of 3.2 h after the start of agitation and ranged from 0-8 h. The maximum total number of 30-min average measurements greater than 30ppb for all sites was an average 5 times and ranged from 3 to 9 times.

**Key Words:** hydrogen sulfide, swine, manure

**339 N- vs. P-based manure nutrient management: A field study of leaching losses of N and P.** J.D. Toth*, Z. Dou, J.D. Ferguson, D.T. Galligan, and C.F. Ramberg, *University of Pennsylvania, Kennett Square*.

Manure applications to croplands have traditionally been made to meet plant requirements for N, which often leads to overapplication of P and elevated P runoff loss. A P-based manure application criterion has been proposed to address this concern. We initiated a field experiment in 1998 to examine leaching losses and soil accumulation of N and P with corn, alfalfa, and orchardgrass receiving N- vs. P-based dairy manure; the crops also received two additional treatments: control (no N or P added) and fertilizer (N applied at rates meeting crop requirements). Passive capillary wick lysimeters were installed underneath the replicated crop strips to collect leachate moving below the crop root zones. Leachate water samples were analyzed for nitrate and P. In the initial year of this long-term study (April 1998-March 1999), flow-weighted annual leachate nitrate-N concentrations from corn and orchardgrass were highest for the fertilizer treatment (23 and 13 mg/L, respectively) and lower for the manured treatments and the control, although these differences were not statistically significant at the 5% level. Mass of nitrate-N lost in leachate was 123 and 57 kg/ha for the fertilizer treatments to corn and orchardgrass, respectively, averaged 88 and 40 kg/ha for the manured treatments of the two crops, and 66 kg/ha for manured alfalfa. Leachate P concentrations from alfalfa were significantly higher for the N-based manure treatment (0.09 mg/L) than for P-based (0.04 mg/L). From April 1999 through January 2000 in the second year of the study, nitrate-N concentrations in leachate below the N- and P-based manure treatments were 19 and 22 mg/L for corn; and 22 and 28 mg/L for alfalfa, respectively. Nitrate-N concentrations from grass did not exceed 8 mg/L. Leachate concentrations and mass in leachate of nitrate and total P from corn and orchardgrass generally have not differed as a result of basing manure application rates on crop N vs. P needs.

**Key Words:** Nutrient Management, Nitrate Leaching, Phosphorus

**340 A new tool to help with sire selection.** A. Perkins*[2], V. LaVoie[1], and J. Stellflug[1], [1]*USDA ARS US Sheep Experiment Station, Dubois ID,* [2]*Carroll College, Helena MT*.

Variation in sexual performance of male mammals can be costly to producers if poor sexual performance sires are selected. Our goal was to develop a drug test that could distinguish between high and low sexual performance males before their use in production. Testosterone is necessary for male sexual behavior, but baseline concentrations are not predictive of libido. Our hypothesis was that when a ram is given an injection of naloxone his testosterone (T) and LH response to the injection can predict his sexual performance. Three experiments were conducted. Variables included dosage of naloxone, season of year, and repeatability within individual rams. Rams previously identified by behavior tests (sexually inactive[asexual; n = 26]and sexually active[n = 26]rams) were treated with naloxone(0.5, 0.75, and 1.5 mg/kg body weight)in November, June, and December. Changes in T and LH were calculated by subtracting prenaloxone average values from postnaloxone values for each ram. The largest difference was considered an individual's response to treatment. Responses to naloxone were compared between ram groups (asexual and sexually active rams) over seasons, years, and dosages using PROC GLM and PROC MIX for repeated measure. The T response to naloxone was greater ($P < 0.05$) in sexually active than in asexual rams, and LH response tended ($P < 0.07$)to differ between sexually active and asexual rams. All dosages were equally effective during the breeding season. In general, greatest values for LH and T were at 15 and 60 min after naloxone, respectively. The same rams were tested over two breeding seasons. Year affected responses ($P < 0.05$) but ram class x year was not significant. Accuracy of predicting asexual rams was 73% and accuracy of predicting sexually active rams was 81%. This test is most effective during the breeding season. We suggest that a refinement of this patent pending protocol be developed for veterinarians and producers to use during routine examination of potential sires.

**Key Words:** Sexual, Breeding, Naloxone

## DAIRY FOODS

**341 Rheological properties of high fat creams containing added whey proteins and homogenized at different pressures.** S. Adapa and K. Schmidt*, *Kansas State University, Manhattan*.

Dairy creams with milk fat levels of 50% and 55%, were compared to study the effect of added whey proteins and homogenization pressures on the rheological behavior. The study was done in two steps. In the first study, whey protein concentrate was added to the creams (50 & 55% fat) at the rate of 0 (control), 1, 2, and 3% (w/w), UHT treated, stored at 4°C and 25°C, and evaluated over a period of 43 days. In the second study, whey protein concentrate was added to the creams (50 & 55% fat) at 2% (w/w) level, UHT treated, homogenized at 0 (control), 500, 1000, and 1500 psi, and evaluated over a period of 43 days. All creams were tested for viscoelastic properties by dynamic testing involving sinusoidal oscillatory tests at frequencies ranging from 1-10 Hz and a strain of 0.7%, using a parallel plate geometry. In both the studies, storage modulus (G′) and loss modulus (G″) of all treatments were slightly dependent on frequency, exhibiting higher values at higher frequencies. In all treatments, G′ was significantly higher than the G″ throughout the frequency range tested without any crossover. G′ and G″ increased significantly over time and also exhibited higher values at lower temperatures (4°C). Tan $\delta$ values (G″/G′) did not change over time. In the first study, level of fat resulted in differences in G′ and G″ rather than the level of added protein. In the second study, homogenization pressures resulted in differences in G′ and G″ values with samples homogenized at higher pressures having higher G′ and G″ values but lower tan $\delta$ values.

**Key Words:** Creams, UHT, Viscoelastic

**342 The concentration of FFA and free amino groups in raw milk from cows fed high or low amounts of concentrate.** H. Alkanhal*, M. Alshaikh, M. Salah, and H. Mogawer, [1]*King Saud University, Riyadh, Saudi Arabia*.

Milk lipase activity, initial concentration of FFA and free amino groups and subsequent lipolysis and proteolysis were measured in raw milk from cows fed either high (70%) or low (40%) amounts of concentrate. Lipase activity (2.36 $\mu$eq of FFA/ml/h) and initial FFA concentration (0.26 meq/100 g of fat) were higher in raw milk from cows fed high amounts of concentrate than those in raw milk from cows fed low amounts of concentrate (2.04 $\mu$eq of FFA/ml/h and 0.14 meq/100 g of fat, respectively). Spontaneous lipolysis at 24 and 48 h was also higher in raw milk from cows fed high amounts of concentrate than in raw milk from cows fed low amounts of concentrate. Initial content of free amino groups (2.12 mmol/100 g of protein) and subsequent proteolysis at 48 h (2.28 mmol/100 g of protein) were higher in raw milk from cows fed a high concentrate ration than those in raw milk from cows fed a low concentrate ration (1.99 and 2.14 mmol/100 g of protein, respectively). Some differences in lipolysis and proteolysis in raw milk were observed between weeks of treatment. Increased lipolysis and proteolysis products in raw milk from cows fed a diet high in concentrate may increase the rate of off-flavor appearance in milk and dairy products.

**Key Words:** Lipolysis, Proteolysis, Concentrate

**343 Effect of lipids supplementation in the ration on production of conjugated linoleic acid (CLA) and milk fat composition of dairy cows.** F.L. Santos*, R.P. Lana, M.T.C. Silva, S.C.C. Brandao, and L.H. Vargas, *Universidade Federal de Vicosa, Vicosa-MG, Brazil*.

The objective of this work was to verify the effects of lipid sources (whole soybean meal and soybean oil) added to the diet on the fatty acids profile of milk fat, specially in the increasing of CLA. Six multiparus 7/8 Holstein-Zebu cows, 30 days after calving, with average body weight of 500 kg and average milk production of 20 l/day were used. The animals were divided in three groups to receive the diets (treatments), all isoproteic and isocaloric, where the control diet had 3 pecent ether extract in total dry matter and the others 7 percent. The treatments containing supplementary lipids, compared to the control, decreased the percent of short chain fatty acids ($P<0.01$) and decreased the content of butiric, caproic, caprilic, capric, lauric, miristic and the percent of saturated fatty acids ($P<0.05$). There was still a trend for reduction in the content of palmitic and palmitoleic acids and a trend for increase in the percent of unsaturated fatty acids and long chain fatty acids ($P<0.10$). For other side, supplementary lipids increased stearic acid ($P<0.05$) and tended to increase the oleic acid and CLA ($P<0.10$). The soybean oil, compared to the whole soybean meal, decreased the content of linoleic and linolenic acids and increased the CLA ($P<0.01$), tended to increase the content of not identified fatty acids and tended to decrease the saturated fatty acids ($P<0.10$). Concluding, the soybean oil, and not the whole soybean meal, to reach 7% lipid in the diet of milking cows, increases the content of CLA in the milk fat.

**Key Words:** Conjugated linoleic acid, Milk fat, Supplementary fat

**344 Properties of docosahexaenoic acid enriched milk, Cheddar cheese and butter.** H. Wang* and A. Hill, *University of Guelph, Ontario, Canada*.

Physical, chemical, sensory and processing properties of docosahexaenoic acid (DHA) enriched dairy products (milk, Cheddar cheese and butter) were investigated. DHA enriched milk was obtained by feeding fish meal supplement to Holstein cows. DHA level in the milk was increased to 0.4% of total fatty acids, while the total fat level in the milk was reduced to 2.43% due to fish meal's inhibition effects on fat synthesis. As drinking milk, no difference was found between DHA milk and control milk in terms of color, flavor and stability. Cheddar cheese made from DHA enriched milk ripened faster after three months of ripening and developed a more desirable texture and stronger Cheddar flavor. The yield efficiencies for DHA enriched and control cheese, $94.4\pm2.44$ and $96.4\pm2.26$, respectively, were not significantly different ($p<0.05$). Relative to controls, average fat globules size was smaller in DHA milk and churning time of DHA cream was longer. DHA butter showed a softer texture and better cold spreadability and butter oils from DHA enriched milk had a lower dropping point compared with control butter oil (32.89 versus 34.06°C). The result of this study showed the feasibility of producing DHA enriched dairy products, such as pasteurized milk, Cheddar cheese and butter, from DHA enriched milk obtained by feeding lactating cows with fish meal supplement.

**Key Words:** DHA, Docosahexaenoic Acid, Functional Dairy Products

**345 Influence of feeding cows fish oil, extruded soybeans, or their combination on the composition of milk, cream, and butter.** N. Ramaswamy*, R. J. Baer, D. J. Schingoethe, A. R. Hippen, L. A. Whitlock, and K. M. Kasperson, *MN-SD Dairy Foods Research Center, South Dakota State University, Brookings*.

Milk was collected from eight multiparous Holstein and four multiparous Brown Swiss cows, which were randomly distributed into four treatments in a replicated 4 x 4 Latin square design with 4 week per period. The four treatments consisted of a control diet (C) with a 50:50 ratio of forage to concentrate, a C diet with 2% added fat from menhaden fish oil (FO), a C diet with 2% added fat from extruded soybeans (ES), and a C diet with 1% added fat from menhaden fish oil and 1% added fat from extruded soybeans (FOES). Milk fat content from the C, FO, ES, and FOES treatments were 3.31, 2.56, 3.47, and 2.94%, respectively. Treatment milks were processed into cream and butter. Raw milk and butter from FO and FOES treatments had a four-fold increase ($P < 0.05$) in the concentration of cis-9 trans-11 isomer of conjugated linoleic acid when compared to the C treatment, and a two-fold increase ($P < 0.05$) when compared to the ES treatment. Butter made from the ES treatment was the softest. A 300 person consumer survey comparing the C and FO milks found no difference in milk flavor.

**Key Words:** Conjugated Linoleic Acid, Milk, Butter

**346 Storage stability of frozen sheep milk.** S.L. Rauschenberger*, B.J. Swenson, and W.L. Wendorff, *University of Wisconsin, Madison*.

Sheep milk is produced only on a seasonal basis, so the possibility to produce sheep milk products year-round lies on the ability to freeze large quantities of milk while maintaining the quality of fresh milk. Fresh sheep milk was divided into two portions, frozen, and stored at two different temperatures, -15°C and -27°C. Over a one-year period, samples were removed every three months, thawed, and analyzed for coliform count, standard plate count, acid degree value, and protein destabilization. Results showed that freezing at -15°C was not adequate for maintaining stability throughout the storage period. In the samples stored at -15°C protein precipitated between six and nine months while no precipitation occurred in the samples stored at -27°C. Samples at both temperatures showed a decrease in coliform and standard plate counts. Acid degree values were significantly higher after six months of storage at -15°C than at -27°C. Various pre-freezing treatments were performed on the whole milk to evaluate their effect of protein stability of samples stored at -15°C. This included lactose hydrolysis (50%), a heat treatment (68°C/ 25 min), and the addition of sodium hexametaphosphate (4 g/ L). At 1,3,6,9 and 12 months of storage, the samples were removed and analyzed for acid degree value and protein destabilization.

**Key Words:** Sheep, Milk, Frozen

**347 Effect of shelf-life and light exposure on acetaldehyde concentration in milk packaged in HDPE and PETE bottles.** M. Van Aardt*, S.E. Duncan, D. Bourne, J.E. Marcy, T. Long, and C.R. Hackney, *Virginia Tech, Blacksburg*.

Whole milk (3.25% milkfat) was packaged in clear glass, high density polyethylene (HDPE), clear polyethylene terephthalate (PETE), clear PETE with UV barrier, and amber PETE containers and exposed to fluorescent light at 1100-1300 lux (100–120 FC) for 18 days at 4°C. Two levels of light exposure (light-exposed or light-protected) were evaluated for each packaging treatment. Control (glass) and treated milks were evaluated by sensory and instrumental methods on day 0, 7, 14, and 18 of storage. Intensity of oxidation, acetaldehyde, and lacks freshness off-flavors was determined by eight experienced panelists. No significant difference was found in acetaldehyde off-flavor between bottle treatments (exposed or protected). The concentration of acetaldehyde that developed over time in any of the containers also never exceeded flavor threshold levels for acetaldehyde in milk. In light-exposed samples, oxidation off-flavor was significantly less when packaged in amber PETE versus the other containers. Milk packaged in HDPE containers also showed a significantly higher level of oxidation off-flavor on day 7 and 18 than milk packaged in PETE containers with UV block, but not from clear PETE or glass containers. Lacks freshness off-flavor increased over time, as expected, in light-exposed and light-protected bottles and was more intense in the light-exposed containers over the first fourteen days. Instrumental analysis (solid phase microextraction gas chromatography) showed increases in compounds associated with light oxidized flavor (acetaldehyde, pentanal, dimethyl disulphide (DMDS), and hexanal) in light-exposed samples over time.

**Key Words:** Acetaldehyde, Oxidation, Polyethylene terephthalate

**348 Rheological properties of aging Monterey Jack goat cheese.** D. L. Van Hekken*[1], M. H. Tunick[1], and Y. W. Park[2], [1]*USDA, ARS, ERRC, Wyndmoor, PA*, [2]*Agric. Res. Station, Fort Valley State University, GA*.

There is a variety of characteristics that define goat cheese quality that needs to be identified and measured. We used different rheological methodologies to characterize the texture of Monterey Jack goat cheese as it aged. Two lots of high moisture Monterey Jack cheese (43.4 and 45.8% moisture) were manufactured at the university's dairy processing facilities. The rheological properties were analyzed after 1, 4, 8, 16, and 26 weeks of storage at 4°C. Cheeses were assayed for meltability and

their rheological properties were determined using torsion, small strain dynamic, and texture profile analyses. All cheeses decreased in hardness and tended to increase in meltability and springiness with storage. Rheological properties measured using torsion and small strain dynamic analyses changed the most within the first two months of storage, then remained fairly constant. As the cheese aged, the samples became softer with decreased hardness, shear stress, and shear rigidity; more elastic with increased elastic modulus; and more viscous with increased viscous modulus and complex viscosity. Texture mapping of torsion data indicated that the cheese became more rubbery as it aged. Rheological properties were influenced by the moisture content of the cheese.

