powder, Storage, Physico-Chemical Properties

92 Influence of pH on flow/meltability of process cheese. M. Sutheerawattananonda* and E. D. Bastian, *University of Minnesota, St. Paul*.

A Rheometrics dynamic stress rheometer (DSR) using 25 mm parallel serrated plate geometry and Schreiber's melt test were used to study meltability of process Cheddar cheese containing disodium phosphate (DSP) or trisodium citrate (TSC) with pH from 5.2 to 5.8. Transition temperature at which tan δ equals 1 decreased as the pH increased. After the transition temperature, tan δ also increased with pH, suggesting that process cheese with high pH would flow and spread better than one with low pH. DSP process cheese at pH 5.2 did not show a transition temperature within the experimental range. DSP process cheese had lower tan δ than TSC process cheese. Tan δ and melting index from Schreiber's test did not correlate. DSP process cheese at pH 5.2 with less emulsified fat did not melt. Scanning electron micrographs did not suggest any differences in size and shape of fat particles. The concentration of calcium in aqueous phase of TSC process cheese was twice that of DSP cheese and increased with decreasing pH.

Key Words: Process Cheese, Meltability, Dynamic Stress Rheometry

93 The use of adjunct cultures and elevated ripening temperatures in reduced fat Edam cheese manufacture. J. D. Woodill* and C. H. White, *Mississippi State University, Mississippi State*.

The objectives of this research were to compare the effects of various adjunct cultures, and an elevated ripening temperature on the flavor and body and texture of reduced fat Edam cheese. Bacteria were isolated from high quality aged Edam cheese. The isolated bacteria, along with other cultures, were evaluated in a slurry system by a descriptive panel (8-10). Bacteria to be used as adjunct cultures were selected based on their performance in the slurry system. Make procedure modifications were implemented in an attempt to increase the moisture content of the reduced fat cheese. After pressing, half of each treatment was stored at 7°C throughout the experiment. The other half of each treatment was stored at 12°C for 1 mo and then at 7°C for the duration of the experiment. An expert panel (8) was used to evaluate the cheese at 2, 4, and 6 mo for flavor and body and texture. A descriptive panel (8-10) and a consumer panel (>50) evaluated selected cheeses at 5 mo. The 2 mo evaluations indicated that the major flavor criticism was flat. As ripening continued, more typical aged flavor descriptors were observed from the results of the expert panel. Cheese with added *Lactococcus lactis* subsp. *lactis* biovar *diacetilactis* consistently had higher flavor scores. The highest scoring treatment at 4 mo was the *L. lactis* subsp. *lactis* biovar *diacetilactis* treatment initially ripened at 12°C. At 6 mo, the highest scoring treatment was *L. lactis* subsp. *lactis* biovar *diacetilactis* ripened at 7°C for the entire study.

Key Words: Reduced Fat Edam Cheese, Adjunct Cultures, Elevated Ripening Temperatures

94 Evaluation of enzyme activity and autolytic properties of adjunct lactobacilli subjected to different attenuation methods. M. El-Soda*, S. A. Madkor, and P. S. Tong, *Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo, CA*.

In recent years, means were developed at both the farm and plant levels to produce milk and cheese with very high sanitary standards. However, this can lead to a reduction in the number of desirable non starter lactic acid bacteria in the cheese. One approach to produce cheese with high organoleptic properties in a reasonable time is using attenuated adjunct cultures. The present work is to evaluate three methods of cell attenuation: freeze shocking, heat shocking and spray drying on the performance of two cultures of adjunct lactobacilli (*L. casei* and *L. helveticus*). The evaluation was based on the following criteria: peptidase and esterase activities as well as the autolytic properties of the attenuated cells in buffer and cheese slurry system. Water soluble nitrogen and free amino acids formation in the Cheddar cheese slurry system were also determined. The results indicate that the highest rate of autolysis was in the following order; freeze shocked cells > untreated cells > heat shocked cells > spray dried cells. Freeze shocking had little or no effect on the intracellular peptidase and esterase activities of the cells. Heat-shocking gave the highest levels of reduction in enzyme activity (∼40-55% reduction in aminopeptidase activity). With heat-shocking reduction in aminopeptidase activity was significantly higher when compared to dipeptidylaminopeptidase or esterase activities. Autolysis and enzyme activities in the cheese slurry containing $\sim 10^7$CFU/gram and incubated at 30°C for 5 days under anaerobic condition showed a similar trend to the buffer system. With both lactobacillus strains, aminopeptidase and esterase activity in freeze-shocked treated slurries were about 80 and 35% higher than that with other treatments. A concomitant increase in the levels of water soluble nitrogen (∼22%) and free amino acids (∼35%) were also obtained in the slurries treated freeze shocked cells compared to that in heat shocked or spray dried cells treated slurries.

Key Words: Adjunct Cultures, Attenuation, Cheese Slurry

95 Proteolysis during ripening of reduced-fat Cheddar cheese. E. Medrano*, L.-B. Zhang, and N. Y. Farkye, *Dairy Products Technology Center, California Polytechnic State University*.

Lower-fat Cheddar cheese containing about 50%-fat reduction was manufactured from milk standardized by A) fortification of whole milk with low-heat nonfat dry milk (NDM) or B) addition of skimmilk to whole milk. The casein to fat ratios in the cheese milks ranged from 1.98 to 2.43. Eight trials were made using 156 kg of milk each for standardization method A, and four trials for method B. Starter was *Lc. lactis* ssp. *lactis* SCO252 (Chr. Hansen's, Inc). Rennet (Chy-max^{TM}II) was used at the rate of 45 ml per 454 kg milk. Cheeses were ripened at 2.2–4.4°C for up to 30 weeks. Samples were taken periodically for determination of proteolysis by gel electrophoresis and water-soluble N. HPLC profiles of the WSN extract were determined also. Generally, levels of proteolysis were lower in cheeses manufactured from milk standardized by method A than those made from milk standardized by method B. For example, %WSN were 7.78, 11.18 and 12.54%, after 8, 14 and 18 weeks of ripening, respectively, for cheese made from milk standardized by method A compared to 16.70, 24.32 and 26.18, during the same period for cheese made from milk standardized by method B. Differences were noted also in the electrophoretic patterns of the cheeses and HPLC profiles of the WSN extract.

Key Words: Proteolysis, Reduced-fat Cheddar Cheese

96 Effect of mode of standardization on the composition and yield of reduced-fat Cheddar cheese. E. Medrano*, L.-B. Zhang, and N. Y. Farkye, *Dairy Products Technology Center, California Polytechnic State University*.

Lower-fat Cheddar cheese containing about 50%-fat reduction was manufactured from milk standardized by A) fortification of whole milk with low-heat nonfat dry milk (NDM) or B) addition of skimmilk to whole milk. The casein to fat ratios in the cheese milks ranged from 1.98 to 2.43. Eight trials were made using 156 kg of milk each for standardization method A, and four trials for method B. Starter was *Lc. lactis* ssp. *lactis* SCO252 (Chr. Hansen's, Inc). Rennet (Chy-max^{TM}II) was used at the rate of 45 ml per 454 kg milk. Average cheese yields using method A was 17.9%. Average yields using method B was 8.8%. Mean composition of the cheeses from milk standardized by fortification with NDM were: protein, 27.25%; fat, 13.85%; FDM, 25.22%; MNFS, 52.55 %; S/M, 3.94%; pH, 5.20 (day 1). Mean composition of the cheeses from milk standardized by addition of skimmilk to whole milk were: protein, 29.93%; fat, 11.55%; FDM, 22.42%; MNFS, 54.80%; S/M, 3.79%; pH, 5.05 (day 1). Cheese made from milk standardized by method A contained higher lactose levels than that made from milk standardized by method B. Lactose concentrations in the cheeses made from milk (method A) were 58.64, 53.41 and 52.35 mg/g in dry matter, respectively, after 1, 3 and 6 months of storage compared to 5.10, 4.82, and 3.87 mg/g in dry matter for cheeses made from milk standardized by method B.

Key Words: Reduced-fat Cheddar Cheese, Standardization, Yield

97 Comparative study of milk standardization methods for milk used to manufacture 50% reduced-fat Cheddar cheese. C. M. Chen*, A. L. Dikkeboom, J. J. Jaeggi, M. E. Johnson, W. A. Tricomi, and M. G. Zimbric, *Wisconsin Center for Dairy Research, Madison*.