**Key Words:** Goat Cheese, Rheology, Texture

**349 Electrophoretic characterization of aging Monterey Jack goat cheese.** D.L. Van Hekken*[1] and Y.W. Park[2], [1]*USDA, ARS, ERRC, Wyndmoor, PA,* [2]*Agric. Res. Station, Fort Valley State University, GA.*

Monitoring the proteolysis in cheese is one way to define and characterize cheese. We used SDS- and urea-PAGE to study the protein profiles of Monterey Jack goat cheese over 6 months of storage. Two lots of Monterey Jack goat cheese were manufactured at the university's dairy processing facilities. Proteins were extracted for electrophoretic analyses after 1, 4, 8, 16, and 26 weeks of storage at 4°C. Samples were homogenized in either Tris-SDS or urea buffers, centrifuged, filtered, and the supernatant lyophilized. The urea preparation included a two hour incubation at 37°C after homogenization and required overnight dialysis before lyophilizing. Extracts were prepared for SDS- or urea-PAGE and separated using the PhastSystem. Stained gels were scanned and the protein profiles quantified using a densitometer. As expected with goat cheese, protein profiles showed that beta-casein was the major protein present, followed by alpha$_{S2}$-, kappa-, para kappa-, and alpha$_{S1}$-caseins. With aging, casein bands decreased or disappeared and peptide bands appeared. Samples, prepared using urea and incubated for 2 hours at 37°C, had more peptide bands, an indication that more proteolysis occurred in these preparations. After 6 months of aging, very little or no alpha$_S$-, kappa-, or para kappa-casein remained and over 40% of the beta-casein had disappeared. SDS-PAGE, which separates proteins based on molecular mass, produced profiles with twice as many peptide bands than profiles produced using urea-PAGE, which separates proteins based on charge/mass ratio. The protein profiles obtained in this study show the proteolysis of beta-casein that is unique to goat cheese and will help in identifying the protein characteristics of quality goat cheese.

**Key Words:** Electrophoresis, Goat Cheese, Proteolysis

**350 Evaluation of sensory and chemical properties of Manchego cheese manufactured from ovine milk of different somatic cell levels.** J.J. Jaeggi*[1], K.B. Houck[1], M.E. Johnson[1], R. Govindasamy-Lucey[1], B.C. McKusick[2], D.L. Thomas[2], and W.L. Wendorff[2], [1]*Center for Dairy Research, University of Wisconsin-Madison ,* [2]*University of Wisconsin-Madison.*

As ovine milk production increases in the United States, somatic cell count (SCC) is increasingly used in routine ovine milk testing procedures as an indicator of flock hygiene and health. Ovine milk was collected from one hundred and fifteen East Friesian-cross ewes milked twice daily. The milk was segregated and categorized into three different average SCC per ml: 6.10 X $10^4$, 1.15 X $10^5$, and 2.19 X$10^6$. Milk was stored in 19 L pails, frozen at -19°C, and held for 4 months. Milk was then thawed at 7°C over a two-day period before pasteurization. Casein decreased with increasing SCC levels from 3.99, 3.97, to 3.72%. Casein to true protein ratio decreased with increasing SCC levels from 81.42, 79.66, and 79.32. Milk fat varied from 5.49, 5.67, and 4.86% respective to the SCC levels of 6.10 X $10^4$, 1.15 X $10^5$, and 2.19 X$10^6$. Manchego cheese was manufactured from the three different SCC lots. A licensed Wisconsin cheese maker subjectively evaluated coagulum development and firmness. As the level of SCC increased, the first signs of flocculation increased resulting in longer times to reach desired firmness for cutting. Yield at one day decreased from 16.03 to 15.97 to 15.09% with increasing SCC levels. There were no differences in cheese composition and pH. Cheeses were coated with a polymeric cheese coating and ripened at 85% humidity at 7°C over the duration of 9 months. Lower yield was attributed to lower casein and fat content of the higher SCC milk. Cheese samples were analyzed at 1 day, 3, 6, and 9 months for soluble nitrogen and both total and individual free fatty acids. Cheese graders noted increased levels of rancidity at the higher SCC level cheeses at each of the sampling points. No differences were noted in cheese texture between the different SCC levels.

**Key Words:** Ovine, Manchego, Somatic cells

**351 Origin and behaviour of acid phosphatase in Cheddar cheese during ripening.** R. Akuzawa*[1] and P.F. Fox[2], [1]*Nippon Veterinary & Animal Science, University, Tokyo, Japan,* [2]*University College Cork, Cork, Ireland.*

Casein is a phosphoprotein, and dephosphorylated proteins/peptides are hydrolysed at a faster rate by lactococcal proteinases/peptidases than phosphorylated proteins or peptides. The combined action of proteinases/peptidases and phosphatases may be important for cheese quality. In preliminary work, the acid phosphatases (AP) from skim milk (SM-I and SM-II), butter milk (BM) and of the cell menbrane (CM) and cell wall (CW) of *Lactococcus lactis* ssp *lactis* 303 were purified or partially purified. The proteinase/peptidase activity of strain 303 on dephosphorylated casein/phosphopeptides [a mixture mainly $\alpha$s2-CN (f1 - 32) and $\beta$-CN (f1 - 28)], which were hydrolysed by the AP from SM-I, SM-II, BM, CM and CW, was determined by liberated amino acids and peptides. The proteinase/peptidase released more peptides and amino acids from the phosphopeptides which had been dephosphorylated by the AP from SM-II or CM. The AP activity in the water-soluble fraction of Cheddar cheese which had been made using strain 303 as starter, decreased slightly during ripening but the total AP activity and that in the water- insoluble fraction increased slightly. The APs SM-II and CM, which were present in water-insoluble fraction, seemed to contribute to the dephosphorylation on phosphopeptides during

**Key Words:** acid phosphatase, cheese

**352 Effect of homogenization pressure and selected additives on some physical properties of retort-processed dairy beverages.** C.A. Lin* and R.L. Richter, *Texas A&M University, College Station, TX.*

The objective of this study was to determine the effect of sodium citrate, locust bean gum, and iota-carrageenan on retort-processed dairy beverages. Changes in particle size distribution, apparent viscosity, and creaming rate during storage were examined. Skim milk, cream, nonfat dry milk, and sucrose were mixed to make beverages with 3% milk fat, 11% milk solids nonfat, and 8% sucrose with either 0.1% sodium citrate, 0.05% locust bean gum, or 0.05% iota-carrageenan. The mixtures were heated to 70°C, homogenized at 20, 50, or 90 MPa, canned, and retorted at 121°C for 6 min. The processed products were stored at 36°C and tested after 1, 10, 20, 30 days of storage. The creaming rate decreased as the homogenization pressure was increased. This change was concomitant to the decreased particle size associated with increased homogenization pressure. The effect of homogenization pressure on Dvs became more apparent as storage time increased. The particle size increased with increased storage time for samples homogenized at 20 MPa but the particle size distribution of samples homogenized at 90 MPa did not change during storage. Samples containing locust bean gum had a slower creaming rate than samples that contained sodium citrate during storage. The viscosity was also higher in samples that contained locust bean gum and were homogenized at 20 MPa. Samples that contained iota-carrageenan and homogenized at 20 MPa had excessive viscosity and coagulated within 10 days of storage. Samples that contained iota-carrageenan and homogenized at 90 MPa were less viscous and did not gel. Samples homogenized at 90 MPa had no or little creaming or coagulation during storage regardless any of the added ingredients.

**Key Words:** Homogenization pressure, Stabilizers, Retorted Dairy Beverages

**353 The effect of bitter flavor on the consumer acceptability of coffee flavored ice cream.** L.F. Osorio* and J.U. McGregor, *Louisiana State University, LAES, LSU Agricultural Center, Baton Rouge.*

Fresh coffee extracts were used to manufacture coffee-flavored ice cream. A medium dark Brazil and dark Espresso roast coffee were extracted by hot pressure and cold brewing methods. Flavored ice cream mixes

were analyzed for total solids, pH, color, and apparent viscosity. A 12-member trained panel performed a triangle test to establish if there was a difference between coffee ice cream flavored before or after pasteurization. A consumer panel of 138 people was also used to evaluate the four treatments for bitterness and sweetness intensity as well as for overall preference. There were no significant differences in pH, viscosity, color and total solids. The trained panel detected significant differences between coffee ice cream flavored before and after pasteurization ($p<0.05$). The espresso-hot pressure extracted coffee ice cream had a more intense bitter flavor and was less preferred as determined by the consumer panel ($p<0.05$). Dairy processors should consider the potential for bitter flavor defects when selecting and processing coffee flavored dairy products.

**Key Words:** Bitter Flavor, Coffee Flavorings, Ice Cream

**354 Acceptable usage levels of textured whey proteins in hamburger patties.** A. Hale*, C. Carpenter, and M. Walsh, *Utah State University, Logan*.

Whey proteins, textured by thermoplastic extrusion, can be used as meat extenders. The objective of this research was to determine the maximum acceptable usage level of textured whey protein (TWP) in hamburger patties. Instrumental and sensory analysis were conducted on 1/4 lb patties containing 0, 30, 40 and 50% by weight hydrated TWP. All patties were standardized to 13.6% total fat. For sensory analysis, cooked samples were assigned random three-digit numbers, rotated on ballot position, and served under red lights, in an open consumer panel. Panelists were asked to evaluate samples on a nine-point hedonic scale for tenderness, juiciness, texture, flavor, and overall acceptability. In all categories, no differences ($p > 0.05$) were observed between the all beef control and patties containing 30 and 40% TWP, but the patties containing 50% TWP scored lower in texture and beef flavor. The peak force needed to break cooked patties was measured using an instrumental probe. Ten patties from each level of TWP were analyzed. There were no differences among the peak break forces of patties containing 30, 40 or 50% TWP. The peak break force was higher ($p < 0.05$) for the all beef control than for patties containing TWP. This indicates that there may be a possible difference in cohesiveness of patties containing TWP although patties made with up to 40% TWP were equally liked as the all beef control.

**Key Words:** Textured whey protein, Hamburger patties, Sensory analysis

**355 Estimating milk density from milk composition and temperature.** A. Ueda and A. Hill*, *University of Guelph, Ontario, Canada*.

Equations to estimate milk density at 4.0°C from fat, protein, and other solids (LOS) contents were developed based on data collected over a whole year from Ontario and Alberta milk producers (N=968). Density was measured using an Anton Par Model DMA 45 density meter. Fat, protein, and LOS contents were determined by Mojonnier, Kjeldahl, and oven methods, respectively. Temperature of 4.0°C was chosen because milk pricing is based on the weight/volume composition of producer milk at 4.0°C. The first objective of this work was to define a model to estimate milk density from weight/weight (w/w) milk composition to provide an accurate weight/volume conversion for the purpose of milk pricing. Current procedures in all Canadian provinces (except Quebec) and many other jurisdictions, use wieght/volume conversion factors (milk density values) which seriously under estimate milk components. The recommended equation is as follows: Density = 0.8469 + 0.03820F + 0.05565P + 0.03274L - 0.01080F*P - 0.007431F*L - 0.0006358P*P - 0.009314P*L + 0.002095F*P*L, where F is %fat(w/w), P is %protein (w/w), and L is other solids (%w/w) Weight over weight (w/w) to weight over volume (w/v) conversions of milk components employing the developed equation were compared to conversions obtained using empirical density values measured at 4.0°C. The average difference between estimated and actual w/v values was 0.000 with standard deviation of 0.002 Kg/hL. The equation could be used to provide more accurate and equitable milk pricing without the requirement for empirical measurement of milk density. Formulae were also constructed to estimate density of producer milk at temperatures of 4.0 to 40.0°C (N=792). The best model required a cubic term for temperature. Standard deviation of residuals (predicted density - empirical density) was .00023 Kg/L.

**Key Words:** Milk density , Weight/volume conversion, Pricing

**356 Stability of vitamin A and D in skim milk delivered by beta-lactoglobulin isolated from whey.** Q. Wang, J. Allen, and H. Swaisgood, *Southeast Dairy Foods Research Center, North Carolina State University, Raleigh*.

We have reported that retinyl-Celite matrix can be used for effective isolation of $\beta$-lactoglobulin ($\beta$-LG) from whey owing to its selective adsorption of $\beta$-LG. The $\beta$-LG obtained in this way has strong binding affinity for fat-soluble nutrients, such as vitamins A, D, E and K and some fatty acids, and might be used to fortify these nutrients in non-fat or low-fat foods. A substitute for oil to deliver the essential nutrients in low-fat and non-fat foods has been sought because fat-soluble nutrients have low stability without a lipid environment. Certain nutritional proteins, such as $\beta$-LG isolated from whey by bioselective adsorption, are attractive for this purpose because of their special structure and ability to reversibly bind the vitamins. The vitamins A and D in skim milk fortified by $\beta$-LG isolated by selective adsorption was determined by saponification of skim milk, extraction of the vitamins from the milk and application of an modified HPLC method to measure the stabilities of the complexes. The skim milk was fortified with vitamins A and D to 1.16 $\mu$g/ml and 10.6 ng/ml, respectively, with vitamin-bound $\beta$-LG to match the levels required by regulation for fortified skim milk. A three-week shelf life study in one half gallon polyethylene bottle showed that both vitamins A and D remained in skim milk without measurable loss. The accuracies of the methods used to determine vitamins A and D were 3 and 6 percent, respectively, in the test period.

**Key Words:** vitamins A and D, beta-lactoglobulin, stability

**357 Selective purification of $\alpha$-lactalbumin from whey protein isolate using a peptide ligand obtained from a combinatorial library.** P.V. Gurgel*, R.G. Carbonell, and H.E. Swaisgood, *North Carolina State University, Raleigh*.

Alpha-lactalbumin is a low molecular weight (14.2 KDa) globular whey protein that acts as a regulator for the enzyme lactose synthase in the biosynthesis of lactose. Because of its high digestibility and lower potential for causing allergic response when compared to beta-lactoglobulin, alpha-lactalbumin is a good candidate as an ingredient for infant formulas. In this work we report a method for purification of alpha-lactalbumin from whey protein isolate (WPI) by bioselective adsorption using ligands from a combinatorial peptide library. A 0.5 mL sample of 5.55 g/L WPI in 50 mM phosphate buffer, pH 7.0 was applied to a 0.6 mL column containing the peptide WHWRKR immobilized onto a polymethacrylate resin. The column was able to bind the target protein, and two fractions rich in alpha-lactalbumin were obtained, eluting in 0.1 M NaCl and 0.25 M NaCl, respectively. The first fraction appears to be slightly contaminated with beta-lactoglobulin and lactoferrin. The second fraction contained alpha-lactalbumin and lactoferrin. Densitometric measurements established that the concentration of alpha-lactalbumin in the original sample of WPI was 30.3%, while in the first fraction it was increased to 97.3%. Thus, the method increased the concentration of lactalbumin 3.3-fold in one step.

**Key Words:** Alpha-Lactalbumin, Peptide Ligands, Bioselective Adsorption

**358 Fractionation of bovine transferrin from whey using immobilized gangliosides.** M.K. Walsh* and S.H. Nam, *Utah State University, Logan*.

Bovine transferrin (BTF) is major-iron transport and regulator protein found in bovine whey. In order to mediate its roles in cell proliferation and differentiation, BTF must interact with eukaryotic cell surfaces. As common components of eukaryotic cell surfaces, gangliosides were used for the affinity fractionation of BTF. The objective of this research was to fractionate BTF from whey protein isolate solution using an immobilized ganglioside matrix. BTF was further purified using Con-A affinity and ion exchange chromatography. BTF was identified by SDS-PAGE and Western analysis. Gangliosides, organically extracted from bovine buttermilk, were covalently immobilized onto controlled-pore glass beads. The immobilized matrix contained 66 micrograms of gangliosides per gram beads. After loading the immobilized matrix, 2 g beads, with a 2% whey solution, the matrix was washed with sodium acetate buffer at pH 4 containing 1 M NaCl before elution of BTF with sodium phosphate at pH 7. SDS-PAGE analysis showed a 74.2% BTF recovery from whey. BTF was enriched to 24% and 61% purity with

Con-A affinity and ion exchange chromatography. The Con-A affinity and ion exchange chromatography steps enriched BTF in the samples and removed other whey proteins from ganglioside purified fractions. BTF appeared in SDS-PAGE and Western analysis as a dimer of 72 and 76 kDa. These results indicate that immobilized ganglioside can be used to easily fractionate BTF from bovine whey.