Milk used for the manufacture of 50% reduced-fat Cheddar cheese was standardized to a casein:fat ratio of 1.70 and 10% solids. Water was added to obtain the desired solids level. In the first experiment, whole milk was standardized with reconstituted NDM (20% solids) and skim milk: 1)whole milk + reconstituted NDM (rNDM), 2)whole milk + 12.5% decrease in reconstituted NDM (−12.5% rNDM) + skim milk, 3)and whole milk + 25% decrease in reconstituted NDM (−25% rNDM) + skim milk. In the second experiment, whole milk was standardized with condensed skim (40% solids)and skim milk and cream when necessary: 1)whole milk + condensed skim (CS), 2)whole milk + 12.5% decrease in condensed skim (−12.5% CS) + skim, 3)and whole milk + 12.5% increase in condensed skim (+12.5% CS) + cream. The control milk was standardized by cream removal. Milk coagula setting times were altered to control cheese moisture levels since preliminary trials indicated higher cheese moisture in experimental vats. The percentage of fat recovered in the cheese was significantly lower for all experimental treatments (range 85.8 − 86.9%) as compared to the control (88.3%). Standardization of whole milk with CS did not affect the percentage of nitrogen recovered in the cheese. However, when milk was standardized with reconstituted NDM the percentage of nitrogen recovered increased: 74.9 (control), 75.5 (−25% rNDM), 75.9 (−12.5% rNDM), and 76.1 (rNDM). Adjusted cheese yields were lower for all experimental treatments. Proteolysis in the cheese was assessed by 12% TCA sol N at 2, 6, 12, and 24 weeks of aging. Experimental cheeses manufactured with rNDM, −12.5% rNDM, and −25% rNDM resulted in 10−20% less 12% TCA sol N at all sampling points. Standardization of milk with condensed skim did not affect the levels of 12% TCA sol N. Cheeses standardized with rNDM had less cheddar flavor and were firmer than the control, −25% rNDM and −12.5% rNDM cheeses. Standardization of milk with condensed skim did not affect the flavor and texture.

98 Acid adaptation in pathogenic bacteria. M. A. Harrison*, *University of Georgia, Athens*.

At times, bacterial foodborne pathogens are able to cope with stressful environmental conditions. There is evidence that many species of bacteria are able to respond and as a result increase the likelihood of their surviving acidic conditions which would typically be considered lethal to the microorganism. This is a concern when one considers that this might result in a microbe with an increased chance for survival in the gastrointestinal tract, food contact surfaces subjected to acidic conditions or in fermented or acidified foods. There have been incidences of foodborne illness related to foods which are typically considered to have sufficient acidity to restrict their growth or to eliminate them from the food (e.g., apple cider, fermented dairy products, salami). Survival mechanisms known to occur in some pathogenic bacteria include pH homeostasis, acid resistance in the enterics, acid shock response, and inducible acid tolerance responses (ATR). ATR is an adaptation response in which a bacterium is able to survive low pH if initially adapted at a relatively mild pH. This response can be grouped into either log phase or stationary phase acid tolerance responses. Bacterial foodborne pathogens which have been shown to have this ability to some degree include *Salmonella typhimurium, Listeria monocytogenes, Escherichia coli* O157:H7, and *Aeromonas hydrophila*. This presentation will consider the mechanism of acid adaptation in foodborne pathogens and the role this phenomenon may play in food safety.

Key Words: Acid Adaptation, Acid Tolerance Response, Foodborne Pathogens

99 New methods for sanitation control. M. W. Griffiths, *Department of Food Science, University of Guelph, Guelph, Ontario, Canada*.

The importance of good sanitation is irrefutable but how can the efficacy of sanitation procedures be monitored in a meaningful way. Culturing of surface swabs or rinse samples provides historical information only and does not allow plant operators to be proactive. The development of ATP bioluminescence-based kits that provide near real-time results has revolutionized the way that sanitation is monitored. Their ease-of-use, and rapidity make them convenient tools for use by the sanitation crew. However, like all analytical methods ATP bioluminescence has its limitations. As it relies on an enzymatic reaction, results are influenced by factors that inhibit the reaction, but commercial kits are designed to minimize these effects. The way in which the results are interpreted is also important for the successful implementation of this technology. Whatever technique is used to monitor sanitation the deficiencies in sampling protocols must also be recognized. Are there ways to overcome these problems? The issues involved in testing the effectiveness of sanitation regimes will be addressed in this presentation.

Key Words: ATP Bioluminescence, Sanitation

100 New methods for the detection of pathogens in dairy products. R. S. Flowers, *Silliker Laboratories Group, Inc*.

In the mid 1980's enzyme-linked-immunosorbent assays (ELISA's) and DNA probe based methods became commercially available for detection of pathogens; e.g. *Salmonella* and *Listeria*, in dairy products and other foods. These methods allow detection of pathogens within 48 h compared to 4–5 days required for detection by traditional culture methods. Several of these methods have been validated and approved by AOAC, and are widely used by both industry and government laboratories for routine analysis. Recently, second and third generation ELISA and Nucleic acid probe methods have been developed that incorporate immunocapture and/or DNA/RNA amplification techniques. In addition, phage based assays and methods incorporating combinations of technologies have been developed, e.g. time-released selective agents, allowing detection of most pathogens within 16–24 h. Currently, all of these rapid methods are screening tests providing rapid negative results, but requiring traditional isolation and identification of isolates for confirmation of positive assays. However, in the future, use of multiple immuno-assays with different reaction sites and/or specific nucleic acid probes may allow for rapid confirmation. Some rapid methods can be performed in most laboratories; but many of the next generation methods require a significant investment in equipment and expertise making them practical only for larger volume laboratories.

Key Words: Rapid Methods, Microbiological Methods

101 Recovery of sublethally injured pathogens. C. W. Donnelly*, *University of Vermont, Burlington*.

Detection of bacterial pathogens in dairy products is often limited by the performance of enrichment media used to support the growth of bacteria to delectable levels. Pathogens such as *Listeria*, *Salmonella*, *E. coli*, and *Staphylococcus* may exist in an injured state in foods or food processing environments as a result of exposure to processing treatments such as heating, freezing, exposure to acids or exposure to sanitizing compounds. The presence of selective agents in enrichment media normally used for recovery of pathogens such as *Listeria* may inhibit the repair and ultimately detection of sublethally injured *Listeria*, which under ideal conditions in foods may go on to repair, grow and regain pathogenicity. We have worked to improve the sensitivity of current detection systems by recognizing the fact that *Listeria* and other pathogens may exist in an injured state in food products and food processing environments, and our work has therefore resulted in development of a new enrichment media, *Listeria* Repair Broth (LRB), which is capable of resuscitating injured *Listeria*. In this presentation, summary data will be provided to show the efficacy of LRB, which results in increased sensitivity of detection in the 97.5–98.8% range, compared to standard regulatory procedures which are only 65–70% sensitive. For recovery of acid-injured *Listeria*, parameters involved in the survival of acid-injured *Listeria* include initial percent injury, storage temperature and the pH of the food. In storage studies which compared standard selective enrichment against use of LRB allowing a four hour repair period prior to the addition of selective agents, LRB was superior (21/54) in its ability to detect injured *Listeria* in apple cider, yogurt, coleslaw and salsa, when compared to UVM (3/54), the medium used in the USDA-FSIS procedure. Similar enrichment strategies, designed to improve detection of injured *E. coli* 0157:H7 in dairy foods, will also be discussed.

Key Words: *Listeria*, Injury, Pathogens

102 Bacteriocins in dairy products: Possibilities and reality. A. E. Yousef, *The Ohio State University, Columbus*.

Some lactic acid bacteria (LAB) produce bacteriocins with powerful inhibitory action against pathogens of concern to the dairy industry. Pediocins, produced by Pediococci, and lantibiotics of Lactococci are potentially useful food additives that may be implemented to control foodborne pathogens such as *Listeria monocytogenes* and *Clostridium botulinum*.

Despite the antimicrobial properties of bacteriocins, there are several issues that should be considered when applying these agents in food processing. Bacteriocins are less effective in food than laboratory media and thus may only be used as a hurdle in a combination processing. The synergy with other food preservation measures makes application of bacteriocins in food processing more feasible. For example, treatments to strip outer membranes of some Gram-negative bacteria make them susceptible to LAB bacteriocins. Novel technologies such as Pulsed Electric Fileds and High Pressure Processing affect the integrity of cell envelop and thus increase susceptibility of some pathogens to bacteriocins. Bacteriocins may bind to some food ingredients and thus become ineffective particularly when used in complex foods. Therefore, carriers that protect bacteriocin against inactivation or binding by food components should be sought.

Because of regulatory constraints in the USA, it is currently more feasible to use bacteriocin-producing strains than purified bacteriocins in food processing. Such bacteriocin-producing strains should be present naturally or have been used traditionally in fermented foods. Production and handling of LAB and their bacteriocins should be subject to food-grade processes. After all these factors considered, application of bacteriocin or producing strains in food preservation should be economically feasible.