**Key Words:** Bovine transferrin, Gangliosides, Purification

**359 Fractionation of peptide mixtures from $\beta$-lactoglobulin enzymatic hydrolysate by means of isoelectric focusing.** P.E. Groleau*[1,2], Y. Pouliot[1], S.F. Gauthier[1], and R. Jimenez-Flores[2], [1]*Centre de recherche STELA, Universite Laval, Quebec City, Canada,* [2]*Dairy Product Technology Center, California Polytechnic State University, San Luis Obispo.*

Enzymatic hydrolysis improves nutritional and functional properties of dairy proteins. Protein hydrolysates often have to be fractionated to obtain peptides with higher functionality or better nutritional value in a more purified form. The fractionation of peptide mixtures can be achieved by nanofiltration (NF), a separation technique using charged membranes. However, the occurrence of peptide-peptide attractive/repulsive interactions in the mixtures will have an impact on NF fractionation since these interactions affect the charge/mass distribution within the peptide mixture. The objective of this study was to investigate the use of isoelectric focusing (IEF) for the separation of a peptide mixture from a tryptic hydrolysate of $\beta$-lactoglobulin, in order to identify peptide-peptide interactions. IEF was performed on a Rotofor cell (Bio-Rad) using the peptide mixture without added ampholytes, so that the amphoteric nature of the peptides would generate the pH gradient for their resolution, based on the isoelectric point (pI) of each peptide. IEF fractionation of the peptide mixture led to 20 peptide fractions having a pH value from 2.5 to 11.6. The results showed that most peptides in the hydrolysate have their pI between pH 4 and 7, and few around pH 11 (10%). Methanol (25%) influences the pH gradient generated. All the fractions were analyzed by RP-HPLC (C18 column) for characterization of its peptidic content. Each peptide fraction was also suspended in phosphate buffer (pH 2.5, 0.1 M) and further separated using capillary electrophoresis (CE) with and without dissociating agents (Urea, DTT) in order to minimize peptide-peptide interactions. Preliminary results showed that urea (1M) has no effect on the electrophoregrams while DTT (70mM) changes only the profile of the fractions having low pI (pH 4).

**Key Words:** $\beta$-lactoglobulin, Peptides, isoelectric focusing

**360 Effect of hydrodynamic conditions on the fractionation of a $\beta$-lactoglobulin peptide mixture by nanofiltration membranes.** J-F. Lapointe*, Y. Pouliot, and S. F. Gauthier, *Centre de recherche STELA, Quebec, Canada.*

The fractionation of a $\beta$-lactoglobulin peptide mixture of by nanofiltration (NF) membranes has been investigated. Enzymatic hydrolysates were prepared by tryptic (TH) hydrolysis of commercial $\beta$-lactoglobulin followed by UF treatment using a 10 000 g.mol$^{-1}$ MWCO membrane in order to remove the enzyme and non-hydrolyzed material from the reaction mixture. The peptide mixture, which was adjusted at pH 9.0, was further fractionated using a SG13 (Osmonics, Minnetonka, MN) cellulose acetate NF membrane with a molecular weight cut-off (MWCO) of 2500 g.mol$^{-1}$. The effect of hydrodynamic parameters was studied by including a total of 16 condition combinations in a complete random design: duration of the filtration (30 and 120 min), recirculation rate (1.5 and 2.5 L.min$^{-1}$), transmembrane pressure (200 and 500 kPa) and peptide concentration (1 and 5 g.L$^{-1}$). All the experiments were performed in triplicate on a 2L peptide solution using a SEPA-CF plate and frame module (Osmonics) in a full recycle mode at 40°C. Permeation rate (L.min$^{-1}$) was measured at 15 min intervals during the filtration and 25 mL permeate aliquots were collected at the end of each trial for further analysis of nitrogen transmission and peptide distribution by reverse phase chromatography (RP-HPLC). Preliminary results show that in high peptide concentration (5 g.L$^{-1}$) conditions, both recirculation rate and transmembrane pressure have an important effect on the membrane selectivity for acid and basic peptides. These results suggest that the control of the hydrodynamic conditions used in NF, in regard of the polarization concentration layer, can potentially modify the selectivity of peptide fractionation.

**Key Words:** Nanofiltration, Peptide fractionation, Enzyme specificity

**361 Modification of rheological properties of whey protein isolate through limited crosslinking with microbial transglutaminase immobilized on porous glass.** C.P. Wilcox* and H.E. Swaisgood, *North Carolina State University, Raleigh.*

The desire of the food industry to convert waste products into value-added, high-priced commodities has inspired a growing interest in the development of processes for the enhancement of whey protein functionality. Physical and chemical methods commonly used for this purpose include thermal treatment and acid hydrolysis. Enzymatic methods are also utilized but are often disregarded due to the cost of continuous replacement of enzyme. Microbial transglutaminase(mTG), which forms isopeptide bonds in proteins, is distinct from its mammalian counterpart in that it is smaller, more stable, and is calcium independent. We developed a process by which mTG was immobilized to a glass bead matrix allowing for limited crosslinking of whey protein isolate. Biotin was covalently bound to 10 mL of controlled-pore glass, followed by adsorption of avidin. The enzyme was then biotinylated using NHS-LC biotin during which there was no significant reduction in activity. An 8% solution of whey protein isolate (WPI) was prepared in 50 mM sodium phosphate containing 2 mM dithiothreitol and 2 mM calcium chloride, pH 6. The WPI was then recirculated over the beads for one to four hours immersed in a water bath held at 40°C. Crosslinked WPI was tested for viscosity using a temperature ramping protocol. Gelation properties were analyzed using a StressTech rheometer. Samples were brought to the desired denaturation temperature, held for 18 min, and cooled to room temperature for gel formation. Small-strain oscillatory measurements were taken to determine gel strength and elasticity. When compared with native WPI, crosslinked WPI exhibited greater viscosity and distinctive gel characteristics. This process allows for recycling of the enzyme, eliminates the requirement for a downstream inactivation step, and permits the precise control over the extent of crosslinking. The functional properties of WPI were repeatedly modified using the same reactor, illustrating the capacity of immobilized enzymes to be used more frequently in applications of this nature.

**Key Words:** Transglutaminase, Immobilization, WPI

**362 Study of protein-polysaccharide interactions, using whey protein-dextran systems.** G. Lemay* and S. L. Turgeon, *Dairy Research Center, Laval University, Quebec, Canada.*

The food we eat every day is mainly made of proteins and polysaccharides. These biopolymers play a major role in the stability and structure of our food products. The study of protein-polysaccharide interactions is therefore essential to the understanding and prediction of food behavior. In aqueous solution, proteins and polysaccharides interact to form co-soluble, incompatible or complex systems. The behavior of a whey protein isolate (WPI) with a neutral or anionic dextran (ratio 10:1; 5:1; 2:1; 1:1; and 1:2) was studied by phase diagrams at pH 5.2 and 7.0. When incompatibility and phase separation occurred, the partition of each phase was identified and quantified by means of polysaccharide and protein dosage using the phenol-sulfuric and the Kjeldahl methods. These dosages revealed that the dextran was found in the superior phase with most of the solvent whereas the WPI was found to be concentrated in the inferior phase. The effects of the different ratios of WPI-dextran sulfate, pH and the presence of NaCl on the WPI behavior was also studied by differential scanning calorimetry (DSC). Dextran sulfate added to WPI seems to have a protective effect on its denaturation temperature and to raise it up by 3 to 7 degrees Celsius. Adding NaCl had a similar effect but adding simultaneous NaCl and dextran sulfate had no additive effect. In this case, only the effect of the salt prevails. This study gave us a better understanding of the behavior of protein-polysaccharide systems and confirmed the electrostatic nature of the WPI-dextran interactions. Further studies of these systems would be interesting to demonstrate the stabilizing role dextrans can play in real food systems.

**Key Words:** Whey Protein Isolate, Dextran and Dextran Sulfate, Protein-Polysaccharide Interactions

**363 Interactions between diatomites or synthetic silicates and calcium phosphocaseinate: effect of adsorbent properties.** N. Martin*[3], Y. Pouliot[1], R. Jimenez-Flores[2], M. Britten[3], and P.S. Tong[2], [1]*Centre de Recherche en Sciences et Technologie du Lait, Ste-Foy, Quebec, Canada,* [2]*Dairy Products Technology Center, San Luis Obispo, California,* [3]*Food Research and Development Center, St-Hyacinthe, Quebec, Canada.*

The ability of diatomite Celite®512, synthetic magnesium silicate Celkate®T21 and synthetic calcium silicate Silasorb® to modify the casein ratio of calcium phosphocaseinate was under investigation. The adsorption treatments were made by mixing 10% of these adsorbents with calcium phosphocaseinate solution (5% total protein) for 30 minutes under constant agitation. Centrifugation was used to separate the adsorbents from the treated solution. Magnesium silicate showed the higher adsorption capacity of all the adsorbents with 13.69g protein per 100g adsorbent. The ratio of caseins to proteins other than caseins was increased from 7.80, in untreated calcium phosphocaseinate, to 17.86 after treatment with this adsorbent. The enrichment in caseins may be explained by the specific affinity of magnesium silicate for whey proteins. The ratio between casein fractions was also affected by treatment with magnesium silicate because of its ability to bind $\beta$-caseins more specifically. Calcium silicate and diatomite adsorption capacities were much lower than magnesium silicate with 5.00 and 1.15g protein per 100g adsorbent respectively. Adsorption treatments with both calcium silicate and diatomite enabled the enrichment of calcium phosphocaseinate in $\alpha$-caseins and $\alpha$-lactalbumins but to different extents depending on the adsorbent used. Moreover, calcium silicate seemed able to specifically remove components other than proteins. In order to get a further comprehension of the adsorbents behaviour, the determination of the active sites at their surface, concurrently with titration analysis were made. The results allowed us to make hypothesis on the chemical and physical state of the adsorbents in aqueous media. However, each adsorption behaviour could not be fully explained only by using results from surface characterization.

**Key Words:** Calcium phosphocaseinate, Adsorption treatments, Active sites

**364 Influence of fractionation sequence and filtration temperatures on the physical and chemical properties of milk fat fractions.** K.E. Kaylegian*[1], [1]*Wisconsin Center for Dairy Research, Madison.*

Milk fat fractions were produced using 3 lots of commercial anhydrous milk fat, in a total of 8 production cycles, each consisting of 2 to 5 fractionation steps. Milk fat fractions were produced on a pilot scale (50 kg) using the Tirtiaux crystallization process and separated with pressure filtration (3 bar). The number of fractionation steps and filtration temperatures were varied to obtain fractions with the most value as food ingredients. Other processing parameters (e.g., cooling profiles and agitation rates) were set to obtain well-formed, filterable crystals, with sufficient yields. First step fractions were obtained at 28, 26, and 21°C, second step fractions at 18, 17, 15, and 12°C, third step fractions at 12, 10, 9, and 8°C, fourth step fractions at 11 and 8°C,and fifth step fractions at 10, 9, and 8°C. The liquid fraction at each step was generally refractionated to improve the yield of the lowest melting fraction in the sequence. Fractions were characterized for yield, dropping point, solid fat content profile, fatty acid composition, and triglyceride composition. The first step solids were all very high melting fractions regardless of filtration temperature. The second step solids were either high or middle melting fractions, depending on the temperature sequence used. Solid fraction obtained at the third through fifth steps were middle or low melting fractions. Distinct difference in properties were observed for fractions filtered at 15 compared with 12°C. Yet, fractions obtained at 11 or 12°C showed similar properties whether they were obtained at the second, third or fourth steps. The differences observed between these fractions were consistent among production cycles, milk fat lots, and the number of fractionation steps used. To obtain a good quality very low melting fractions, a minimum of three steps were required. Processing was easier and fraction characteristics were better when the previous step had a filtration temperature no greater than 12°C.

**Key Words:** Milk fat, Fractionation, Milk fat fractions

**365 Low cholesterol Mozzarella cheese obtained from homogenized and beta-cyclodextrin-treated milk.** H. S. Kwak*, C. G. Nam, and J. Ahn, *Sejong University, Seoul, Korea.*

The effects of homogenization conditions and $\beta$-cyclodextrin ($\beta$-CD) on cholesterol removal of Mozzarella cheese was examined. The homogenization pressure influenced markedly on the cholesterol removal in milk, and 75.64% of cholesterol, the highest rate was removed at 1000 psi. In addition, an increase in temperature resulted in an increase of cholesterol removal in the range of 71.75% to 78.22%. Among different concentrations of $\beta$-CD addition, 1.0% showed 78.21% of cholesterol removal. Therefore, cholesterol-reduced Mozzarella cheese was made by cheese milk treated with 1000 psi homogenization at 70°C and 1% $\beta$-CD addition for a subsequent study. In results, meltability, stretchability, and oiling off in cholesterol-reduced cheese were significantly lower than those in control. Hardness, gumminess and chewiness were significantly reduced, while cohesiveness and elasticity increased. Appearance and flavor of the cheese were superior, but texture inferior to the control. This study showed a possibility in the manufacture of cholesterol-reduced cheese by the application of $\beta$-CD.

**Key Words:** Cholesterol removal, Beta-cyclodextrin, Mozzarella cheese

**366 Configuration of an unconventional bioreactor for milk lactose hydrolysis.** A.N. Genari[1], F.M.L. Passos*[1], and H.E. Swaisgood[2], [1]*Universidade Federal de Vicosa, Vicosa, Brazil,* [2]*North Carolina State University, Raleigh.*

Permeabilized microbial cells can be used as a crude enzyme preparation for industrial level processes. Immobilization and subsequent reuse of the bioreactor can compensate for the low specific activity of this preparation. Alginate beads are a common support for biomass immobilization; however, this configuration has a low surface area and requires low biomass concentration for bead strength. We have designed a biocatalyst consisting of a paste of permeabilized *Kluyveromyces lactis* cells gelled with manganese alginate over a semi-circular stainless steel screen. A ratio of wet permeabilized biomass to alginate of 50:4 (w/w) resulted in a paste with maximum gel biomass retention. The biocatalyst was more stable when stored in milk at 4°C than in 50% glycerol. The unused biocatalyst stored in milk did not lose activity after 50 days. However, operational use through 40 cycles resulted in 50% loss of activity. An unconventional bioreactor for milk lactose hydrolysis was designed using this biocatalyst. Semi-circular screens were stacked in a column with circumferential cuts placed alternately to permit continuous serpentine flow of milk through the column, thus producing maximum contact between the milk and biocatalyst surface during the hydrolysis process. The bioreactor was operated as a packed bed or with recirculation (essentially a CSTR) at 40°C. The maximum activity was observed when operated with recirculation. The continuous system with recirculation resulted in 82.9% lactose hydrolysis at a flow rate of 2.0 mL/min, indicating the potential of this system for processing low lactose milk. This system could be used for processing other substrates using an appropriate biocatalyst.

**Key Words:** Lactose hydrolysis, Immobilized cell bioreactor, Kluyveromyces lactis cells

**367 Application of optical light microscopy to monitor air cell changes in ice cream during freezing in a batch freezer.** Y.H. Chang*, R.W. Hartel, and R.W. Hartel, *University of Wisconsin, Madison.*

Air cell size is one of the important aspects of ice cream structure that determines product quality. An optical light microscopy method was developed as an alternative to scanning electron microscopy to monitor dynamic air cell size changes in ice cream during freezing in the batch freezer. Effect of fat, stabilizer, and emulsifier content on the change in air cell size during freezing were studied. The effects of whipping temperature, under both freezing and nonfreezing conditions, were also studied. Air cell size decreased progressively during batch freezing. High concentration of stabilizer affected air cell size distributions at early drawing times when ice cream was still in slurry state because of higher mix viscosity. Formula changes had relatively low impact on air cell size changes in ice cream at later stage of freezing. Freezing conditions were necessary to obtain high overrun and small air cell sizes, since whipping of ice cream mix at elevated temperatures (0 and 20°C) resulted in much less overrun.

**Key Words:** Ice cream, Air cell, Freezing

**368 Isolation and characterization of lactococcal bacteriophages infecting EPS-producing strains.** H. Deveau* and S. Moineau, *Universite Laval, Quebec, Canada.*

Starter cultures used to manufacture buttermilk are composed of *Lactococcus* and *Leuconostoc* strains. The *Lactococcus* strains are mainly employed to acidify milk and to produce exopolysaccharides whereas the *Leuconostoc* strains are responsible for the flavor development. It is well documented that milk acidification can be slowed down by lytic phages. Here, we report that exopolysaccharide production can also be impaired by lytic lactococcal phages. Forty-seven buttermilk samples, containing phages, were obtained from 13 North American plants. Eight distinct lytic phages, named Q61 to Q68, were isolated from these samples and their genomes were characterized by restriction patterns (*Eco*RI and *Eco*RV). Some of these phages were isolated from only one plant (Q63, Q66 and Q68) while others were found in several plants, including phage Q64 which was present in 8 of the 13 dairy plants tested. Using a multiplex PCR method, the 8 phages were classified within the 936 species (small-isometric heads). The host range of these phages was determined on 19 *Lactococcus* strains including 7 EPS-producing and 12-acidifying cultures. Three phages attacked the mucoid strain RA whereas the 5 other phages infected only the mucoid strain UO. The five other $EPS^+$ strains (H414, MLT2, MLT3, VFK, W12D) as well as the 12 acid strains were insensitive to the 8 phages. These results showed that $EPS^+$ producing strains are also sensitive to phages and that these phages are members of the most dominant lactococcal phage species.