Key Words: Bacteriocins, Pathogens, Feasibility

103 An elective whey based medium for the differential enumeration of *Lactobacillus delbrueckii* subspecies *bulgarius* and *Streptococcus salivarius* subspecies *thermophilius* in yogurt. S. A. Ibrahim*[1] and M. I. Yamani[2], [1]*University of Minnesota, St. Paul,* [2] *University of Jordan, Amman, Jordan.*

A whey based medium for the differential enumeration of *Lactobacillus delbrueckii* subspecies *bulgarius* and *Streptococcus salivarius* subspecies *thermophilius* was developed. The efficacy of the whey medium was compared to MRS Agar and M 17 agar using commercial yogurt cultures. Bromocresol green whey agar (BGWA) was prepared by mixing one part of sterile agar solution (115 C/15 min) containing 3% yeast extract, 1.2% K_2HPO_4, 0.004% bromocresol green and 4% agar with two parts of whey. Morphology of *L. bulgarius* colonies in BGWA pour plates were light in color and formed an iregular mass with twiced filament projections, while the morphology of *S. thermophilius* colonies were green lenticular with smooth edges. BGWA performed generally better than MRS and M 17 agar when cultures were enumerated in commercial yogurt samples. BGWA performed significantly better than MRS agar in seven (33.3%) cultures of *L. bulgarius* and better than M 17 agar in 14 (66.6%) cultures of *S. thermophilius*. Wider ranges were noted in the counts of MRS and M 17 agar than those of BGWA.

Key Words: Yogurt, Whey Based Medium

104 Characterization of flavor components produced during Kefir storage. Z. Güzel-Seydim[1]*, A. C. Seydim[2], A. K. Greene[1], and P. L. Dawson[2], [1]*Clemson University, Department of Animal and Veterinary Sciences, Clemson, SC 29634* [2]*Clemson University, Department of Food Science, Clemson, SC 29634.*

Kefir is an exotic fermented milk beverage produced by culturing a mixture of lactic acid bacteria and yeasts in milk. Kefir was prepared by inoculating 5 g kefir grains into 800 ml pasteurized whole milk and incubated at 25 ± 1°C. Incubation was completed upon reaching the desired pH of 4.5 − within 22 to 24 hrs incubation. After incubation, samples were sieved to sterile jars and stored at 4°C. After 1, 7, 14 and 21 days of storage, the pH, organic acid and volatile flavor component content were determined to monitor possible flavor changes during storage. Previous studies have been conducted in this laboratory to determine these parameters during fermentation. Stored samples were analyzed for organic acid (orotic, citric, pyruvic, lactic, uric, acetic, propionic, butyric and hippuric) content by HPLC with UV detection at 275 nm. Acetoin, ethanol, acetaldehyde and diacetyl production were monitored using gas chromatography equipped with headspace autosampler. Lactic acid concentration increased during storage reaching a maximum of 7738.96 ppm by 21 d. Orotic and citric acids slightly increased during storage. Although pyruvic and hippuric acids were produced during fermentation, neither was detected during storage. Acetic, propionic and butyric acids were not detected during kefir production or storage. Ethanol concentrations increased during storage and reached 0.8 % by 21 d. The amounts of acetaldehyde and acetoin, common flavor substances in many cultured dairy products, increased during fermentation. The amount of acetaldehyde was increased by 51.35% by Day 21 of storage as compared to Day 1. During storage, the concentration of acetoin decreased from 25.2 ppm (Day 1) to 16.1 ppm (Day 21). However, diacetyl, another common flavor component in cultured dairy products, was not detected during fermentation or storage. At Day 21 of storage, the average pH of samples reached to 4.4.

Key Words: Kefir, Organic Acids, Fermentation

105 Influence of incubation temperatures and starter culture strains on yogurt quality. Z. Güzel-Seydim*[1] and E. Sezgin[2], [1]*Clemson University, Clemson, SC* [2]*Ankara University, Ankara, Turkey.*

Different strains of yogurt starter cultures and different incubation temperatures were investigated in the production of plain, unflavored yogurt. Milk samples were inoculated with 2% CH-1 (non-filant type) and B-3 (filant type) (Chr. Hansen), and then incubated at 35°C and 45°C until the pH reached 4.6. Total solids, fat content, pH, viscosity, consistency, whey separation, organoleptic evaluation and content of lactic acid, acetaldehyde, volatile fatty acids and tyrosine were measured as quality criteria on the yogurt samples at Day 1 and Day 14 of storage. Lactic acid content and pH values of samples were more significantly affected by starter culture strain than by incubation temperature ($p<0.01$). Among these treatments, use of different culture strains affected tyrosine and acetaldehyde contents of yogurts. Yogurt samples made from milk inoculated with CH-1 culture and incubated at 35°C had the highest acetaldehyde content. The differences in volatile fatty acid content of yogurt samples made by different methods were statistically insignificant ($p>0.01$). Rheological properties of yogurts were affected by using different incubation temperatures and different cultures. Consistency and viscosity of yogurts increased when made from milk inoculated with B-3 and incubated at 35°C; whereas, whey separation was decreased more with B-3 yogurt produced at 35°C than with yogurt made with CH-1 at the same incubation temperature. During the 14 d storage, the rheological properties of all yogurts were scored higher, and the content of lactic acid, volatile fatty acids, and tyrosine increased while pH values and amount of acetaldehyde were decreased. In the organoleptic evaluation, at all days of storage, the scores for consistency of yogurts made with B-3 culture were higher than the scores for yogurt made with CH-1. In contrast, the flavor scores were higher in yogurts made with CH-1 than for samples made with B-3 over all days of storage.

Key Words: Yogurt, Incubation Temperature, Filant Type

106 Effect of added freeze-shocked adjunct lactobacilli on ripening indices and flavor development in Cheddar cheese. S. A. Madkor*, P. S. Tong, and M. El-Soda, *Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

Adjunct cultures of lactobacilli are used to improve and accelerate flavor development during cheese ripening. These adjuncts are thought to provide enzymes that promote proteolysis and lipolysis in cheese. The objective of this study was to compare the effectiveness of selected strains of adjunct lactobacilli attenuated by freeze-shocking at -20°C for 48h on the chemical and sensory attributes during ripening of Cheddar cheese. The Cheddar cheese curds were made using lactococcus starter and traditional milled curd cheese-making procedure. After milling the curds were inoculated with no (control) or one of six freeze-shocked adjunct cultures ($\sim 10^7$cfu/gram) of lactobacilli (4 strains of *L. helveticus* and 2 strains of *L. casei*). Proteolysis as measured by water-soluble nitrogen was higher mainly for *L. helveticus* than *L. casei*. All the adjunct-treated cheeses showed higher levels of free amino acids, as well as more protein breakdown in polyacrylamide gel electrophoretograms when compared to control. Lipolysis as measured by total free fatty acids were consistently highest in adjunct-treated cheeses throughout ripening period (0-6 months). Incorporation of adjunct lactobacilli in Cheddar cheese curd positively influenced the flavor intensity and prevented bitterness throughout the entire ripening period. Taste panel indicated that adjunct-treated cheese rapidly developed a typical flavor and highest flavor intensity after 3 months of ripening compared to control cheese. The present findings are consistent with the results of previous studies of the selected lactobacillus strains in buffer and cheese slurry systems. The capability of selected strains of adjunct lactobacilli to enhance ripening and flavor intensity in cheese might be linked to the action of enzymes released by added freeze-shocked cells that lyse during aging. Further cheese making trials with the adjunct cultures subjected to different attenuation treatment are in progress.

Key Words: Lactobacilus Strains, Cheese Ripening

107 Manufacture and characterization of low-fat dairy spreads prepared with diatomaceous adsorbent-treated creams. I. Echeverry*[1] and R. Jiménez-Flores[2], [1] *University of Illinois, Urbana-Champaign* [2] *California Polytechnic State University, San Luis Obispo.*

Dairy creams were treated with diatomaceous adsorbents to explore the possibility of inducing changes in their functional properties. The treated creams were used to manufacture low-fat dairy spreads and their textural properties evaluated and compared to those of control and commercial spreads. Dairy creams of 32% fat content were subjected to an adsorption treatment with three different types of diatomaceous silicates. The raw standardized cream was divided into six samples (five treatments plus a control). The level of adsorbent used was 7.5% per gram of fat in the creams. Low-fat dairy spreads of 40% fat and 8% SNF (no stabilizers added) were prepared with the treated creams by double stage homogenization. Textural properties (hardness, adhesiveness and cohesiveness)of the treated dairy creams and low-fat dairy spreads were evaluated and compared to those of commercially available spreads. The low-fat dairy spreads presented lower hardness and greater adhesiveness and cohesiveness than the commercial spreads. Treated creams of 70% fat had lower hardness, greater adhesiveness and similar cohesiveness than commercial spreads of similar fat content. Transmission and electron microscopy analyses pointed towards structural differences in fat globules of treated creams and low-fat dairy spreads as compared to the untreated controls. Treated cream samples were found to have different size and distribution of fat globules compared to the control.