**Key Words:** *Lactococcus*, Exopolysaccharides, Bacteriophages

**369 Identification and characterization of the anti-receptor gene of *Streptococcus thermophilus* bacteriophages.** M. Duplessis* and S. Moineau, *Universite Laval, Quebec, Canada.*

*Streptococcus thermophilus* is a thermophilic lactic acid bacterium widely used for the production of fermented milk products such as Italian cheeses and yogurt. The manufacture of these dairy goods has risen sharply over the past years. It is known that increased productivity within existing facilities will lead to milk fermentation failures due to lytic bacteriophages. *S. thermophilus* phages are currently classified into two groups based on their number of major structural proteins (MSP) and their mode of DNA packaging. Phages with two MSP and cohesive genome extremities (cos-type) appear to be predominant. Recently, our laboratory sequenced the first complete genome of a cos-type lytic phage (DT1). Sequence analysis suggested that one open reading frame (ORF18) may be the anti-receptor protein, which is responsible for host specificity. Homologous regions were sequenced in five other cos-type *S. thermophilus* phages (DT2, DT4, MD2, MD4 and Q5). The N-terminal (494 amino acids) and the C-terminal (189 amino acids) regions were highly conserved among the six phages. The conserved regions were interspersed by a heterologous segment located in the middle of the protein. As for the lambdoid and T-even phages, the presence of conservative and non conservative regions are characteristics of anti-receptors. One to four collagen-like repeats $(Gly\text{-}X\text{-}Y)_n$ were also found in ORF18 and they could represent recombination hot spots. A host range study was also conducted with the six phages on thirty-eight strains of *S. thermophilus*. Phages DT4 and Q5 had identical host range and ORF18 whereas the other phages had a distinct host range and ORF18. These results strongly suggest that ORF18 is the anti-receptor of *S. thermophilus* bacteriophages.

**Key Words:** Anti-receptor, Bacteriophages, *Streptococcus thermophilus*

**370 Survivability of lactic acid bacteria and bifidobacteria in a spreadable yogurt cheese product.** T. Pritchard*, M. Guo, A. Zielinski, and P. Kindstedt, *Northeast Dairy Research Center, University of Vermont, Burlington.*

A newly developed dairy-based product containing pro-biotic cultures was evaluated over an 8 week period to determine the survivability of Lactic Acid Bacteria (LAB) and Bifidobacteria (BIF) within the product. Six batches of the spreadable yogurt cheese were manufactured and evaluated at 1 week intervals. Chemical analysis of the yogurt cheese determined the product was: moisture, 75%; fat, 6.9%; protein, 12.4% and pH 4.64. Following production, the yogurt cheese was placed in plastic bags, vacuum packaged and held at 4°C. Viable LAB and BIF were enumerated via incubation under anaerobic conditions using M17 and RAF 5.1 media respectively. Analysis of the yogurt cheese revealed an average intitial inoculum of $6.36 \times 10^6$ +/- 5.28 X $10^6$ LAB and 2.57 X $10^7$ +/- 7.29 X $10^6$ BIF. Following drainage of the curd, the LAB had increased 400 fold to 2.74 X $10^9$/g while the BIF increased 2 fold to 5.41 X $10^7$/g during the same period. Average levels of LAB remained above 2 X $10^9$/g for four weeks. A steady decline in viable LAB was noted from week 5 (1.8 X $10^9$, +/- 3.73 X $10^8$/g) to week 8 (1.1 X $10^9$, +/- 7.62 X $10^8$/g). Average levels of BIF dropped to initial inoculum levels (2.79 X $10^7$/g) by week 3. The number of viable BIF remained stable between weeks 4 (2.87 X $10^7$, +/- 9.05 X $10^6$/g) and week 8 (2.21 X $10^7$, +/- 9.41 X $10^6$/g). The results of the survey indicate that the newly developed yogurt cheese is an effective matrix in which to supply LAB and BIF to consumers. The yogurt cheese product shows promise as a means of delivering BIF at levels of 10 to 20 fold higher that that which the food industry has presently targeted (i.e. $10^6$/g).

**Key Words:** Lactic Acid Bacteria, Bifidobacteria, yogurt-cheese

**371 Enhancement of AbiK anti-phage activity on low-copy plasmids.** J. D. Bouchard* and S. Moineau, *Universite Laval, Quebec, Canada.*

Phages of lactic acid bacteria are the main cause of fermentation failures in the dairy industry. Anti-phage mechanisms can be introduced into industrial strains of *Lactococcus lactis* to limit this problem. It is expected that a phage resistance system will be fully effective in different strains and under various conditions. AbiK is a putative protein of 599 amino acids, encoded by the 7.8-kb natural lactococcal plasmid pSRQ800, that leads to abortive infection of small isometric-headed phages (EOP $10^{-6}$). Upstream of the *abiK* gene, two small open reading frames of 116 (ORF3) and 94 (ORF4) amino acids are preceeded by a putative promoter and followed by a putative terminator. Unlike the *abiK* gene, this operon has a G+C content similar to typical lactococcal DNA sequences. ORF4 posseses a helix-turn-helix motif and has low homology with several transcription regulators. When the three genes were cloned on a low-copy plasmid, a transcript encompassing *orf3-orf4-abiK* was detected by RT-PCR. Deletion of the ORF3-ORF4 operon or presence of ORF4 *in trans* on a high-copy vector reduced the anti-phage activity of AbiK (EOP $10^{-2}$). On a medium-copy plasmid, RT-PCR indicated that *orf3*, *orf4* and *abiK* are not cotranscribed and deletion of ORF3-ORF4 had no effect on AbiK activity. Based on these results, ORF4 may act as a repressor on its promoter but only when cloned on medium- and high-copy plasmids. Under those conditions, *abiK* is sufficiently expressed from its own promoter to ensure a strong phage resistance phenotype (EOP $10^{-6}$). On low-copy plasmids, *abiK* is also transcribed from the *orf3-orf4* promoter which thereby increases the efficacy of AbiK from EOP $10^{-2}$ to EOP $10^{-6}$. Thus, surrounding genes and *cis*-acting sequences can play a role in the modulation of anti-phage activity.

**Key Words:** *Lactococcus lactis*, Bacteriophages, Abortive infection

**372 Sensory aroma characteristics of milk spoilage by *Pseudomonas* species.** W. W. Hayes*, C. H. White, P. D. Gerard, and M. A. Drake, *Mississippi State University, Mississippi State.*

A long shelf-life with consistent high quality is desired for fluid milk. *Pseudomonas* species are normally associated with fluid milk spoilage with *P. fluorescens*, *P. fragi*, and *P. putida* being the most common. By having a better insight into the spoilage characteristics of pseudomonads, detection and prevention of these organisms may be improved. The objectives of this study were: 1) to determine sensory aroma profiles of spoilage aromas for milk with varying fat contents spiked with these pseudomonads; and 2) to determine differences in growth rates at 5°C. Two strains each of *P. fluorescens* (P27 & P11), *P. fragi* (P24 & P9), and *P. putida* (P14 & P15) were evaluated. Milk (skim and whole) was double-steamed and either initially inoculated with $10^3$ CFU/mL of each pseudomonads or not inoculated as a control. Milks were held at 5°C for one month. Plate counts were conducted every three days to determine growth rates. Descriptive aroma analysis was conducted weekly using a trained panel (n=8). Experiments were replicated in triplicate. All six strains grew at refrigeration temperature (5°C). Within three days of storage all treated milks had reached $10^7$ CFU/mL and by the first descriptive analysis panel all treatments had reached $10^8$ CFU/mL. All strains were lipolytic on spirit blue lipase agar. On skim milk agar strains P27 and P11 were proteolytic. Spoilage was detected in milks containing strains P27, P14, and P15 following one week storage, while spoilage in milks treated with strains P11, P24, and P9 was not detected

until two weeks storage. "Fruity" aromas predominated after one week spoilage. After two weeks spoilage, "cheesy", "putrid", and "barny" aromas predominated. Types and onset of spoilage differed among the strains ($P<0.05$). This information may aid in shelf-life prediction of milk.

**Key Words:** *Pseudomonas*, Milk, Spoilage

**373 Manufacture of hard cheese inoculated with pathogenic bacteria in a Bio-Safety Level 3 pilot plant.** J. E. Schlesser *[1], A. Teo[2], and D. Englehardt[2], [1]*Food and Drug Administration, NCFST, Summit-Argo, IL*, [2]*Illinois Institute of Technology, Chicago, IL*.

The Bio-Containment lab was modified to upgrade the lab to Bio-Safety Level 3 for small scale cheese-manufacturing from raw milk inoculated with food pathogens. Environmental sampling was conducted before the experiment and after decontamination to evaluate these procedures. The finalized procedures were incorporated into the Bio-Containment Operating Manual. Initially, cheese was made from raw milk with naturally occurring *Escherichia coli* to test the cheese-making equipment,and to monitor levels during the cheese-making process, subsequent aging period, and the decontamination in the bio-containment lab. Environmental sampling of surfaces showed that no naturally occurring *E. coli* was present on the equipment after decontamination. Later, cheese was made with raw milk inoculated with *E. coli* K-12 (ATCC 35695). This streptomycin-resistant strain of *E. coli* was used to monitor levels during the cheese-making process, subsequent aging period, and the decontamination in the bio-containment pilot plant. Cheese, made with raw milk inoculated with $10^{5}$ *E. coli* K-12, showed the surrogate was incorporated at approximately the same levels as the initial inoculum. Environmental sampling of surfaces showed that no surrogate was present on equipment after decontamination. Environmental sampling using an Anderson Air Sampler showed that no *E. coli* K-12 was present in the cubicle air or into the external environment filtered through a 0.3 micron HEPA filter. After Bio-Containment Lab and procedures were certified by an external consultant, hard cheese inoculated with 5 strains of *E. coli* O157:H7 were manufactured. Air and surface sampling showed similar results.

**Key Words:** Pathogenic Bacteria, Raw Milk Cheese, *E. coli*

**374 Effects of incorporation of proteolytic strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* in commercial ABT cultures on EPS production, textural properties of yogurt and survival of bacteria.** A. Shihata and N.P. Shah*, *Victoria University of Technology, Melbourne, Australia*.

Use of polysaccharide producing ABT (*L. acidophilus*, *Bifidobacterium* spp., and *S. thermophilus*) starter cultures are gaining popularity among yogurt manufacturers. Polysaccharide-producing yogurt bacteria are important determinants of yogurt viscosity and texture. These starter cultures improve the viscosity of yogurt, leading to resistance to mechanical damage. *S. thermophilus* is the main fermenting organism in ABT starter cultures. In previous studies, *S. thermophilus* in commercial ABT starter cultures was found to be much less proteolytic as confirmed by o-pthaldialdehyde-based spectrophotometric assay. Among several strains of *L. delbrueckii* ssp. *bulgaricus* screened, two strains were found to be highly proteolytic. The aim of this study was to determine the effects of incorporation of proteolytic strains of *L. delbrueckii* ssp. *bulgaricus* to ABT (ABT-LB) starter cultures on EPS production and the textural properties of yogurts. The yogurt made using commercial ABT starter cultures was used as a control. The firmness of yogurts was measured with a cone-penetrometer and the apparent viscosity with a Brookfield viscometer. EPS was extracted and quantified by phenol sulfuric method. Sugar analysis of the EPS fractions was determined by HPLC. Survival of the three (in ABT) or four (in ABT-LB) groups of bacteria was determined over a 4-week storage period. Results have shown that yogurts made with ABT-LB were the firmest and most viscous and produced the highest amount of exopolysaccharide (3g/100g) as compared with those prepared with ABT starter cultures. The EPS fractions extracted from yogurt samples composed mainly of galactose and glucose. Viable counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, *L. acidophilus* and *Bifidobacterium* spp. improved by over 2 log cycles in yogurts made with ABT-LB starter cultures during 4 weeks storage period.

**Key Words:** ABT starter cultures, EPS, Texture properties

**375 Casein and whey proteins degradation patterns by selected lactic bacteria.** A. Shihata and N.P. Shah*, *Victoria University of Technology, Melbourne, Australia*.

Caseins and whey proteins are the main nitrogen source for yogurt bacteria *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* and probiotic bacteria *L. acidophilus* and *Bifidobacterium* spp. These bacteria utilize caseins and whey proteins with the help of extra-cellular proteinases and membrane bound amino-peptidases as well as intracellular proteinases and peptidases. The objective of this study was to investigate caseins and whey proteins degradation patterns by selected yogurt and probiotic bacteria. Capillary electrophoresis (CE) was used for analysis of milk protein degradation patterns owing to its advantages over electrophoresis and chromatographic methods. Sterile reconstituted skim milk (RSM) was inoculated with individual strains of yogurt and probiotic bacteria and incubated for 6 h. Samples were withdrawn and levels of hydrolysis of various caseins and whey proteins determined. CE was carried out using a Beckman P/ACE system 5010 with a Gold Software data system version 810. The separations were performed using a hydrophilic-coated fused silica capillary column. Complete amino acid analyses of RSM before and after 6 h incubation with individual bacterium were also carried out. The proteolytic enzyme profiles of the bacteria, particularly their amino-, di-, tri-, and endo-peptidases were determined. Yogurt bacteria possessed higher levels of proteolytic enzymes than probiotic bacteria and the extent of degradation of caseins and whey proteins by yogurt bacteria was much higher. The patterns of caseins and whey proteins hydrolysis varied with the strain and the organisms showed no definite trend. Yogurt bacteria released higher levels of free amino acids and demonstrated greater amino peptidases and di-peptidases activities than probiotic bacteria. Both groups of bacteria utilized aspartic acid, glutamic acid, leucine and lysine from RSM, whereas appreciable amounts of cystine and cysteine were detected in RSM after 6 h incubation. The levels of cysteine and cystine were higher in RSM after incubation with probiotic bacteria than when inoculated with yogurt bacteria.

**Key Words:** Protein degradation, Lactic bacteria, Proteinases

**376 Quantitation of a proteinase secreted by a strain of *Pseudomonas fluorescens* using rocket immunoelectrophoresis.** A. Zahran*[1] and B. Ward[2], [1]*Minia University, Egypt*, [2]*University of Edinburgh, UK*.

Rocket Immunoelectrophoresis (RIE) constitutes a simple method for quantitating protein based on the cross reaction between antigen and antibodies raised against the enzyme. RIE technique was used to monitor the synthesis of proteinase protein during growth in glucose basal medium in the presence and absence of calcium chloride. Measurement of protein synthesis rather than enzyme activity was very sensitive and enzyme protein could be detected down to 45ng. Preliminary work showed that the quality of the antibodies raised against the enzyme when mice were used was better than using rabbit antibodies. *Pseudomonas fluorescens* R8 synthesized a constitutive enzyme as protease synthesis was not greatly affected by the presence of proteins or protein degradation products in the medium. The enzyme was synthesized at the early stages of growth and was detected in the glucose basal medium. The amount of proteinase secreted in complex medium was between 2.6-2.8 fold higher compared to the enzyme produced in basal medium with either glucose or glutamate in the presence of calcium chloride.

**Key Words:** *Pseudomonas fluorescens*, Proteinase, Immunoelectrophoresis

**377 Purification and characterization of a heat stable proteinase secreted by *Pseudomonas fluorescens* R8.** A. Zahran*[1] and B. Ward[2], [1]*Minia University, Egypt*, [2]*University of Edinburgh, UK*.

An extracellular protease from *Pseudomonas fluorescens* R8 was purified to electrophoretic homogeneity in three steps. This protease was a major component in the culture supernatant. Two peaks were eluted after ion exchange chromatography. Peak A contained about 60% of

the total activity whereas peak B contained about 15%. The two peaks were found to have the same molecular weight (45K daltons) and their immunochemical identity has been determined. Characterization of peak A showed it to be a metallo-alkaline protease which contained calcium and zinc. The enzyme had an optimum temperature around 40°C and optimum pH = 7.5. The activity of the protease was inhibited by o-phenanthroline, EDTA and EGTA. It was more sensitive to o-phenanthroline than EDTA and EGTA. The amino acid composition showed that the enzyme contained high levels of aspartic acid and glycine but lacked cysteine. The total carbohydrate content was very low revealing that the protease was not a glycoprotein. The protease was heat stable, the D-value at 140°C was 1 min. and 28% of the starting activity remained after heating at 74°C for 17 sec. Heating the enzyme at 55°C led to autolysis. The enzyme attacked casein, its fractions, bovine serum albumin and cytochrome c. It also attacked $\beta$-lactoglobulin.