Key Words: Low-Fat Dairy Spreads, Diatomaceous Adsorbents, Fat Globules

108 Heat classification of skim milk powders based on soluble protein determined by FPLC. K. Munsell* and R. Jiménez-Flores, *California Polytechnic State University, San Luis Obispo.*

Skim milk powders are used in various food products such as baked goods, dairy products, and processed foods. The functionality of the powder in each product is dependent on the heat treatment received by the skim milk during processing. A preheat treatment to the skim milk can lower the solubility or can improve the water absorption of the final powder. Changes in powder functionality are due to the denaturation of whey proteins. As the intensity and duration of the preheat treatment increases, more whey proteins are denatured. Therefore, assessing the heat treatment of a skim milk powder is important in determining its functionality and subsequent use. The most common method currently used is the whey protein nitrogen index (WPNI). The WPNI method classifies powders as low, medium, or high heat and has shown to be inaccurate, unreliable, poorly reproducible, and time consuming. The objective of this work was to establish a more rapid and reliable method to heat classify skim milk powders. The developed method used fast protein liquid chromatography (FPLC) with a 20 mM bis-tris buffer system at pH 6.5 to separate the soluble proteins in reconstituted skim milk. All samples were reconstituted to 9% solids, diluted with buffer, centrifuged to obtain the supernatant, then further diluted. The soluble proteins, contained in the supernatant, were injected into an anion-exchange column, the UNO-Q. The protein peaks eluted were identified by SDS-PAGE and integrated for a comparison of peak areas between samples. Increasing heat treatments to a raw milk sample indicated a gradual non-linear decrease in total peak area of α-lactalbumin and β-lactoglobulin peaks. A survey of several low and high heat skim milk powders showed decreasing total peak areas of the major whey proteins corresponding to decreasing WPNI values. Further studies will establish protein concentrations of the undenatured whey proteins in skim milk powders for a more useful heat classification.

Key Words: Skim Milk Powder, Heat Classification, FPLC

109 Influence of ultrafiltered sweet buttermilk and emulsifying salt level on the microstructure and free oil formation of reduced fat Process cheese. D. M. Raval* and V. V. Mistry, *Dairy Science Department, Minnesota-South Dakota Dairy Foods Research Center, South Dakota State University.*

The objective was to evaluate the influence of ultrafiltered sweet buttermilk **(UBM)** and varying emulsifying salt levels on the microstructure and free oil formation of reduced fat Process cheese. Reduced fat Process cheese was manufactured using reduced fat Cheddar base cheese from pasteurized milk (1.37 to 1.38% fat) (control treatment) and the same pasteurized milk with 5% UBM (buttermilk treatment). Sixteen and 8 wk base cheeses in 1:1 blend and disodium phosphate and trisodium citrate (1:1 blend) at 0.50, 1.25 and 2.00% wt/wt were used for each Process cheese treatment. Processing was carried out in an open steam-jacketed kettle with indirect heating to 71°C for 3 min, cooling to room temperature and storing at 4 to 6°C. It was observed with scanning electron microscopy that at 0.50% emulsifying salts, Process cheeses from the control treatment were characterized by a small number of large fat particles of round to uneven shapes, while those from the buttermilk treatment displayed a honeycomb-like structure within the large fat particles, possibly because of further clustering of fat into smaller individual particles. Higher magnifications showed the honeycomb-like structure to be a membrane-like material most likely to be composed of the emulsifying protein and residual fat globule membrane material left behind on the removal of fat during sample preparation. At 1.25% emulsifying salts, fat in control cheeses existed as chains of connected circular particles but in cheeses from the buttermilk treatment they were present as more finely and evenly distributed individual particles, indicating greater emulsification. At 2.00% emulsifying salts, the differences in microstructure between treatments diminished. At similar emulsifying salt levels Process cheese from the buttermilk treatment had less ($P < 0.05$) free oil than control cheeses which also suggest better emulsification of the former.

Key Words: Reduced Fat Process Cheese, Buttermilk, Microstructure

110 Application of ultrafiltered sweet buttermilk in the manufacture of reduced fat Process cheese. D. M. Raval* and V. V. Mistry, *Dairy Science Department, Minnesota-South Dakota Dairy Foods Research Center, South Dakota State University.*

The objective was to evaluate the application of ultrafiltered sweet buttermilk **(UBM)** for the manufacture of reduced fat Process cheese. Reduced fat Cheddar cheeses, manufactured from pasteurized milk (1.37 to 1.38% fat) as control or from the same pasteurized milk supplemented with 5% UBM as buttermilk treatment cheese, were used to manufacture reduced fat Process cheese. Sixteen and 8 wk old reduced fat Cheddar cheese in 1:1 blend and disodium phosphate and trisodium citrate (1:1 blend) at 0.50, 1.25 and 2.00% wt/wt were used for each Process cheese treatment. Processing was carried out in an open steam-jacketed kettle with indirect heating to 71°C for 3 min, cooling to room temperature and storing at 4 to 6°C. There were no differences ($P > 0.05$) between treatments in moisture (48%) and moisture in non fat substance of the Process cheeses. Because of compositional differences between base reduced fat Cheddar cheeses, Process cheeses from the buttermilk treatment had lower fat ($P < 0.05$) than the controls (14.48 vs. 15.10%) and were therefore harder. Ash and pH were not affected by UBM but increased ($P < 0.05$) with emulsifying salt level while hardness decreased. Reduced fat Process cheeses from the buttermilk treatment had lower ($P < 0.05$) meltability and higher ($P < 0.05$) apparent viscosity than the control cheeses. Meltability increased in cheeses from the buttermilk treatment with increasing emulsifying salt up to 1.25%, while it was highest in the controls at 1.25%. Sensory attributes were not influenced by UBM, while emulsifying salt level only influenced mouthfeel and appearance, both being judged better at 2.00% than at 0.50% emulsifying salt. The application of UBM to tailor functionality of reduced fat Process cheese appears to be promising.

Key Words: Reduced Fat Process Cheese, Buttermilk, Functionality

111 Comparison of nutrient and mineral composition among different commercial caprine milk products. Y. W. Park, *Fort Valley State University, Fort Valley, GA*.

Although a variety of scattered reports are available on nutritional values of caprine milk products, comprehensive research data on basic nutrient and mineral composition in commercial caprine fluid, condensed, and powdered milk products are extremely limited. Levels of basic nutrients and twelve major and trace minerals in commercial caprine fluid milk, condensed, powdered, yogurt, and cheese products manufactured in the U.S. were quantified and compared for compositional differences among these products. Mean total solids content (%) of fluid milk, condensed milk, powdered milk, yogurt, plain soft and Cheddar cheeses were:11.3, 20.9, 94.1, 11.5, 40.2, and 58.3, respectively. Mean protein (%) and fat (%) of the corresponding products were: 2.92, 3.40; 6.11, 6.75; 27.0, 28.2; 3.99, 2.25; 18.9, 22.5; 30.3, 26.6, respectively. Calcium and phosphorus contents (ppm) of the corresponding products were: 103, 125; 440, 393; 7715, 7471; 161, 144; 691, 1105; 3492, 3067, respectively. Iron and zinc contents (ppm) of the same products were: 0.062, 0.349; 1.518, 1.635; 3.33, 30.21; 0.117, 0.388; 7.16, 3.64; 8.86, 3.81, respectively. The levels of potassium (K) in cheeses were lowest among all products including fluid goat milk, suggesting that a considerable amount of K was lost during cheese manufacturing processes. Levels of all trace minerals were higher in yogurt and cheeses than the fluid milk, indicating that retention rates of these minerals were high in the two manufactured products. The levels of trace minerals in cheeses were even greater than those in yogurt products.

Key Words: Caprine Milk Products, Composition, Minerals

112 Effect of milkfat concentration on sensory properties of chocolate ice cream. E. A. Prindiville, R. T. Marshall*, and H. Heymann, *University of Missouri, Columbia*.

Most studies of ice creams with lowered fat content have focused on vanilla ice cream; however, cocoa introduces variables that affect flavor and texture that vanilla does not introduce. Nonfat and lowfat chocolate ice creams have been rated as harsh and bitter when compared to their full-fat counterparts. The objective of the study was to determine the effect of milkfat on the sensory properties of chocolate ice cream. Concentration of milkfat was varied to represent full-fat (9.0%), reduced-fat (6.0%), lowfat (4.0%), and nonfat (0.6%) ice creams. Frozen products were held at -30°C (control) or were heat-shocked by storing at -12°C. Eight trained panelists conducted descriptive sensory analyses of the samples at 0 wk and 4 wk of storage. Attribute ratings were analyzed by ANOVA and LSD methods, with a design of 4 trt x 3 rep x 2 samples x 8 judges and alpha = <0.05. Attributes showing significant differences among fresh ice creams were sweetness, intensity of cocoa flavor, cocoa aftertaste, firmness, chalkiness, creaminess, melting rate, viscosity, iciness, smoothness of texture, smoothness of appearance, brown color, and foaminess. Milkfat at concentrations of 9% and 6% produced more creaminess and smoothness than it did at 4% and 0.6%. The lower fat samples had more brown color, initial cocoa flavor, and cocoa aftertaste. Consumer acceptance (n=98) did not differ among the fresh ice creams. Compared with samples stored at -23°C, the heat-shocked samples were scored higher in iciness and sweetness but lower in smoothness of texture, smoothness of appearance, and firmness. Samples with the lower concentrations of fat suffered the greatest heat shock damage. The heat-shocked samples of nonfat and lowfat ice creams were scored higher in cocoa flavor, off flavor, and chalkiness than were the higher fat samples. They were also scored lower in cooked milk flavor, smoothness of appearance, smoothness of texture, and viscosity. Data clearly showed that milkfat concentration in chocolate ice cream has a major effect on texture and flavor attributes. Ice creams containing higher fat concentrations are better protected against heat shock damage.

Key Words: Ice Cream, Chocolate, Milkfat

113 Torsion properties of Mozzarella cheeses with added caseinate or NFDM. D. L. Van Hekken*, M. H. Tunick, P. W. Smith, E. L. Malin, and V. H. Holsinger, *USDA, ARS, Eastern Regional Research Center Wyndmoor, PA*.

Concern over the possible use of substitutes for natural cheeses in federal food programs initiated our study of the differences in textural properties of part skim Mozzarella cheese and Mozzarella substitutes. Cheeses were made from fresh milk (9% solids) with 1 or 2% added calcium caseinate or NFDM. Cheeses containing 9, 10, or 11% solids were also made using only reconstituted NFDM and cream. Composition of finished cheeses ranged from 12 to 19% fat and 51 to 58% moisture by weight. At week 1 and 6, sample plugs were tested in a torsion gelometer to obtain shear stress, shear strain, and shear rigidity at the point of fracture. After 6 weeks of storage, all cheeses had lower rigidity and tended to fracture at lower shear stresses and higher shear strains. When compared to the part skim Mozzarella control (9% solids) at the same age, most of the other cheeses had similar shear stress and strain values, although cheese made from milk with 2% added NFDM fractured at lower stress at week 1, and the cheeses made from milk with either 1 or 2% NFDM or 2% calcium caseinate fractured at higher strain at week 6. Cheeses made from milk with 1% added calcium caseinate had significantly higher rigidity than cheeses made from milk with 1 and 2% added NFDM. Cheeses made from milk with 2% calcium caseinate had significantly lower rigidity than all cheeses made exclusively of reconstituted NFDM. When relating torsion properties to texture, data showed that all cheeses tended to become more rubbery as storage increased and cheeses made entirely from reconstituted NFDM were tougher than cheeses made from fresh milk with added NFDM. This study shows that torsion analysis may be useful in evaluating the rheological differences between natural Mozzarella and Mozzarella substitutes.

Key Words: Cheese, Mozzarella, Torsion

114 Effect of elevated storage temperature on fat stability (free oil) of Mozzarella cheese. F. L. Lee*, M. R. Guo, and P. S. Kindstedt, *Northeast Dairy Foods Research Center, University of Vermont, Burlington*.

A detailed understanding of the factors that control Mozzarella cheese emulsion stability is currently lacking. Previous research showed that elevated storage temperature (14°C v. 4°C) resulted in increased fat instability and free oil release. The objective of this study was to identify physico-chemical factors that may affect fat stability and the release of free oil from Mozzarella cheese that has been aged at 14°C. On three different occasions, three 2.3 kg blocks of low moisture part-skim Mozzarella cheese were obtained from a commercial manufacturer on the day after manufacture (d 1) and immediately cubed, subdivided into 10 subsamples, and vacuum packaged. Five subsamples were stored at 4°C and five at 14°C. On d 2, 4, 6, 8 and 10 after manufacture, a subsample from each temperature treatment was randomly chosen and analyzed for pH, free oil and expressible serum. Serum samples were analyzed for crude protein, pH 4.6 soluble protein, and urea-PAGE. Data were analyzed as a split-split-plot ANOVA. Cheese pH was not significantly affected by storage temperature or duration. Free oil increased and expressible serum decreased significantly during storage at both temperatures; however, levels of both free oil and expressible serum were significantly higher at 14°C. Crude protein in the expressible serum increased significantly during storage at both temperatures; however, expressible serum from cheese stored at 14°C contained significantly less crude protein than that from cheese stored at 4°C. In contrast, pH 4.6 soluble protein was not significantly affected by storage temperature. Urea-PAGE showed increasing concentrations of α_s- and β-caseins in the expressible serum during storage at both temperatures; however, levels appeared to be higher at 4°C. In summary, the amount of expressible serum and its protein content were affected by storage temperature, which may be related to the observed differences in fat stability and free oil release at different storage temperatures.

Key Words: Mozzarella Cheese, Free Oil, Expressible Serum

115 Effects of pH reduction and lactic acid bacteria cell population on the color of nonfat milk. J. L. Brewer, S. L. Owens, and S. A. Rankin*, *University of Maryland, College Park*.

Research has demonstrated that lactic acid fermentation of nonfat milk can result in increases in measurements of milk whiteness. However, little is known of the mechanisms responsible for whiteness development in fermented milk. The objective of this study was to determine the effects of pH reduction and bacterial cell population increases on whiteness development in nonfat milk. Nonfat milk was acidified with lactic acid to pH values typical of fermentation. Bacterial cells of *Lactococcus lactis* ssp *lactis* (SCO 230) were grown in Elliker broth and harvested by centrifugation. Cell pellets were washed by suspending in peptone buffer and centrifuged to obtain a final cell mass of approximately 5 g. Pellets were suspended in nonfat milk to achieve a final increase in bacterial cell population of approximately 10^9 CFU/ml. Colorimeter values of acidified milks (AM) and milks with high bacterial cell populations (HBM) were conducted in triplicate. The entire study was repeated three times and analyzed with ANOVA; Fisher multiple comparisons were conducted where appropriate ($\alpha = .05$). Untreated nonfat milk (NM) and 2% reduced fat milk (TM) were included for comparison. Reflectance L*, a*, and b* values for AM reached maxima at pH 4.6, 4.0, and 4.0, respectively, each representing statistically significant increases relative to NM. Interestingly, L*, a*, and b* minima were obtained at pH 5.0, with values statistically less than the NM control. HBM exhibited significant increases in L*, a*, and b* values relative to NM but lower than TM values. Relative to NM, HBM milk exhibited increases in % reflectance from 500–700 nm and decreases in % transmission from 400–700 nm. These results demonstrate that both pH reduction and high bacteria cell populations can increase instrumental measurements of milk whiteness.

Key Words: Milk, Fermentation, Color

116 Antioxidant activity of whey, whey permeate, mother liquor, and taurine in selected antioxidant systems. X. Li* and R. L. Richter, *Texas A&M University*.

Cheese whey and whey permeate have been demonstrated to have antioxidant activity but the source of the activity has not been identified. Taurine is the fourth most abundant free amino acid in milk and has antioxidant properties. The objective of this research was to determine if some of the antioxidant activity in whey was caused by taurine. Raw milk was converted to cheese curd and the whey drained at pH 6.2. The whey was clarified, pasteurized, and subjected to ultrafiltration to obtain whey permeate. Permeate was concentrated by evaporation at 75°C and cooled to crystallize the lactose which was removed by centrifugation. The mother liquor was collected for antioxidant determinations. Antioxidant activity of the fractions was tested in a liposome system made from phosphatidylcholine using either $FeCl_3$ or lipoxidase as the catalyst and in a hemoglobin antioxidant model system. Antioxidant activity was observed for all whey fractions in the liposome antioxidant system regardless of the catalyst. Taurine did not exhibit antioxidant activity in the liposome system but did have antioxidant activity in the hemoglobin system. These results indicate that taurine in whey and whey fractions might have antioxidant activity but it is not responsible for the antioxidant activity observed using the liposome model system. Antioxidant activity present in mother liquor indicates that the mother liquor might be useful for as a food ingredient as an antioxidant.

117 High pressure homogenization and peptidase activity of lactic acid starter culture. M. L. Gonzalez* and R. L. Richter, *Texas A&M University College Station*.