**Key Words:** *Pseudomonas fluorescens*, Proteinase, Characterization

**378 Application of a model system to evaluate the effect of pH on the texture of Cream cheese.** M. Almena-Aliste* and P.S. Kindstedt, *University of Vermont, Burlington, VT/USA.*

A model system that uses ammonia to increase the pH of cheese was used to study the effect of pH on Cream cheese texture. Full-fat Cream cheese and one-third reduced-fat (Neufchatel) cheese were obtained from a retail source. Cheeses were sectioned into samples (10x40x65mm) and the samples were randomly assigned to three groups. Control samples were vacuum packaged and stored at 4°C for up to 5 d. Treatment samples were exposed to ammonia for either 5 or 10 min in a desiccator cabinet to increase the pH. Treated samples were then immediately vacuum packaged and stored at 4°C for up to 5 d. Triplicate samples of control and treatment cheeses were randomly chosen after 1, 2, 3, 4 and 5 d of storage and analyzed for pH and texture. Texture was evaluated objectively by rheological analyses using a Texture Analyzer machine (TA-XT2) fitted with a 12.5 mm cylinder probe, directly on sample cheese (6°C). The parameter *maximum force*, evaluated on the force-displacement curve during a penetrometric test (7 mm depth), was used as an index of the mechanical resistance of the cheese. The entire experiment was repeated once using different batches of cheese. A RCB split-plot ANOVA was used to evaluate the effects of exposure time to ammonia and storage time on pH and texture. The relationship between cheese pH and texture was evaluated by linear regression. Exposure to ammonia significantly increased the pH of both full-fat and reduced-fat cheeses, and significantly decreased the texture values of full fat cheese. Texture of reduced fat cheese was not significantly affected by exposure to ammonia. Strong negative correlations between the pH and texture of full fat cheeses were observed in both experimental trials (r = -.89; -.94). Correlations between the pH and texture of reduced fat cheese were not as strong (r= -.51; -.80). Presumably, textural differences were caused by pH-induced changes in the interactions among water-fat-protein-stabilizer which define the structure of Cream cheese, but the nature of those interactions are not well understood.

**Key Words:** Cream cheese texture, Rheology, Cheese pH

**379 Impact of $CO_2$ addition to milk on selected analytical testing methods.** Y. Ma*, D. Barbano, J. Hotchkiss, and S. Murphy, *Northeast Dairy Food Research Center, Cornell University, Ithaca, NY.*

Addition of $CO_2$ to raw milk and dairy products can be used to control the growth of bacteria at refrigeration temperatures. The objective of this study was to determine the effects of dissolved $CO_2$ in milk on the performance of four routine testing methods: antibiotic residue test, freezing point test, infrared milk composition analysis, and alkaline phosphatase test. Raw or pasteurized whole milk was carbonated at $< 4$°C to contain 0 (control), 200, 400, 600, and 1,000 ppm added $CO_2$. Addition of $CO_2$ to raw milk (up to 1,000 ppm) had no effect on the performance of the three antibiotic ($\beta$-lactam) tests, namely, SNAP, Charm II Sequential Tablet, and Delvo-P. Carbonation of milk to 1,000 ppm decreased milk pH from 6.61 (control) to 6.15 (measured at 38°C). Milk freezing point decreased in a linear fashion with increasing concentrations of dissolved CO2, from -0.543°H (control) to -0.595°H (1,000 ppm). The effect of $CO_2$ on milk pH and freezing point was reversible upon removal of dissolved $CO_2$ by vacuum. Increased $CO_2$ levels in milk shifted the IR absorption spectrum at the lactose reference wavelength (7.651 to 7.788 $\mu$m), which caused the corrected lactose readings to decrease and the corrected fat B readings to increase. Changes in fat A and protein readings, both uncorrected and corrected, were relatively small compared to those of fat B and lactose. For the alkaline phosphatase test, pasteurized milks with six levels of carbonation (0 to 1,000 ppm) were intentionally adulterated with 0, 0.05, 0.1, and 0.2% added raw milk. Addition of $CO_2$ did not influence the ability of Fluorophos, Charm, and Scharer Rapid tests to differentiate between a pasteurized milk and milk that had been contaminated with raw milk or was equivalently under-pasteurized.

**Key Words:** carbon dioxide, analytical testing methods

**380 Development of colonic pre-cancerous lesions in rats fed synthetic and natural sources of conjugated linoleic acids and nordihydroguaiaretic acid.** D. D. Gallaher*[1], C. M. Gallaher[1], H-J. Cho[1], A. Saari Csallany[1], and R.J. Baer[2], [1]*MN-SD Dairy Foods Research Center, University of Minnesota, St. Paul, MN,* [2]*MN-SD Dairy Foods Research Center, South Dakota State University, Brookings.*

Conjugated linoleic acid (CLA) is a naturally occurring fatty acid produced by microbial fermentation in the rumen that is thought to have chemoprotective properties. We hypothesize that CLA acts by inhibiting 15-lipoxygenase activity. Two studies were conducted to determine the effect of synthetic CLA and a natural source of CLA, normal and high CLA containing butter, on the development of colonic precancerous lesions (aberrant crypts, AC) and bile acid excretion on carcinogen treated rats. In Study I weanling rats were fed a modified AIN93-G containing 20% corn oil (C), C + 0.1% CLA, C + 0.4% CLA, C + 0.8% CLA, C + 0.1% NDGA (nordihydroguaiaretic acid, a known 15-lipoxygenase inhibitor), or 20% butter fat (B) for 10 days. Rats were then administered dimethylhydrazine (15 mg/kg B.W.) once a week for two weeks by gavage, and continued on their diets for 8 weeks. Animals fed C + 0.8% CLA, C + 0.1% NDGA, and 20% B developed significantly fewer AC and AC foci compared to C. The 20% B had increased total bile acid excretion. In Study II the same protocol was used, but the diets fed were C, B, and 20 % high CLA butter (HB). High CLA butter was produced by feeding Holstein cows a 50:50 ratio of forage to concentrate with 2% added fat from Menhaden fish oil. Butter contained 2.5 g CLA/100 g fat. In addition 20% B was fed post initiation. Feeding 20% B post initiation did not alter AC compared to C. However, 20% HB significantly decreased AC and AC foci. These results suggest CLA has chemoprotective properties in this colon cancer model and supports the hypothesis that CLA acts by inhibiting 15-lipoxygenase.

**Key Words:** Conjugated Linoleic Acid, Colon Cancer, Butter

**381 Sensory characteristics of milks with different casein to serum protein ratios.** D.M. Barbano*, M.A. Rudan, and Y. MA, *Northeast Dairy Foods Research Center, Cornell University, Ithaca, NY.*

Whole milk was separated into skim and cream. The skim was microfiltered using a 0.1 $\mu$m ceramic membrane to produce a 3 X casein concentrate and a permeate containing milk serum proteins. The protein portion of the MF permeate was concentrated about 15 X by ultrafiltration (UF). Combinations of MF retentate, cream, UF retentate from MF permeate, and lactose were used to make a matrix of 20 different pasteurized, homogenized milk samples. There were 4 fat levels (0.1, 1.0 , 2.0, and 3.3%) and 5 target ratios of casein to serum proteins (approximately, 80/20, 65/35, 50/50, 35/65, and 20/80). The true protein (3.11%) and anhydrous lactose (4.70%) content was constant for all 20 samples. The calcium content decreased from about 1200 mg/kg at an 80/20 ratio to 600 mg/kg at a 20/80 ratio at all fat levels. The experiment was replicated 3 times with different batches of milk. Hunter color measurements, relative viscosity, and a quantitative descriptive analyses (14 panelists) were done. As casein to serum protein ratio decreased, L-value decreased at all fat levels, but the magnitude of the decrease was larger at lower fat levels. The impact of the decrease in casein to serum protein ratio from 80/20 to 20/80 on relative viscosity was larger than the impact of a decrease in fat from 3.3 to 0.1%. Relative viscosity at 4°C increased with increasing fat content and increasing casein to serum protein ratio. Change in fat content and casein to serum protein ratio influenced the scores for all appearance descriptors. The decrease in casein to serum protein ratio caused the appearance scores to decrease

greatly at low fat levels but not at high fat levels, cream aroma scores to decrease at high fat levels and not low fat levels, thickness scores to decrease at all fat levels. Mouth coating and drying scores decreased as casein to serum protein ratio decreased. The overall quality ratings for skim and 1% fat milks decreased as casein to serum protein ratio decreased, but overall quality rating was not influence by casein to serum protein ratio at 2%. The overall quality rating of full fat milk was higher for lower casein to serum protein ratio than for high to lower the positive impact of lower mouth coating and lower mouth drying scores.

**Key Words:** Microfiltration, Fluid milk, Casein to serum protein ratio

**382 Quality attributes of vanilla ice cream in the North Carolina market.** A.P. Hansen* and M.D. Keziah, *North Carolina State University, Raleigh*.

Vanilla ice cream samples were obtained from local dairies and grocery stores. These samples were collected over a two-year period and analyzed for body, texture and flavor. A panel of 10-15 dairy judges were trained according to ADSA guidelines. The body and texture was evaluated first by placing a spoonful in the roof of the mouth and evaluating the coldness and degree of ice crystals present. The major body defects of the premium ice creams were slightly coarse, icy and gummy. Several samples had a perfect body and texture. The major flavor defects were cooked and too high flavor. Several samples had no flavor defect. The mid-range priced ice creams body and texture was coarse and icy, weak and gummy. Several samples had an excellent body. The major flavor defects were cooked, too high flavor, unnatural flavor, lacks flavor and syrup flavor. The lower priced ice creams exhibited many defects. The major body defects going to the least were coarse and icy, gummy, weak, crumbly and sandy. The flavor defects were cooked, lacks flavoring, whey, old ingredients, syrup flavor, unnatural flavor and oxidized. It was evident that all three classes of ice cream could be improved in quality. Several of the low cost ice cream samples were unsaleable from a flavor standpoint. This is where the greatest improvement needs to be made in evaluating raw ingredients before making the mixes.

**Key Words:** Vanilla ice cream, Sensory evaluation, Quality

**383 Quality attributes of cottage cheese in the North Carolina marketplace.** A.P. Hansen* and M.D. Keziah, *North Carolina State University, Raleigh*.

The per capita consumption of cottage cheese continues to decrease each year. This may be related to the quality of cottage cheese available to the consumer in the retail market. Cottage cheese samples were evaluated over a two-year period from N.C. dairies and grocery stores. The evaluation was conducted with at least 10-14 trained dairy judges. Attributes in appearance body/texture and flavor were evaluated according to ADSA protocol. Eight-six percent of the samples had slight to definite shattered curd. The other 14% had the following defects: free whey, free cream, matted and lacks cream in that order. The body and texture defects were gelatinous, pasty, weak and soft approximately 7% for each defect followed by firm and rubbery and overstabilized at 14% each. Approximately 29% were mealy and grainy and 15% of the samples had no defect. The major flavor defect was high acid and diacetyl. Other minor flavor defects that were observed are bitter, flat, lacking freshness, oxidized, rancid and whey. It is apparent from evaluating commercial cottage cheese samples that there is great room for improvements in flavor body and texture. If dairies would produce better quality cottage cheese, the per capita sales would increase.

**Key Words:** Cottage cheese, Sensory evaluation, Quality

**384 Modification of pizza sauce to limit changes in composition and melted consistency of pizza cheese.** R.H. Ouellette* and P.S. Kindstedt, *University of Vermont, Burlington*.

Previous studies demonstrated that Mozzarella cheese lost calcium, gained moisture, and changed in melted consistency during contact with pizza sauce. The objective of this research was to determine whether fortification of pizza sauce with calcium lactate, NaCl and a humectant (FRUITRIM ®) would simultaneously limit the loss of calcium, uptake of moisture and undesirable changes in melted consistency. On three separate occasions, LMPS Mozzarella cheese was obtained from a commercial cheese plant on the day after manufacture and stored for 1 wk at 4°C. Then, test samples (1x1x3 cm) were prepared and randomly assigned to sterile petri dishes containing no pizza sauce (control), pizza sauce, or pizza sauce fortified with 0.5% calcium, 0.3% NaCl and 5% humectant. Petri dishes containing cheese or cheese plus sauce in a 1:1 (w/w) ratio were vacuum packaged and stored at 4°C for up to 12 d. On day 1, 2, 4, 8 and 12 of cheese:sauce contact, cheese samples were analyzed for pH, moisture, salt and calcium contents and apparent viscosity. Data were analyzed according to a RCB split-plot ANOVA. Salt content and pH decreased significantly and similarly in cheese samples that were exposed to unfortified or fortified sauce. In contrast, cheese exposed to fortified sauce did not decrease in calcium content, whereas, cheese exposed to unfortified sauce decreased significantly. Cheese exposed to fortified or unfortified sauce increased significantly in moisture content. However, moisture uptake was reduced by ca. 55% in the fortified treatment. Exposure to unfortified sauce caused significant decreases in apparent viscosity, symptomatic of a loss of structure and function. In contrast, cheese exposed to fortified sauce showed only small decreases in apparent viscosity, indicating significantly less change in structure and function than that caused by the unfortified sauce. The data suggest that fortifying the pizza sauce to minimize the loss of calcium from, and uptake of moisture by, the cheese may enhance the stability of Mozzarella cheese in refrigerated pizza.

**Key Words:** Mozzarella cheese, Pizza, Functionality

**385 Development and application of a model system to increase the pH of Mozzarella cheese.** P.S. Kindstedt, A.B. Zielinski*, and M. Almena-Aliste, *University of Vermont, Burlington*.

It is generally accepted that the pH of Mozzarella cheese influences functional characteristics. However, it is very difficult to study the independent effect of pH because attempts to change the pH of cheese by changing manufacturing conditions often result in other changes, such as in mineral content or proteolytic activity, that confound the pH effect. Therefore, our objective was to develop a post-manufacture model system to study the independent effect of pH (while minimizing confounding effects) on the characteristics of LMPS Mozzarella cheese. The model system achieves incremental increase in the pH of cheese by exposing test samples to an ammonia atmosphere for varying lengths of time. Preliminary studies indicated that a large surface area to volume ratio, such as in shredded cheese, is needed to achieve uniform increase in pH throughout the cheese sample. In numerous experimental trials, the pH of commercially-manufactured shredded LMPS Mozzarella cheese increased in a linear manner ($r > .90$) with time of exposure to ammonia in the pH range of ca. 5.2 - 7.0, with an apparent buffering region at ca. pH 5.9 to 6.0. An exposure time of ca. 10 min was needed to increase the cheese pH by 1.0 pH unit. Cheese total solids percentage did not change significantly during exposure times of up to 18 min. The model system was employed in numerous experiments to study the relationship between cheese pH and apparent viscosity. Apparent viscosity increased in a linear manner ($r > .90$) with increasing cheese pH in the range of ca. 5.2 up to 6.5. Raising the cheese pH by exposure to ammonia resulted in a noticeably more fibrous and elastic melted consistency, which may have been caused by pH-induced shifts in calcium distribution. The model system was scaled-up so that larger batches of cheese (up to 2-kg) could be exposed to ammonia to achieve a controlled increase in pH. The scaled-up system may offer a useful approach to study the effect of pH on the physico-chemical, enzymatic, functional and structural characteristics of cheese.

**Key Words:** Mozzarella cheese, Functional characteristics, Cheese pH

**386 Development and application of a model system to decrease the pH of Mozzarella cheese.** P.S. Kindstedt*, A.B. Zielinski, C. Ge, and M. Almena-Aliste, *University of Vermont, Burlington*.

Previous studies demonstrated that it is possible to incrementally increase the pH of Mozzarella cheese by exposing the cheese to an ammonia atmosphere for varying lengths of time. The objective of this study was to use a similar approach to decrease the pH of Mozzarella cheese. The model system achieves incremental decrease in the pH of cheese by exposing the cheese for varying lengths of time to an atmosphere equilibrated with acetic acid. Preliminary studies indicated that much longer exposure times are needed to decrease the pH by exposure to volatile acetic acid than to increase the pH by exposure to ammonia. It was necessary to expose commercially obtained shredded LMPS Mozzarella cheese to the acetic acid atmosphere for 24 h at 4°C, in order to achieve

a 0.6 unit decrease in pH; e.g., a decrease from pH 5.3 to 4.7. Decreasing the cheese pH to ca. 4.7 by exposure to volatile acetic acid resulted in a perceptible increase in the whiteness of the cheese and a dramatic change in melting characteristics. Specifically, commercially obtained LMPS Mozzarella cheeses that had pH values reduced from ca. 5.3 to 5.4 to ca. 4.7 to 4.8 using this model system assumed a granular non-cohesive consistency with a complete loss of ability to flow and stretch when melted at 60°C. The results demonstrated that decreasing the pH of Mozzarella cheese has a dramatically different effect on melting characteristics than increasing the pH, which may be caused by pH-induced shifts in calcium distribution. A scaled-up version of the model system may offer a useful approach to study the effect of pH on the physicochemical, enzymatic, structural and functional characteristics of cheese. In the present studies, the commercial test cheeses had already developed peak functionality at the time of pH adjustment. Future studies should focus on altering the cheese pH immediately after manufacture in order to evaluate the effect of pH on changes during aging.

**Key Words:** Mozzarella cheese, Functional characteristics, Cheese pH

**387 Effect of frozen storage on functional properties of Mozzarella and non-pasta-filata style pizza cheeses.** M.-I. Kuo* and S. Gunasekaran, *University of Wisconsin, Madison*.

Meltability, free oil formation, and stretchability were used to assess the effect of aging time, ripening before (2, 7, and 14 d at 4°C) or after (7 and 14 d at 4°C) freezing, and frozen storage (1 and 4 wk at 20°C) on properties of Mozzarella and non-pasta-filata style pizza cheeses. Cheeses were cut into 7×10×5-cm blocks and vacuum-sealed one day after manufacture. The results obtained were compared with unfrozen control samples, aged at 4°C between 7 to 21 d. A modified squeeze flow method was used to measure cheese meltability. The amount of free oil released from cheese upon heating was measured using a computer image processing system. The stretchability of the melted cheese was measured by a uniaxial horizontal extension test. The results indicate that frozen storage significantly affects the functional properties of Mozzarella cheese. However, the effect depends on the aging time before and after freezing. In contrast, frozen storage does not have a significant effect on the functional properties of non-pasta-filta style pizza cheese. The optimal freezing treatments for minimal loss of desirable functional properties were determined. The Mozzarella cheese should be aged one week at 4°C before freezing and storing it for 4 wk at 20°C. The cheese should be thawed and aged for one week at 4°C before consumption. The non-pasta-filata style pizza cheese can be stored up to 4 wk at -20°C followed by thawing and aging at 4°C for 9 to 16 d.

**Key Words:** Cheese, Freezing, Functional Properties

**388 Comparison of melt profiles of LMPS Mozzarella manufactured by pasta filata and stirred curd methods.** C.M. Chen, A.L. Dikkeboom, M.E. Johnson, and M.G. Zimbric*, *Wisconsin Center for Dairy Research, Madison*.

The melt properties of pasta filata (brined) and stirred curd LMPS Mozzarella cheese were compared at 10, 30, 60, and 90d of aging using the thermal melt tube test and the newly developed melt profile analysis. Cheeses had similar melt characteristics as measured by a thermal tube test. The thermal tube test assesses cheese meltabilty by measuring the distance a disc of cheese flows over time. Yet, the cheeses had different melt characteristics when baked on a pizza. After 10d, the pasta filata Mozzarella tended to flow off the crust, while the stirred-curd Mozzarella did not over-flow until it was at least 30d. A newly developed melt profile analysis simultaneously measures the changes in cheese height and temperature, providing information on softening time (s), softening temperature (°C), rate of flow (V/s), complete melt time (s), complete melt temperature (°C) and extent of flow (% decrease in cheese height at complete melt). The manufacturing methods did influence the softening temperature and complete melt temperature and the extent of cheese flow. The cheese softening temperatures for the pasta filata and stirred curd at 10 and 30d were 42.5, 40.5°C and 43.7, 41.2°C, respectively. There were similar trends for complete melt temperature. The extent of flow for pasta filata style was greater when compared to stirred curd at 10 and 30 d. This indicates that the stirred curd cheese requires a higher cheese temperature to begin flow, completely melt and to exhibit a greater extent of flow. Cheese composition (46.6 % moisture, 22.9% fat, 25.46% protein, and 1.57% salt), pH and degree of proteolysis did not significantly differ It is proposed that differences in the melt profiles are related to the microstructure of the cheese as influenced by the manufacturing technique.

**Key Words:** Mozzarella, Stirred Curd, Melt

**389 Comparative study of *Lactobacillus acidophilus* strains for probiotic characteristics.** S. Oh*[1], C. H. Chai[2], S. Kim[2], Y.-J. Kim[1], R. H. Liu[1], H. S. Kim[3], and R. W. Worobo[1], [1]*Department of Food Science & Technology, Cornell University,* [2]*Department of Animal Science, Korea University,* [3]*Culture Systems Inc, Misawaka, IN*.

*Lactobacillus acidophilus* has been studied for use as a probiotic culture such as dairy products and dietary supplements. *Lb. acidophilus* is considered to have a higher tolerance to gastric juice and bile acid than other yogurt starter cultures. Some *Lb. acidophilus* strains can assimilate cholesterol during growth in the presence of bile under anaerobic condition. The objectives of this study were to compare the probiotic characteristics of twelve *Lb. acidophilus* strains for their ability of cholesterol assimilation, production of CLA (conjugated linoleic acid), and viability under artificial gastrointestinal fluids. Strains were variable with regard to production of CLA and acid tolerance. The ability of cholesterol assimilation had some variation among *Lb. acidophilus* strains, but strains could be classified into two groups of assimilation levels ($P<0.05$). The group of high cholesterol assimilation exhibited significantly higher resistance to 0.3 and 0.5% bile acid compared to the group of low cholesterol assimilation ($P<0.05$). However, acid tolerance was not related to the ability of cholesterol assimilation but the ability to assimilate cholesterol of *Lb. acidophilus* may be related to bile-resistant.

**Key Words:** *Lactobacillus acidophilus*, Probiotics, Cholesterol Assimilation

**390 Purification and partial amino acid sequence of a acidocin 30SC, a bacteriocin produced by *Lactobacillus acidophilus* 30SC.** S. Oh*[1], S. Kim[2], J. J. Churey[1], and R. W. Worobo[1], [1]*Department of Food Science & Technology, Cornell University,* [2]*Department of Animal Science, Korea University*.

Acidocin 30SC, a bacteriocin produced by *Lactobacillus acidophilus* 30SC, was purified by ammonium sulfate precipitation, hydrophobic interaction chromatography (Octyl-Sepharose CL4B), and reverse-phase chromatography (Resource RPC). Active fractions from Octyl-Sepharose column chromatography were pooled and applied to FPLC on a reverse-phase column. The active fractions from the reverse-phase column were pooled and reinjected onto the FPLC column with a linear gradient of acetonitrile. Elution of Acidocin 30SC coincided with the absorbance peak at 34% acetonitrile. The molecular weight of Acidocin 30SC was estimated to be 3.5 kDa by tricine-SDS-PAGE. The sequence of 23 N-terminal amino acid residues from purified Acidocin 30SC was identified as follows: NH2-Ile-Gln-Gln-Leu-Gly-Phe-Gly-Phe-Met- Leu-Glu-Gly-Glu-Ala-X-Gly-Ile-Gly-Pro-Glu-X-X-Phe. Acidocin 30SC did not contain any modified amino acids such as lanthionine or methyllanthionine. The N-terminal sequence of Acidocin 30SC was compared with the others of the NCBI database using the Blast program. No sequence similarity was found to any bacteriocins. Acidocin 30SC appears to be a new bacteriocin with activity against a range of bacteria that warrants further investigation.

**Key Words:** Acidocin 30SC, Bacteriocin, Purification

**391 Fermentation of fructooligosaccharides by lactic acid and probiotic bacteria.** H. Kaplan* and R. Hutkins, *University of Nebraska, Lincoln*.

Utilization of fructooligosaccharides (FOS) by lactic acid bacteria and bifidobacteria may play an important role in determining which organisms become established in the intestinal tract. Therefore, FOS fermentation may be an important criteria for selection of bacterial strains to be used as probiotics. The objectives of this research were to develop a cultural method for identifying bacteria that ferment FOS and to screen commercial dairy starter culture and probiotic strains for this property. Identification of FOS-fermenting strains was based on the development of acid in MRS agar to which glucose was replaced by 2% purified FOS. The purified FOS was comprised of three components - a

glucose monomer (G) linked $\alpha$-1,2 to two or more $\beta$-2,1-linked fructosyl units (F), to give $GF_2$, $GF_3$, and $GF_4$. Of the 28 strains of lactic acid bacteria and bifidobacteria we examined, 12 of 16 lactobacilli and 7 of 8 bifidobacteria were able to ferment FOS. Included among the FOS-fermenting strains were several commercial probiotic strains, although the widely used strain *Lactobacillus* GG was a non-fermenter. None of the four streptococci fermented FOS. Growth studies were also done for bacteria grown in MRS broth containing glucose, FOS, or no added carbohydrate. Only strains that gave a positive FOS fermentation reaction by the agar method were able to reach high cell densities and to lower the pH to below 4.0 in the MRS broth containing FOS. Analysis of the fermentation broths by HPLC revealed that the FOS-fermenting strains consumed only $GF_2$ and $GF_3$, and none fermented $GF_4$. In contrast, growth experiments in which *Escherichia coli*, *Salmonella* and *Enterobacter* were inoculated into minimal broth cultures containing pure FOS revealed that these bacteria had limited capacity to utilize FOS as a carbon source. These results demonstrate that FOS utilization is a property unique to specific strains of probiotic bacteria.

**Key Words:** Fructooligosaccharide, Probiotic, Lactic Acid Bacteria

**392 Acid tolerance of *Lactobacillus acidophilus* increases following exposure to supernatant from early stationary phase cells.** R. Hage* and P. Courtney, *The Ohio State University, Columbus*.

*Lactobacillus acidophilus* must overcome the stresses imposed on it by the food system and the digestive tract of the host in order to function as a probiotic. *L. acidophilus* cells at stationary phase have demonstrated an increased resistance to stress compared to cells at earlier stages of growth. The objective of this study was to find evidence of extracellular signaling in response to stress, and to assess whether or not such signals are involved in acid stress resistance. Supernatants were collected from *L. acidophilus* NCFM cultures grown to mid-log and early stationary phases in MRS pH 6.5. These were adjusted to pH 7.0 with NaOH and filter-sterilized. Part of the early stationary phase supernatant was heated to 75C for 5 minutes. Cells grown to mid-log phase in MRS pH 6.5 were then incubated in each of the three supernatants for two hours. They were then centrifuged, washed, suspended in MRS pH 1.6 and plated on MRS agar immediately and at 15 minute intervals for 60 minutes. After 45 minutes at pH 1.6, the mean log reduction for cells exposed to unheated early stationary phase supernatant was 3.10 compared to 5.49 for cells in mid-log phase supernatant and 4.43 for cells in heat-treated early stationary phase supernatant. After 60 minutes at pH 1.6, only cells incubated in unheated early stationary phase supernatant formed viable colonies on MRS agar. This indicates that early stationary phase cell supernatants contain a heat-sensitive factor(s) that appear to confer acid-stress resistance on mid-log phase cells.

**Key Words:** *Lactobacillus acidophilus*, Acid-tolerance, Stress

**393 Translocation and clearing of *Lactobacillus acidophilus*.** M. G. Conde, C.L.L.F. Ferreira*, I.D.P. Marlieri, E. Teshima, and L. M. Borba, *Universidade Federal de Vicosa*.

Probiotics are functional foods carrying large numbers of beneficial bacteria from the host's intestinal tract. The most commonly applied microorganisms as probiotics are those of the *Lactobacillus* and *Bifidobacterium* genera. *Lactobacillus acidophilus* NCFM is a strain isolated from humans, present in several probiotics. Despite its wide use, there is little information on its translocation to the host's organs. To investigate this process, 110 animals comprising two groups of 50 weaned Wistar rats (control and test), and one basal group (10 animals) were used. The control group received a standard diet (AIN-93, "ad libitum") and 0.1 ml skimmed milk/day. Besides the standard diet, the test group received 0.1 ml skimmed milk containing 1.0 x $10^{10}$ CFU/ml of a strain of *Lactobacillus acidophilus* of human origin for 14 days. After this time, 10 animals were killed and had their spleens, hearts, livers and kidneys screened for *Lactobacillus* (time 0). The same procedure was repeated at 7, 14, 21, and 28 days. The highest translocation was observed for the spleen. The average translocation for the other organs did not differ significantly from each other. However, the counting at 28 days after the end of the experiment indicated a elimination (% clearing) of the microoganims translocated from the spleen (100), liver (100), heart (100), kidney (98.32). The evaluation of the organs from the control and basal groups did not indicate the presence of *Lactobacillus* in MRS agar. The numbers translocated cells, besides being low, were constantly eliminated during the evaluation period. The time required for the complete elimination of *Lactobacillus* was calculated for the different organs according to the regression models adjusted and corresponded to 25.7; 27.5; 27.7; and 32.1 for the spleen, liver, heart and kidneys, in that order. The current data suggested that 32.1 days after the end of a 14 day period of probiotic consumption containing 1.0 x $10^{10}$ CFU/ml of *Lactobacillus acidophillus*, the organs here in studied were cleared from the translocated cells. Since the implications of microbial translocation in the host with different degrees of debility is not known, the translocation capacity is suggested to be an important parameter for the selection of strains to be used as probiotics.

**Key Words:** Probiotics, Translocation, *Lactobacillus acidophilus*

**394 Impact of starter culture on whey flavor variability.** R.M. Tomaino*, D.L. Larick, and L.G. Turner, *North Carolina State University, Raleigh*.

Although the knowledge pertaining to the functional and nutritional properties of whey has greatly increased, research focused on the development of the characteristic flavor of whey is lacking. The characteristic whey flavor has been considered a limiting factor in full utilization of whey ingredients in food products. Off flavor development in dried whey products has traditionally been attributed to a combination of Maillard browning and lipid oxidation reactions with little emphasis on the contribution of start culture activity. To study the effect of starter cultures on whey flavor, three commercially available single strain lactococcus lactis cultures were each used to produce Cheddar cheese whey, along with a control that utilized glucono-$\delta$-lactone for acid development. Whey was drained, filtered, clarified, pasteurized (77°C/16s), bottled and frozen until analysis. Flavor characteristics were evaluated by descriptive sensory analysis using trained sensory panelists. Dynamic headspace analysis, coupled with gas chromatography-mass spectroscopy, was conducted, to quantitatively and qualitatively characterize volatile compounds. Assessment of proteolysis and lipolysis were performed using an o-phthaldialdehyde spectrophotometric method and gas chromatography to identify free fatty acids, respectively. Greater volatile production ($p<0.05$) occurred in the whey produced with cultures compared to the control, and differences could be detected between the whey samples and the control. The degree of proteolysis was greater ($p<0.05$) among the whey produced with a culture compared to the control, with differences detected between the cultures. Results have indicated that the starter cultures contribute lipolytic enzymes, which is evident when comparing free fatty acid data between starter culture samples and the control. Sensory data substantiated the analytical differences. The ability of starter culture to impact the flavor profile of the resultant whey has been demonstrated. Further research should focus on elucidating the impact of starter cultures in the production of whey ingredients with the goal of inhibiting or limiting the production of the characteristic whey flavor.

**Key Words:** Whey, Off-flavor, Lactococcus lactis

**395 Inhibition of *Lactococcus lactis* ssp. *lactis* c2 bacteriophage proliferation in *L. lactis* ssp. *lactis* C2 grown in medium containing heat treated *L. lactis* ssp. *lactis* c2 phage-peptide.** C.L. Hicks*, *University of Kentucky, Lexington*.