Previous studies have proven that homogenization can cause the release of soluble protein from cells such as Saccharomyces cerevisiae by disruption of the cells. The disintegration of large quantities of cell permits the extraction of substances such as enzymes that are found within the cell. The objective of this study was to determine the effects of high pressure homogenization of lactic starter culture on the peptidase activity of the lactic acid bacteria cells. Concentrated lactic acid starter culture was subjected to repeated homogenization at 90 MPa at an initial temperature of 10°C. Concentrated culture was circulated through the homogenizer for 0, 1, 3, 6, and 9 cycles. Lactic acid bacteria survival and a stress test were performed on the homogenized culture. Aminopeptidase activity was determined at 43°C at pH 7. Endopeptidase activity was measured using N-Glu-L-Phe-pNA and L-acetyl-L-Ala-pNA and performed at 37°C, pH 7.2 and 45°C pH 7.6. Peptidase activity was determined by measuring the increase of free nitroaniline at 410 nm. The Lactic acid bacteria concentration in the homogenized cultures decreased 8 $\log_{10}$ CFU/ml from the initial cell count after homogenization for 9 cycles. There was no indication of stressed cells from culture after 6 cycles through the homogenizer. Bacteria cells that were homogenized for 1 and 3 cycles grew when inoculated into non- selective media. Endopeptidase activity aminopeptidase activity were affected by the number of cycles performed on the starter culture. Endopeptidase and aminopeptidase activity increased with the number of homogenization cycles. Higher peptidase activity was obtained when the treated culture was assayed at a pH of 7.6 and 45°C. High pressure homogenization liberates enzymes within the bacteria cell without inactivating them.

118 Yield stress deteminations of yogurt firmness using the Vane method. P. M. Jaar*, R. Moreira, and R. L. Richter, *Texas A&M University*.

The firmness of yogurt is an important parameter of yogurt quality and acceptability. The objectives of this experiment were to determine the firmness of yogurt using the Vane method and to compare the Vane method with penetrometry. Yogurt was formulated to contain 11% milk solids nonfat and 2% or 0% milkfat with 0% or 0.5% emulsifier. The samples were heated to 90°C and homogenized at 30, 60 and 90 Mpa. The samples were incubated at 400°C until the pH of the samples was 4.2. The samples were stored overnight at 40°C. The dynamic and static yield stresses were determined with the Vane method using a Brookfield HBDVIII rheometer with a four blade vane spindle. Three vane spindles of different heights were used for each sample to eliminate end effects. Determinations were made at 40°C. Firmness of yogurt containing 2% milkfat and no emulsifier increased as homogenization pressure increased. No significant difference was found between nonfat samples regardless of composition or homogenization pressure. A texture analyzer with a cone shaped probe was used to measure peak force which was compared to results obtained using the Vane method. Measurements obtained using the Vane method were less variable than the penetrometer method. The Vane method is an accurate and economical alternative to the other methods used for measuring yogurt firmness.

Key Words: Yogurt, Yield Stress, Vane Method

119 Cheese making properties of milk from cows of different milk protein genotype. A. L. Dikkeboom[1]*, C. M. Chen[1], J. J. Jaeggi, M. E. Johnson[1], W. A. Tricomi[1], M. G. Zimbric[1], J. A. Lewandowski[2], and R. Bremel[2], [1]*Wisconsin Center for Dairy Research, Madison* [2]*Department of Animal Science, University of Wisconsin, Madison.*

Cheddar cheese was manufactured from milk obtained from Holstein cows with the AA or BB kappa-casein genotype. The milks were pooled from three to six individual cows and from at least two separate milkings. Five vats of cheese were made from each type of milk over a period of three months. Only one vat of cheese was made from each milk each day. The milk from cows with the AA genotype were higher in protein (3.06 vs 2.96), casein (2.49 vs 2.42), and fat (3.59 vs 3.39) than milk from cows with the BB genotype. The higher casein and fat resulted in higher cheese yield (9.89 vs 9.60). There were no differences in moisture in the cheeses made with either genotype of milk (ca. 38.5%). The milk from cows with the BB genotype had slightly higher non-protein nitrogen as a percentage of the total nitrogen than milk from cows with the AA genotype, but the percent casein as a percent of the true protein was higher (81.42 vs 80.43%). Fat retention was higher in cheese made from milk from BB genotype cows (91.3 vs 88.5). The coagula were cut at the same firmness, but the milk from cows of the BB genotype clotted faster than milk from cows with the AA genotype (19 vs 33 min). Sensory analysis of the Cheddar cheese indicated no differences between the cheeses that could be attributed to the genotype of the cow. Similarly, thermal melt tests did not indicate any differences between cheeses that could be attributed to the milk from which the cheese was made. A new test that measures the softening point of the cheese indicated a trend towards a slightly higher softening point in cheese made with milk from cows of the AA genotype. The significance of this, if any, has not yet been elucidated.

120 Manufacturing skim milk mozzarella Cheese. M. G. Zimbric*, C. M. Chen, A. L. Dikkeboom, J. J. Jaeggi, M. E. Johnson, and W. A. Tricomi, *Wisconsin Center for Dairy Research, Madison.*

The key factors in the manufacture of skim milk mozzarella cheese are producing a cheese with high moisture(58-62%) and manipulating the protein matrix to produce the desired stretch and melt when heated. These were achieved by a shortened manufacturing time using preacidified milk and a cold water rinse. Use of a starch-based fat mimetic increased moisture from 60% to 63%. Skim milk cheese was manufactured using milk with .12% fat, 3.22% total protein and 2.48% casein. The milk was preacidified to pH 6.25 using acetic acid. Addition of high level of starter (1.5% wt/wt) accelerated the production of acid. Coagulant was added at pH 6.05. The curd was cut at pH 6.00 approximately 70 minutes after the addition of coagulant. The curd and whey mixture was heated to 41°C. After reaching cook temperature, the whey was drained and the curd cheddared until pH 5.30. The curd was milled and soaked in 21°C water. After draining the water, 2% salt by curd weight was added to the curd. The curd was stretched in a mixer/molder with a water temperature at 88°C. The temperature of the blocks of skim milk mozzarella was 57°C. The blocks were cooled in cold water for about 40 minutes and brined for 30 minutes. The final skim milk mozzarella cheese produced had an average 1.10% fat, 60% moisture, 1.30% salt and 34% protein. Sensory analysis of the cheese and shreds was done at one week, four weeks and eight weeks. Shreds were also evaluated on pizzas cooked in a convection oven at all time points. The cheese had acceptable melt, flavor, shreddablity and chewiness. Skinning and transparent color were observed on the pizzas. Addition of titanium dioxide to the milk during cheese making corrected the transparent color.

121 Use of cheese model systems in developing a desirable aroma profile for regular and low fat Cheddar cheeses. J. Wang* and G. A. Reineccius, *University of Minnesota, St. Paul.*

Cheddar cheese is a complex food system, consisting of moisture, milk fat, whey proteins, polysaccharides, sodium, minerals, and vitamins embedded in a casein matrix. Interactions between volatile and non-volatile compounds underscore the importance of developing a model cheese system or analog to evaluate aroma compounds in a medium similar to the real cheese matrix. In this study, we developed bland regular and low fat cheese models and used them to evaluate the sensory profile of key aroma compounds in mild Cheddar cheeses. The model cheese systems were made from native calcium phosphocaseinate prepared by membrane microfiltration, low heat skim milk powder, vegetable oil shortening, glucono-delta-lactone, and sodium chloride. The concentrations of those components were adjusted to represent the regular (33%) and low fat (8%) Cheddar cheeses (pH=5.3). Nine potent aroma components in mild Cheddar cheeses had previously been identified and quantified by instrumental techniques. They were added into cheese models at their respective concentrations. Eleven panelists gave descriptive analysis of the flavored cheese models with other three commercial Cheddar cheeses (regular fat, 50% fat reduction, and fat free) on fifteen attribute terms. Rotatable response surface designs were conducted to estimate the optimum concentrations of key aroma compounds in the cheese models that could gave a desirable aroma profile of mild Cheddar cheese. The results showed that the original flavored cheese models lacked sour, moldy, and sulfide notes. They were also weak in aroma intensity and were not well balanced compared to the real Cheddar cheeses. Modification of their concentrations and incorporation of additional aroma compounds improved the aroma quality of the flavored regular-fat cheese models. This study is still continuing. The next step will be focused on determining the aroma compounds and their quantities required to give the low fat Cheddar cheese model an equivalent flavor quality as a regular fat model.

Key Words: Cheese Model, Cheddar Cheese, Low Fat

122 The use of adjunct cultures in reduced fat Jarlsberg-type cheese manufacture. K. N. Jensen* and C. H. White, *Mississippi State University, Mississippi State.*

The purpose of this research was to compare the effects of several adjunct cultures on the flavor and texture of reduced fat Jarlsberg-type cheese. Bacteria were isolated from high quality aged Jarlsberg cheese. The isolates, along with additional cultures were evaluated in a slurry system by a descriptive panel (8-10). Bacteria which produced aroma notes most similar to high qualtiy aged Jarlsberg-type cheese were chosen as adjuncts. Make procedure modifications were utilized to increase moisture and decrease acidity of the cheese. Expert (8) evaluation was performed on the cheese at two, four, and six months. A descriptive panel (8) and consumer panel ($\geq$50) evaluated the cheeses at four months. Experts at two months found the major criticism to be "acid". As the cheese aged the panel described the cheese with "acid" and "nutty" being the major criticisms. At two months, *Lactococcus lactis* ssp *lactis* biovar *diacetylactis* was the highest scoring adjunct and significantly higher ($p \leq 0.05$) than the reduced fat control. At four months of age there were no significant differences($p \leq 0.05$) between the reduced fat control and the highest scoring adjuncts. At six months of age *L.lactis* ssp *lactis* biovar *diacetylactis* was again the highest scoring adjunct and significantly higher($p \leq 0.05$) than control cheeses.

Key Words: Reduced Fat Jarlsberg Type Cheese, Adjunct Cultures

123 Effect of homogenization on immunoglobulins' agglutination response in presence of lactic cultures. C. L. Hicks* and Z. Tabeidie, *University of Kentucky, Lexington.*

Immunoglobulins were prepared from skimmed (8000 x g for 20 min at 4°C) colostrum (first milking from three Jersey cows). Skimmed colostrum was acidified with 1N HCl to pH 4.6 and centrifuged (3000 x g for 20 min) to remove the coagulate. Supernatant was neutralized with 1N NaOH to pH 7.4. An immunoglobulin precipitate was developed by adding ammonium sulphate (33% saturation) to the supernatant. The precipitate was reconstituted in simulated milk ultra filtrate (SMUF) buffer (pH 6.6) to its original volume. Excess salts were removed by diafiltration (SMUF buffer) through an ultrafiltration 10,000 molecular weight cutoff system. Isolation of immunoglobulins was accomplished using a DEAE batch process. Resulting immunoglobulins were freeze dried and stored at -20 C. Immunoglobulins were added (0.4%) to skim milks that were unhomogenized or homogenized (18.6 MPa) and heated or combined with skim milk heated to various temperatures. Agglutination-sensitive lactic cultures (CH970 or E72) were grown in these skim milks. Severity of culture agglutination was monitored by observing top to bottom pH differentials of the milk columns. When skim was heated (32, 49, 65, and 82°C) then homogenized, the homogenized samples were less agglutinated than the raw skim control. No differences were observed in the severity of culture agglutination (CH970) for skim treated at 32, 49, and 65°C. No agglutination occurred in the 82°C treated skim. When cultures were grown in homogenized and unhomogenized skim milk with immunoglobulins present, homogenized skims were again less agglutinated than unhomogenized skim as expected. In mixtures of raw homogenized or unhomogenized skim and heat treated skim (50/50), mixtures containing homogenized skims produced less agglutination. In mixtures of homogenized and unhomogenized skims with immunoglobulins added and mixed with heat treated skim (50/50), mixtures containing homogenized skims agglutinated slower than mixtures containing unhomogenized skims. Homogenization appeared to have little effect on the reactivity of immunoglobulins in the culture agglutination mechanism.

Key Words: Homogenization, Immunoglobulin, Culture Agglutination

124 Effect of heat treatment on immunoglobulins' agglutination response in presence of lactic cultures. Z. Tabeidie and C. L. Hicks*, *University of Kentucky, Lexington.*

Immunoglobulins were prepared from skimmed (8000 x g for 20 min at 4°C) colostrum (first milking from three Jersey cows). Skimmed colostrum was acidified with 1N HCl to pH 4.6 and centrifuged (3000 x g for 20 min) to remove the coagulate. Supernatant was neutralized with 1N NaOH to pH 7.4. An immunoglobulin precipitate was developed by adding ammonium sulphate (33% saturation) to the supernatant. Precipitate was reconstituted in simulated milk ultrafiltrate (SMUF) buffer (pH 6.6) to its original volume. Excess salts were removed by diafiltration (SMUF buffer) through an ultrafiltration (10,000 molecular weight cutoff) system. Isolation of immunoglobulins was accomplished using a DEAE batch process. Immunoglobulins were freeze dried and stored at -20°C. Immunoglobulins were added (0.4%) to skim milks that were heated at various temperatures. Agglutination-sensitive lactic cultures (CH970 or E72) were grown in skim milks. Severity of culture agglutination was monitored by observing top to bottom pH differentials of the milk columns. Control experiments where cultures were grown in raw, homogenized (18.6 MPa), and heat treated (77°C for 30 min) skim determined that the maximum pH differentials were 0.87, 0.32, and 0.00 for CH970 and 0.34, 0.27, and 0.00 for E72, respectively. When skim was either heat treated or homogenized, culture agglutination was eliminated or reduced. However, when heat treated (77°C for 30 min) skim was added to homogenized skim, culture agglutination was greater than observed for homogenized skim or heat treated skim alone. When heat treated immunoglobulins (65, 70, 75, 80, and 85°C) were added to heat treated skim and combined with homogenized skim (50/50 mixture), agglutination could be observed after 1 h in those skims that were heated to 75°C, compared to cultures grown in skim containing immunoglobulin heated to 65°C (agglutinated after 2 to 3 h), and compared to homogenized skim (agglutinated after 3 h), and heated skim (no agglutination within 6 hr). Temperatures at and above 75°C enhanced immunoglobulin's response and increased the severity of culture agglutination when homogenized skim milk was present.

Key Words: Immunoglobulin, Culture, Agglutination

125 Study of the possible mechanisms involved in the mucosal immune system activation by Lactic Acid Bacteria (LAB). G. Perdigón*, S. Alvarez, E. Vintiñi, M. Medina, and M. Medici, *Centro de Referencia para Lactobacilos, Universidad Nacional de Tucuman, Chacabuco 145, 4000 Tucuman, Argentina.*

To induce mucosal immune response is not easy due to oral tolerance. In some conditions bacteria could activate this immune system. The antigens orally administered can interact with M cells of Peyer patches (PP) or bind to the epithelial cells inducing inflammatory immune response. We have demonstrated that certain LAB are able to induce specific secretory immunity and others to enhance the gut inflammatory response. The aim of this work was to establish the reason of his different behaviour and the possible mechanisms involved in the interaction of LAB at intestinal level. We studied IgA+ and IgM+ B cells comparatively in bronchus and intestine, CD4+ T cells and the IgA anti LAB in the intestinal fluid induced by oral administration of *L. casei, L. delbrueckii* ssp.*bulgaricus, L. acidophilus, L. rhamnosus, Lactococcus lactis* and *S. salivarius* ssp. *Thermophilus.* The increase in the IgA+ at the bronchus level mean an enhancement the number of the IgA+ cells that enter in the IgA cycle by PP or epithelial interaction. The IgM+ cells are increased when the stimulus does not induce the switch of IgM+ to IgA+. The increase in the CD4+ cells means PP interaction, and enhancemnt of the B and T cells migration. The antibody anti LAB is related to the precessing and presentation of the microorganisms to the immune cells. We demonstrated that only *L. casei* would be able to interact at PP level showing an increase in IgA+, CD4+ cells and specific antibodies for itself. *L. bulgaricus* and *L. acidophilus* would induce gut mucosal activation by interaction with the epithelial cels without significant increase in the immune cell associated to the bronchus. While *L. rhamnosus* and *S. thermophilus* would also interact with epithelial cells that process and present these microorganisms evoking immune response against their epitopes and *Lac. lactis* induced an increase of IgA+ cells to enter to the IgA cycle but not CD4+ cells, thus, it would bind to the epithelial cells that would activate B lymphocytes without processing and presentation of itself because we did not determine specific antibodies against *Lac. lactis.*

Key Words: Lactic Acid Bacteria, Mucosal Inmune System, Mechanisms

126 Proteolytic breakdown of casein by whole cell, intracellular and cell wall extracts of probiotic and yogurt bacteria. N. P. Shah* and A. Shihata, *Victoria University of Technology, Australia.*

Probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium* spp.) and yogurt bacteria (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) possess a complex system of proteinases and peptidases which enable them to use milk casein as an additional source of amino acids and nitrogen. Several proteolytic enzymes have been purified and biochemically characterized. The aim of this study was to investigate the hydrolytic patterns of α-, β-, κ-, and α_s-1 casein by probiotic and yogurt bacteria. Preliminary screening of various probiotic and yogurt bacteria for their proteolytic activity has been carried out using the o-phthaldialdehyde spectrophotometric assay. Seven strains of *S. thermophilus*, 10 strains of *L.delbrueckii* ssp. *bulgaricus*, 9 strains of *L. acidophilus* and 7 strains of *Bifidobacterium* spp. have demonstrated presence of proteolytic activity. The proteolytic activity of these selected strains was further investigated to examine their ability to hydrolyse various caseins into peptides and amino acids. Casein degradation was evaluated by SDS PAGE for various incubation periods. The reaction mixture consisted of cell suspension obtained by harvesting cells grown in MRS broth and casein (2 mg/mL). The incubation was carried out at 37°C. Proteolysis of casein and its fractions in whole cell, intracellular and cell wall extracts of probiotic and yogurt bacteria was also studied using SDS PAGE. Furthermore the proteinase and peptidase activity of yogurt and probiotic bacteria was also studied. Preliminary results have shown identical patterns of hydrolysis of α- and β-casein by *L.delbrueckii* ssp. *bulgaricus* and *S. thermophilus.* β-casein hydrolysis by *L. acidophilus* has also been observed.