Preparation of phage-peptide was problematic because of contamination by bacteriophage (phage). A modification was needed to prepare an uncontaminated phage-peptide. Peptides from c2 phage were heat treated and tested for phage inhibition activity. Peptides were prepared by hydrolyzing *Lactococcus lactis* ssp. *lactis* c2 phage (1 x $10^8$ pfu/ml) with ficin (0.01%) for 6 h at 31°C. Hydrolyzed phage were micro-filtered (MiniKross, 0.2 $\mu$m) to remove cellular debris. Filtrate was ultra-filtered through a YM3 (Millipore) UF membrane (3000 MW Cut-Off) at 5°C. Portions of the permeate (250 ml) were heat treated at 101, 111, or 121°C for 15 min. Heat treated permeates were cooled and freeze dried. Sterilized M17 media with $CaCl_2$ were prepared (8 tubes containing 25 ml medium) to which the three heat treated peptides were added (2.5% peptide w/w, added to 6 tubes) and inoculated with *L. lactis* ssp. *lactis* C2 (4%). At 1 h four tubes were infected with *L. lactis* ssp. *lactis* c2 phage (1 x $10^2$ pfu/ml). Culture growth and lysis was monitored spectrophotometrically ($\lambda_{600nm}$). Culture grown in media containing the heat treated phage peptides were not inhibited by the peptide. When C2 culture in medium without peptide was infected with c2 phage lysis of the culture occurred after 270 min of incubation. Lysis of the C2

culture also occurred after 270 min of incubation when the media contained phage peptide heat treated at 101°C irrespective of whether it had been infected with c2 phage. These data indicated that bacteriophage survived the heat treatment and contaminated the freeze dried peptide. However, when C2 culture was grown in media containing the 111 and 121°C heat treated peptide and infected with c2 phage lysis did not occur. These heat treated phage-peptides may have effectively prevented the infective phage from attaching to C2/s antigenic binding sites better than the 101°C heat treated peptides.

**Key Words:** c2 Bacteriophage, *Lactococcus lactis*, Peptides

**396 Characterisation of *Bifidobacterium* isolates using amplification of the transketolase/transaldolase intergenic spacer region in combination with temporal temperature gel electrophorisis.** J. P. Burton* and G. W. Tannock, *University of Otago, Dunedin, New Zealand*.

Comparisons of the V regions of 16S rRNA sequences has proved to be an essential tool in the classification of bacteria. These 16S rRNA sequences are relatively conserved in the case of some bacteria, such as in the case of *Bifidobacterium* species isolated from human faeces. We have detected an intergenic region within the *Bifidobacterium* chromosome that may prove useful in at least characterising, and perhaps identifying, isolates of these bacteria. The region varies in size between isolates, but is approximately 100-200 bp in length and is located between the transketolase *(tal)* and transaldolase *(tal)* genes. To test the degree of sequence variability of this region in different bifidobacterial species, we compared the migration of PCR amplicons of the region by temporal temperature gel electrophoresis (TTGE). Nine *Bifidobacterium* type cultures of species detected in human faeces were characterised by this method. The DNA fragments generated from *Bifidobacterium angulatum* and *B. catenulatum* had the same migration properties in the gel, as did those of *B. infantis* and *B. longum.* However, *B. adolescentis, B. breve, B. bifidum, B. dentium* and *B. pseudocatenulatum* could be differentiated according to the thermal stability of their respective amplicons.

**Key Words:** *Bifidobacterium*, TTGE

**397 Study of exopolysaccharide production by *Lactobacillus rhamnosus* ATCC 9595M in a supplemented whey permeate medium.** M. Macedo*[1], C. Lacroix[1], and C-P. Champagne[2], [1]*Dairy Research Centre STELA, Quebec, Canada,* [2]*Food Research and Development Centre, St-Hyacinthe, Canada*.

The aim of this work was to study exopolysaccharide (EPS) production during pH controlled batch fermentations of *Lactobacillus rhamnosus* ATCC 9595M in a whey permeate (WP) medium supplemented with Yeast Nitrogen Base (YNB). A central composite design consisting of 20 fermentations, 14 unique combinations (8 factorial points, 6 axial points) and 6 replications (central points) and response surface methodology were used. This experimental design allows to study in interaction the effect of three factors: incubation temperature (22-42°C), WP concentration (1.6-8.4%) and YNB supplementation (0-2.0%). Fermentation were conducted in two 4 L fermentors, with agitation set at 100 rpm and pH controlled at 6.0 by addition of $NH_4OH$ 3N. Optical density (625nm), cell counts (CFU/mL), lactic acid production (g/L), lactose consumption (g/L), apparent viscosity of the broth medium (Pa.s) and EPS production expressed as total sugar (mg/L) were measured. Biomass response surface analysis showed not significant difference for the main factors and only the WP∗T interaction was significant ($p<0.05$). The YNB factor exhibited a limited effect on maximum biomass and EPS production, indicating that YNB may not be suitable as nitrogen source, for the culture. Data showed that EPS production was not growth-associated. Maximal EPS production varied from 40 to 257 mg/L. The EPS productivity calculated for maximum EPS production varied from 0.75 to 6.0 mg EPS/L.h. The EPS quality was measured by the apparent viscosity of the broth at 200 $s^{-1}$. Apparent viscosity decreased for long incubation times in the range of optimal growth temperature, without a parallel decrease in EPS concentration, which may indicate a possible hydrolysis of EPS. The EPS production by *Lb. rhamnosus* ATCC 9595M in this study, even with a limited nitrogen source, is close to maxima reported in the literature for lactobacilli and optimal culture medium.

**Key Words:** exopolysaccharide, whey permeate, lactobacilli

**398 Capsule formation by nonropy yogurt cultures affects its viscoelastic properties.** A. Hassan*, M. Corredig, and J. Frank, *The University of Georgia, Athens*.

Little information is available on the development of gel structure resulting from acidification of milk by starter cultures. Furthermore, no previous studies have focused on the effect of nonropy capsule-forming cultures on the viscoelastic properties of yogurt gels. This work was designed to study the structure formation of yogurt made with cultures containing ropy *lactobacillus delbrueckii* ssp. *bulgaricus* (R), capsule-forming nonropy *streptococcus thermophilus* (CNR) and noncapsule-forming nonropy (NCNR) cultures. Reconstituted (11% w/v), steamed (95°C for 15 min) skim milk was inoculated with 5% of R, CNR or NCNR and yogurt was prepared at 37°C. Inoculated milk was transferred to a controlled stress rheometer (Rheometric Sci. Piscataway, NJ) and samples were oscillated at a frequency of 0.1 Hz at 1% strain. The pH was monitored simultaneously as the rheometer readings were recorded, by measuring the pH in a parallel control cup held at the same temperature in a water bath. Fermentation was terminated at pH 4.5. The gelation behavior was plotted against pH. Gelation point which was defined at G' > 1 Pa occurred at pH 5.3 in milk fermented with R and NCNR cultures. On the other hand, milk fermented with CNR cultures showed structure development at higher pH values ( 5.5). This was in agreement with our previous observation using confocal scanning laser microscopy. Milk containing CNR showed higher complex shear moduli ($G^*$) than that containing R or NCNR. The loss tangent of all gels increased to a maximum shortly after gelation. When $G^*$ was normalized against the value of its own $G^*$ at the final pH (4.5) and plotted vs. pH, the gelation profiles of milk fermented by different strains fell into a single master curve. In conclusion, the minimal deformation applied in our experiment showed that the gelation profile of milk fermented by ropy or nonropy lactic cultures was similar. The presence of bacterial capsules affected the casein aggregation behavior, which was different from that of milk fermented by R or NCNR. °

**Key Words:** Bacterial capsules, Yogurt, Viscoelastic properties

**399 Characterization and differentiation of *Lactobacillus acidophilus* strains for use as probiotics.** S. McKechnie[1], N.P. Shah*[1], and M.L. Britz[2], [1]*Victoria University of Technology Melbourne, Australia,* [2]*University of Melbourne, Victoria, Australia*.

There is increasing evidence that *Lactobacillus acidophilus* plays a health-promoting role by contributing to the maintenance of the intestinal flora. However, despite their use in probiotic products, the relative therapeutic value of various strains has yet to be determined. In addition, the onset of molecular techniques has led to the re-classification of presumed *L. acidophilus* species. Therefore it is important to confirm the identity of candidate strains. The aim of this study was to assess a variety of techniques for the characterisation and differentiation of a group of probiotic strains. A culture collection previously identified as *L. acidophilus* based on phenotypic criteria were screened to select strains with functional properties typical of probiotic bacteria. Among the selection criteria were the ability of strains to withstand low pH and high bile concentrations which are commonly encountered in the gastrointestinal tract. Those strains that met initial selection criteria were further characterised by traditional biochemical techniques and molecular methodologies including SDS-PAGE of whole cell proteins and Pulsed-Field Gel Electrophoresis of chromosomal DNA digested with the restriction enzyme *Sma1.* Those strains that differed considerably to the reference strain were further characterised using 16S rRNA sequence analysis. Whilst 16S rRNA sequence analysis confirmed the identity of isolates as members of the *L. acidophilus* species, the use of a polyphasic taxonomy approach, encompassing biochemical and physiological characteristics with that of molecular based phenotypic tests broadened the classification of *Lactobacillus* species and provided valuable information relating to the therapeutic value of these strains.

**Key Words:** *L. acidophilus*, Pulsed Field Gel Electrophoresis, 16S rRNA

**400 Pediocin production by recombinant *Streptococcus thermophilus*.** G.A. Somkuti*, P.E. Coderre, and D.H. Steinberg, *Eastern Regional Research Center, ARS-USDA*.

Pediocin production by *Streptococcus thermophilus* ST128 was induced by transformation with recombinant plasmids carrying the pediocin gene cluster (*ped*) from *Pediococcus acidilactici*. The amount of pediocin produced (U/ml) was measured by an agar spot-test with *Listeria innocua* as the test organism. Level of pediocin production was influenced by the length and temperature of incubation and the type of medium used. Pediocin production by ST128(*ped*) was at a maximum (up to 12,800 U/ml) in tryptone-yeast extract-lactose medium after 8 h of incubation at 40°C and remained unchanged during the next 16 h. Several other full strength or diluted media (Elliker's, MRS, skim milk, cheese whey) were also suitable for pediocin production by ST128(*ped*). At and above 45°C, pediocin titers dropped and bacteriocin was undetectable at a growth temperature of 50°C. Level of pediocin production remained essentially unchanged in skim milk diluted 1:10 (v/v, 25,600 U/ml), and in cheese whey diluted 1:5 (v/v, 12,800 U/ml). In the course of 10-12 daily transfers of recombinant ST128 cultures, the amount of pediocin produced was reduced. The gradual loss in bacteriocin activity was caused by structural and segregational instability of the transforming plasmid vector with the *ped* gene cluster in the new host. It is concluded that pediocin production is possible in relatively inexpensive whey-based media. Findings also suggest that recombinant lactic acid starter cultures with stably maintained *ped* may have direct application in controlling the growth of deleterious bacteria (*Listeria*) in dairy foods.

**Key Words:** Pediocin, *Streptococcus thermophilus*

**401 Identification and characterization of PepO2 from *Lactobacillus helveticus* CNRZ32, an enzyme involved in the hydrolysis of a β-casein derived bitter peptide.** Y.S. Chen*[1], J.E. Christensen[2], and J.L. Steele, [1]*Department of Food Science,* [2]*Department of Bacteriology*.

Analysis of the peptides formed by cell free extracts of *Lactobacillus helveticus* CNRZ32 with the bitter peptide β-casein fragment 193-209 (β-CN(f193-209)) indicated that an endopeptidase and/or carboxypeptidase was involved in its hydrolysis. Peptides derived from a carboxyl blocked β-CN(f193-209) suggested that the enzyme involved was an endopeptidase. To identify this enzyme a gene bank of *Lb. helveticus* CNRZ32 constructed in *Escherichia coli* DH5α was screened for activity with β-CN(f203-209) which was N-terminally blocked and had a C-terminal ρ-nitroanilide group. A total of 188 pools of clones (10 clones each) was screened and 2 isolates with activity were identified. DNA sequencing of the isolate revealed an open reading frame which could encode a peptide with 56% identity and 72% similarity to PepO of *Lb. helveticus* CNRZ32. Therefore this gene was designated *pepO2*. The open reading frame contained 1947 bp, encoding for a peptide containing 649 amino acid residues, with a putative Shine-Dalgarno sequence and a putative transcriptional terminator sequence. Northern hybridization was performed and the results suggested that *pepO2* is monocistronic. To examine the physiological role of PepO2 and its role in the hydrolysis of β-CN(f193-209), a PepO2$^-$ derivative of CNRZ32 will be constructed.

**Key Words:** bitterness, peptidases, *Lactobacillus helveticus*

**402 Amplification of the core streptavidin and β-galactosidase genes and construction of a core *Stp-LAC 4* fusion gene.** L.M. Damasceno[1], F.L.M. Passos*[1], V.G. Janolino[2], and H.E. Swaisgood[2], [1]*Universidade Federal de Vicosa, Vicosa-UFV, MG, Brazil,* [2]*North Carolina State Unversity, Raleigh*.

Advances in biotechnology have increased the potential use of enzymes to modify or develop food processes or functionalities of food ingredients. There are many advantages in using immobilized enzyme bioreactors, including the capability of continuous and automated processing. Nevertheless, bioreactor preparation and regeneration are major economic factors limiting commercial use. Therefore, isolation, purification and immobilization of an enzyme in a single step is an ideal strategy. This can be accomplished by adding an affinity tail to the enzyme of interest. The enzyme β-galactosidase, which has application in the dairy industry, was used as a model to demonstrate this approach to bioreactor preparation. The core streptavidin gene (core *Stp*), from *Streptomyces avidinii*, and the β-galactosidase gene (*LAC 4*), from *Kluyveromyces lactis*, were amplified and modified by PCR to facilitate cloning into a *Pichia pastoris* expression and secretion vector. The *LAC 4* gene was cloned in-frame to the core *Stp* gene in a two-step procedure. *P. pastoris*, the methylotrophic yeast, was transformed by electroporation with the resulting plasmid and recombinant cell lysates and culture media were passed through biotinylated glass beads to prepare the immobilized enzyme bioreactor.

**Key Words:** Fusion protein, Streptavidin-beta-galactosidase fusion, Enzyme bioreactor

**403 Occurrence of *Bacillus sporothermodurans* and the influence of the thermal processing procedure on its presence in Brazilian UHT milk.** P.B. Zacarchenco*[1], M. F. F. Leitao[1], M. T. Destro[2], and C. Andrigheto[2], [1]*Faculdade Engenharia de Alimentos-UNICAMP, Campinas, Sao Paulo/Brazil,* [2]*Faculdade Cincias Farmaceuticas-USP, Sao Paulo, Sao Paulo/Brazil*.

*Bacillus sporothermodurans*(BSP) has been isolated worldwide, but mainly in European countries as a usual contaminant of UHT milk. Even being nonpathogenic, generally its presence results in product rejection based on international microbiological standards limiting the counts to a maximum of 100 cfu/ml. In this research work, the occurrence of BSP was evaluated in UHT milk samples produced in different regions of Brazil (Central, South and Southeast) and processed by the indirect thermal treatment system (plate or tube exchangers) or by the direct system (steam infusion or steam injection). A total of 100 commercial samples were analysed both quantitatively by spread plate technique using Brain Heart Infusion Esculin Agar (BHI-E agar) and qualitatively after thermal shock treatment (115°C/7min), followed by incubation and plating in that medium. The results showed a total of 45 per cent of the examined samples with counts above 100 cfu/ml, varying from 2 $.10^4$ and 9.5 $.10^5$ cfu/ml (20,000 and 950,000 cfu/ml). However, 71.4 per cent of the samples processed by indirect system were above limits, while none of the samples heat treated by the direct system, that allows a more drastic temperature condition, were rejected. A similar picture was observed in the qualitative evaluation, with contamination levels of 71.7 and 22.2 per cent for the indirect and direct system, respectively. A total of 300 cultures were isolated from contaminated samples, showing cultural, morphological and biochemical patterns similar to BSP, except a late glucose fermentation capacity showed by most of the isolated strains. When 24 representative strains were submitted to PCR-RAPD evaluation and compared to the reference BSP strain (DSMZ 10599), they were confirmed as *Bacillus sporothermodurans* based on their DNA pattern.

**Key Words:** Uht milk, Bacillus sporothermodurans, Heating system

**404 Optimizing of beta-cyclodextrin recycling process for cholesterol removal in cream.** H. S. Kwak*, H. M. Suh, J. Ahn, and H. J. Kwon, *Sejong University, Seoul, Korea*.