Key Words: Proteolytic Activity, Casein Degradation, Probiotic Bacteria

127 DNA motif(s) from *Lactobacillus gasseri* induce lymphocyte activation. H. Kitazawa*, S. Itoh, K. Konno, T. Yamaguchi, T. Saito, and T. Itoh, *Tohoku University, Sendai, Japan*.

The *Lactobacillus acidophilus* group, a member of gastrointestinal lactic acid bacteria (LAB) widely used in dairy foods, is expected to develop as a probiotic LAB because of the ability to stimulate host immune responses. We have evaluated and assessed the mitogenic responses of the LAB to lymphocytes as an incidence of biological activity among them. The present study was conducted to determine the mitogenic factors from the LAB, particularly focusing on mitogenic DNAs in the LAB. In 10 of 16 strains of *Lactobacillus acidophilus* group LAB, the DNA components significantly stimulated mitogenic responses to murine splenocytes. The mitogenic activity to lymphocytes from peyer's patches, a gut-associated lymphoid tissue, was also observed in some DNAs. The active DNA (DNA1131) of *Lactobacillus gasseri* JCM1131^T was selected to analyze the active components. Plasmid DNAs from the positive clones obtained by subcloning, using the pUC119 vector ligated with 1131DNA partially digested with Sau3AI, were purified by the precipitation method with polyethylene glycol. The plasmid DNA templates were amplified by using the polymerase chain reaction (PCR) with primers for the multicloning site of the vector. In the 46 of the 192 positive colonies significant mitogenic activity was detected. The active nucleotides which stimulated mitogenic responses were sequenced by a DNA sequencer MODEL 4000(LI-COR, Lincoln, USA). High homologous DNA sequence of 8 mers (designated as AT5AC) were found in the active DNA, but not in the non-active DNA. Chemically synthesized-AT5AC induced a mitogenic response and was characterized as a B-cell specific mitogen. The binding of AT5AC to lymphocytes were demonstrated by FACS and laser microscopy. These findings indicated that a novel DNA motif inducing mitogenic activity, different from CpG motifs from *E. coli* recently reported as a B-cell mitogen, exists in DNAs from *L. gasseri*.

Key Words: Lactobacillus Gasseri, Mitogenic Factor, DNA Motif

128 Genomic sequence of the lytic phage DT1 from *Streptococcus thermophilus*. D. M. Tremblay* and S. Moineau, *Department of Biochemistry and GREB, Université Laval, Québec, Canada*.

Streptococcus thermophilus is widely used for the manufacture of Italian cheeses and yogurt. Increased productivity have led to milk fermentation failures due to bacteriophages. To date, all *S. thermophilus* phages have a small-isometric head and a non-contractile tail. Recently, these phages were classified into two groups based on their mode of DNA packaging and the number of major structural proteins (MSP). One group included phages which had 3 MSP and packaged their DNA via a pac mechanism. The complete genomic sequence of one member (temperate phage O1205) of this group was reported last year. The other (more predominant) group included phages with two MSP and packaged their DNA via a cos site. Here, we report the first complete nucleotic sequence of a lytic *S. thermophilus* phage which belong to the 2 MSP/cos group. Phage DT1, isolated from a Mozzarella whey sample in 1996, has a linear genome of 34821 nucleotides long with a GC content of 39.1% and 47 open reading frames (ORFs) of 40 codons or more. All phage genes appeared to be transcribed in only one orientation. Database searches revealed putative functions to some ORFs including holin, lysin, primase, helicase and the two major structural proteins (MSP). The N-terminal sequence of the two MSP (31.9 kDa and 21.8 kDa) were also determined and the first 10 amino acids were AIVGLKLVKL and LLDSKTDHSG. Direct and inverted repeats were identified in an 254 bp intergenic region, suggesting a phage origin of replication. Highly conserved regions were found between many putative proteins of the lytic phage DT1 and the temperate phage O1205. This study provides new fundamental knowledge on *S. thermophilus* phages.

Key Words: *Streptococcus thermophilus*, Bacteriophage, Genome

129 Multiplex PCR method for detection and identification of *Lactococcus lactis* phages of the 936 and c2 species. S. Labrie* and S. Moineau, *Université Laval, Québec, Canada*.

Bacteriophages are still the leading cause of milk fermentation failures worldwide. *Lactococcus lactis* phages are classified in numerous species but only three are commonly encountered in dairy plants, namely, 936, c2 and P335. Methods currently available for detection of phages include activity tests and plaque assays. These methods do not provide information on the species of the phage detected. As the next generation of phage-resistant strains and starter rotations are introduced into the market, a method for rapid identification of phage species would be an asset. The aim of this project was to develop a PCR-based method to simultaneously detect and identify lactococcal phages. The first objective was to identify a highly conserved DNA region within the genome of each phage species. For this purpose, the gene coding for the major capsid protein (MCP) was targeted.
The DNA sequence of the *mcp* gene was determined for 3 phages of the c2 species (eb1, Q38 and Q44) and compared with the *mcp* gene sequence of the reference phages c2 (l5) and bIL67 (ORF26). An identity of 86.4% was observed over the entire 1437-bp *mcp* gene of the 5 phages, whereas an identity of 93% was observed at the protein level. The N-terminal sequence of the MCP appeared more conserved than the C-terminal. For the 936 species, the DNA sequence of the *mcp* gene was also determined for 3 phages (p2, Q7 and Q42) and compared with the *mcp* gene sequence of the reference phages sk1 (ORF11) and F4-1. An identity of 84.9% was observed over the entire 906-bp gene of the 5 phages whereas an identity of 86.4% was observed at the protein level. Two sets of primers (one for each species), based on the conserved regions were designed. A multiplex PCR was optimized for detection and species assignment. PCR products of 182-bp and 423-bp were obtained for c2 and 936 phages, respectively.

Key Words: *Lactococcus Lactis*, Bacteriophages, PCR

130 Microbiological effects of the novel and powerful abortive infection mechanism Abi900 from *Lactococcus lactis*. E. Dion*, E. Emond, and S. Moineau, *Université Laval, Québec, Canada*.

Bacteriophages are known to hamper lactic acid fermentation. This phenomenon can be partially alleviated by using phage-resistant cultures. Natural phage exclusion systems in *Lactococcus lactis* are used to construct phage-resistant strains. The appearance of new lytic phages has fueled the search for novel anti-phage systems. We have isolated a natural 11-kb plasmid (named pSRQ900) from the *Lactococcus lactis* W-37 which confers very high resistance (EOP $< 10^{-8}$) against the phage species 936 and c2. The genetic determinants were identified and one open reading frame was involved in the phenotype. The deduced protein did not show any homology to known proteins in databases. Here, we characterized the mechanism encoded on pSRQ900 and its microbiological impact on the phage lytic cycle.
Phages adsorbed to the same level on sensitive and resistant cells. Extracts from cells harboring pSRQ900 showed no endonucleolytic activity on genomic DNA of phage p2 and *Lactococcus lactis* LM0230. The presence of pSRQ900 had no impact on cell survival. Phages p2 (936 species) and c21 (c2 species) were used to determine burst size and efficiency to form center of infection (ECOI). Sensitive cells released on average 58 progeny p2 phages whereas the burst size dropped to 1, and the ECOI to 0.8 percent in cells carrying pSRQ900. Similarly, pSRQ900 reduced the burst size of phage c21 from an average of 89 to 3 and the ECOI to 24.5 percent. No difference was observed on the phage resistance phenotype when tested at 21, 30 and 38°C. Based on all the above results and the current classification of phage defense systems, pSRQ900 encodes an abortive infection mechanism. This powerful system was named Abi900.

Key Words: Abortive Infection, Bacteriophage, Mechanism (Abi)