This study was designed to find optimum conditions of β-cyclodextrin recycling process by applying four different factors (ratio of solvent to cholesterol-β-cyclodextrin complex, mixing speed, mixing temperature, and mixing time) in cholesterol dissociation from cream. Using the ratio of 6 to 1 (solvent to the complex) showed the highest cholesterol dissociation rate (82.50%) when mixed at 1000 rpm at 50°C for 1 h. Mixing speed did not significantly affect the cholesterol dissociation. Also, mixing time appeared to be insignificant. The optimum mixing temperature was 50°C, and mixing at 40°C resulted in a significantly lower rate, compared with that at 50°C. In a subsequent experiment, using recycled β-cyclodextrin only showed 75.07% of cholesterol removal in cream, while 6 to 4 mixture of recycled to unused β-cyclodextrin increased cholesterol removal to 95.59%, which is highly close to that of 100% unused β-cyclodextrin.

**Key Words:** Beta-cyclodextrin recycling, Cholesterol removal, Cream

**405 Binding bile salts by soluble fiber: potential use in dairy products containing a probiotic culture.** E.P. Cuesta* and S.E. Gilliland, *Oklahoma State University, Stillwater*.

Soluble fibers are used in the food industry as stabilizers and thickeners. It has been widely reported that soluble dietary fibers can be hypocholesterolemic. One of the mechanisms for cholesterol reduction

relates their ability to bind bile salts, enhancing steroid excretion from the body.
Binding of sodium taurocholate, sodium glycocholate and sodium cholate by soluble fibers (guar gum, xanthan gum and soluble fiber extracted from oat meal) was measured.
The fiber solutions were incubated in presence of the individual bile salt for 2 and 4 hours at 37 C, then the fiber was precipitated and the bile salt remaining in the solution was analyzed by HPLC to determine the percentage of binding.
None of the fibers exhibited the ability to bind sodium glycocholate at the concentration tested but all of them were able to bind sodium taurocholate and sodium cholate with different range of percentage depending of the concentration of bile salts.
The major conjugated bile salt in the human intestine is sodium glycocholate and at least part of it can be deconjugated by selected probiotic cultures. Thus, the combined use of a selected probiotic culture that deconjugates sodium glycocholate with a selected soluble fiber that will bind the free sodium cholate could be useful in improving the control of serum cholesterol levels.

**Key Words:** Soluble Fiber, Bile Salts

### 406 Cholesterol recovery from beta-cyclodextrin complex of cream using a new combined method with immobilized cyclomaltodextrinase of alkalophilic *Bacillus* sp. KJ133 and solvent extraction.

H. J. Kwon, H. J. Jung, and H. S. Kwak*, *Sejong University, Seoul, Korea*.

A new combined method with enzymatic hydrolysis of $\beta$-cyclodextrin ($\beta$-CD) and solvent extraction of cholesterol from the hydrolyzed mixture was developed to recover the cholesterol of $\beta$-CD-cholesterol complex that was prepared from dairy products such as cream, milk, and cheese. Alkalophilic *Bacillus* sp. KJ133 was isolated from soil as a cyclomaltodextrinase (cyclomaltodextrin dextrin hydrolase, EC 3.2.1.54, CDase) producer. The intracellular CDase was prepared from cells by sonication and showed the optimum $\beta$-CD hydrolyzing activity at pH 6.0 and 50°C. The CDase efficiently hydrolyzed the $\beta$-CD of $\beta$-CD-cholesterol complex and free cholesterol was easily extracted from the hydrolyzed mixture using non-polar solvent such as ethyl acetate. To increase the stability of a free CDase, we also developed an immobilized CDase using sodium alginate as a carrier. Immobilized CDase showed a high yield of cholesterol recovery in a time dependent manner than that of free one. Gas chromatography analysis showed that over than 70% of cholesterol was recovered from the $\beta$-CD-cholesterol complex of cream while only 3 and 29% of cholesterol were recovered from solvent extraction and free CDase treatment, respectively. The cholesterol recovered can be used as raw material for steroid systhesis and this method will provide an efficient way to recover the cholesterol or other organic compound from the $\beta$-CD-cholesterol or -organic compound complex.

**Key Words:** Cholesterol recovery, Cyclomaltodextrinase, Beta-cyclodextrin

### 407 Increase in conjugated linoleic acid (CLA) in fermented milk by *Lactococcus lactis*.

Y.-J. Kim*[1], S.T. Lee[1], and R.H. Liu[1], [1]*Dept. of Food Science, Cornell University, Ithaca, NY*.

Conjugated linoleic acid (CLA) is a strong anticarcinogenic agent and dairy products are good sources of CLA. Increase in CLA concentration will improve the value of dairy products. The objective of this work was to elevate CLA concentration in fermented milk (e.g. yogurt). Fifteen strains of dairy starter culture were screened for CLA production using sunflower oil (containing 70% linoleic acid) as a substrate in culture medium. Among the screened strains, *Lactococcus lactis* IO1 showed the highest CLA producing ability. Sunflower oil in culture medium inhibited bacterial cell growth and the optimal concentration of sunflower oil for CLA production was 0.2 g/L which accounted for 0.5% of total fat in milk. Moreover, additions of dry powder and glucose in the culture also increased CLA concentration, and the CLA production was directly proportional to the bacterial cell number. The elevated bacterial cell number reduced the time to lower the pH in culture medium, but low pH inhibited isomerase reaction and enhanced biohydrogenation, which led to the decrease in CLA accumulation. The addition of potassium phosphate buffer enhanced CLA production by increasing buffer capacity and keeping culture medium for a longer period of time. Using this technique, CLA production could be increased up to 2.3 times higher than control fermented milk ($p< 0.01$). In conclusion, our results have demonstrated that CLA formation in fermented milk could be elevated. The maximum CLA formation in fermented milk was affected by various factors such as a bacterial strain, cell number, optimal substrate concentration and period of incubation time at neutral pH.

**Key Words:** Conjugated linoleic acid, *Lactococcus lactis*, Sunflower oil

### 408 Factors affecting the filtration of nonfat milk through diatomaceous earth to reduce *Bacillus* endospore contamination.

A. Bienvenue*[1] and R. Jimenez-Flores[1], [1]*DPTC, Cal Poly State University, San Luis Obispo, CA*.

Endospore forming bacteria are naturally occurring in milk as a result of contamination from the environment and the equipment. In addition, endospores are a concern in food products because of their exceptional heat resistance during processing. Therefore, endospore forming bacteria can affect both the quality and the safety of foods. Previous studies on bio-silicate performance in endospore removal resulted in the selection of Hyflo Super-Cel®. The objective of this study was to determine the variables within milk that affect endospore removal efficiency during filtration. Eight milk samples (defatted early lactation raw, defatted late lactation raw, defatted bulk tank raw, and five commercial pasteurized nonfat milk) were enriched in *Bacillus* endospores to a concentration of 1 x $10^5$ CFU/ml. Simultaneously, a filtration cake was built by recirculation of a bio-silicate slurry according to a set pattern (i.e. flux and time). The spiked milk was then filtered through the diatomaceous earth filtering cake. Samples of milk before and after filtration were characterized for their microbial load, protein, fat, ash, citrate, calcium, and chloride content. Moreover, SDS-PAGE was used to compare the proteins adsorbed to the diatomaceous earth. Results showed that milk filtered through a Hyflo Super-Cel® cake removed greater than 90% of the aerobic mesophilic endospore population added in raw late lactation and in early lactation defatted milk. In addition, the temperature history of the raw milk samples and the centrifugation process used to remove the fat affected the endospore removal efficiency. High endospore removal was associated with the presence of a 55 kDa protein adsorbed to the bio-silicate. None of the remaining milk constituents studied could be correlated to the endospore removal efficiency obtained.

**Key Words:** Bio-silicates, Endospore removal, Milk

### 409 Water-soluble nitrogen accumulation and *Lactococcus* cell viability after high pressure processing of Cheddar cheese.

U. Nienaber, T.H. Shellhammer, W.J. Harper, and P.D. Courtney*, *Ohio State University, Columbus*.

High pressure processing is a novel manufacturing technology capable of activating and/or inactivating many food enzymes and causing bacterial cell leakage and death. Application of high pressure to Cheddar cheese for short periods of time early in the ripening process has the potential to shorten ripening time. Applying higher pressures later during ripening may allow cheese manufacturers to arrest ripening at a desired level. The objective of this study was to determine the effects of high pressure treatments on cheese ripening, as inferred by water soluble nitrogen content.
Cheddar cheese was manufactured on site with a commercial mesophilic starter culture, divided into one pound blocks (6x6x14cm), and vacuum packed in high barrier flexible pouches. Duplicate samples of two-week old cheese were pressure treated using an ABB Quintus Food Processor QFP-6 Cold Isostatic Press. Applied pressures ranged from 100 to 800 MPa for one or five minutes at 25°C. *Lactococcus* cell viability was determined immediately after pressure treatment of cheeses by plating serial dilutions on M17-lactose medium. Cheeses were stored at 4°C throughout the study, and sampled periodically. WSN was extracted using standard methods and measured by the Kjeldahl method.
*Lactococcus* cell viability was only slightly decreased from the non pressure-treated control ($8.0X10^8$ cfu/g) at pressures of 100 to 300 MPa for one minute. One-minute treatments at 400 and 500 MPa caused 3.4 and 4.6 log cycle reductions respectively. Less than $10^3$ cfu/g was detected after 600-800 MPa treatments. One month after pressure treatment, WSN content was determined in control and pressure-treated cheeses. These analyses revealed the highest WSN level in the 400 MPa-treated cheese and the lowest level in the 700 MPa-treated cheese. In addition, a five-minute treatment of two week old cheese at 800 MPa dramatically arrested the evolution of water soluble nitrogen over time when compared to the control cheese. High pressure treatment impacts

the extent of water soluble nitrogen accumulation over time in Cheddar cheese, and thus may have potential for use in controlling cheese ripening.

**Key Words:** High Pressure, Cheese, Ripening

**410 Influence of cream homogenization and protein supplementation of Cheddar cheese milk by ultrafiltration on functionality of whey protein concentrates.** M. G. Nair, V. V. Mistry*, and B. S. Oommen, *MN-SD Dairy Foods Research Center, South Dakota State University, Brookings*.

Cheddar cheese was made from milks that were supplemented with (**C1**) or without (**C0**) ultrafiltered skim milk of 16.2 to 16.9% protein and standardized to 0.7 casein-to-fat ratio with unhomogenized (**P0**) or homogenized (**P1**) cream (35% fat). The four treatments were **P0C0** and **P1C0** (3.2% protein) and **P0C1** and **P1C1** (6.0% protein). Whey pasteurized at 62.8° C for 30 min was ultrafiltered at 55°C to 9:1 for C0 and 5:1 for C1, separated, standardized to 0.4% fat, and spray dried. The protein contents of whey protein concentrates (**WPC**) of C1 (51.8%, P0C1 and 51.2%, P1C1) were higher ($P < 0.05$) than those of C0 (50.2%, P0C0 and 47.7%, P1C0). Moisture of WPC was approximately 4% and fat ranged from 2.9 to 3.2%. Foaming capacity of 10% protein solution expressed as overrun increased ($P < 0.05$) from 399 (C0) to 582% (C1), but decreased ($P < 0.05$) from 639 to 415% as the pH increased from 4.5 to 10.0. Foam stability, measured as percentage of intact foam after 5 min, increased ($P < 0.05$) as pH of the solution was raised from 4.5 (16.1%) to 10.0 (61.8%). Emulsification activity index measured as the amount of light absorbed at 500 nm by a 3:1 emulsion of 2% protein solution at pH 7 and corn oil homogenized with a Polytron homogenizer at 19,000 rpm for 3 min decreased ($P < 0.05$) from 0.525 (P0) to 0.411 (P1) and from 0.635 (C0) to 0.302 (C1). Stability of the emulsion was reduced ($P < 0.05$) from 9.57 (P0) to 6.51% (P1). The fracturability (load at yield point) of heat induced gel (10% protein solution (pH 7) heated at 80°C for 30 min) increased ($P < 0.05$) from 12.81 (P0) to 14.14 Newton (P1) and decreased ($P < 0.05$) from 14.36 (C0) to 12.59 Newton (C1). Gel firmness (load at 80% compression) increased ($P < 0.05$) from 19.54 (C0) to 22.24 Newton (C1). Cheese milk process modifications such as use of homogenized cream and ultrafiltered milk have potential to influence functionality of WPC.

**Key Words:** Whey protein concentrate functionality, ultrafiltration, cream homogenization

**411 Influence of rennet source on casein peptide formation in low-fat Mozzarella cheese.** E.L. Malin*, M.H. Tunick, and P.W. Smith, *Eastern Regional Research Center, U.S. Department of Agriculture, Wyndmoor, PA*.

Rennets from four different sources were used to prepare very low fat Mozzarella cheese to determine the specific proteolytic attributes of each rennet. The four rennets, widely used in commercial production of Mozzarella cheese in the U.S., included calf stomach rennet, fermentation produced chymosin, and fungal aspartic proteinases from *Cryphonectria parasitica* and *Rhizomucor miehei*. SDS-PAGE of cheese extracts showed that the greatest loss in intensity of the alpha-s1 casein band after 6 weeks of storage at 4°C occurred in cheese made with calf rennet, whereas *C. parasitica* was more active on beta casein. An intense band at the gamma-1 position in the *C. parasitica* extract may represent a different beta casein fragment created by the action of *C. parasitica* aspartic proteinase. HPLC of the four cheese extracts at 6 weeks showed many major peptides with similar or identical renetion; some peaks occurred in clusters. More peptides were produced by *R. miehei* and calf rennet than by fermentation produced chymosin and *C. parasitica*. Although x-ray crystal structures suggest that the four rennet enzymes have almost identical active sites, very small sequence differences in other portions of each enzyme may induce slight alterations in binding site and/or turnover rate that result in the proteolytic variations observed by SDS-PAGE and HPLC.

**Key Words:** Mozzarella, Rennet, Peptide

**412 Cheese yield and standardization of milk for cheese making: comparison of predictive cheese yield equations.** C.M. Chen, A.L. Dikkeboom*, M.E. Johnson, and M.G. Zimbric, *Wisconsin Center for Dairy Research, Madison*.

The physical characteristics of cheese are influenced to a great extent by cheese composition. Standardization is the process of changing milk composition to produce a cheese of desired composition. Accurate standardization requires a formula that not only predicts cheese yield but also fat in the dry matter (FDM) of the cheese. All yield equations incorporate cheese moisture, milk composition (casein and fat) and use factors to express the recovery of casein and fat in milk in cheese. The main difference between predictive cheese yield formulas is how they measure the contribution of non-fat, non-casein solids (other solids) to cheese yield. One type of yield formula (Emmons, general formula Type H, J. Dairy Sci. 76:914-920) bases the contribution of other solids to cheese yield on non-fat, non-casein solids of whey, salt in the cheese, moisture of the cheese and a solids exclusion factor (sef). The Van Slyke formula uses fat and casein recovery and cheese composition to calculate the contribution of other solids to cheese yield. If the casein recovery is held constant at .96, the Van Slyke puts a premium on the conversion of milk fat to cheese to determine the contribution of other solids to cheese yield. We analyzed the data from 92 vats of reduced-fat Cheddar cheese. The milk casein to fat ratio was standardized by cream removal or the addition of reconstituted NDM. Percentage of total milk solids was varied from 9.53 to 14.31. Yield was calculated using the Van Slyke and Emmons equations, and data from all the vats of cheese. An average Van Slyke equation was determined and compared to the Emmons Type H equation using either a sef factor of 0 or .5. Average milk composition and fat recovery were used in both equations. Using a sef of 0, the estimation of yield by the Emmons equation was equivalent to the actual average cheese yield. Using a sef of .5 underestimated cheese yield. The average Van Slyke equation also precisely predicted cheese yield. The Emmons yield equation (sef=0), gave a more accurate prediction of cheese yield when fat recovery values were varied and incorporated into both yield equations.

**Key Words:** cheese yield, reduced fat

**413 Characteristics of reduced fat Edam cheese with adjunct cultures.** W. Tungjaroenchai*, M. A. Drake, and C. H. White, *Mississippi State University, Mississippi State*.

The influence of four adjunct cultures [*Brevibacterium linens* (BL2), *Lactococcus lactis subsp. diacetylactis*, *Lactobacillus helveticus* (LH212), and *Lactobacillus reuteri* (ATCC23272)] on chemical and sensory characteristics of reduced fat Edam cheese was studied. Mean fat and moisture contents of reduced fat cheese were 20.85 ± 0.76 and 42.95 ± 0.43, respectively. Fat and moisture of full fat control cheese were 30.06 ± 0.78 and 39.11 ± 0.60. Titratable acidity increased in all cheese with aging while pH initially decreased but increased in cheese after 5 mo. aging at 7°C ($p<0.05$). Lactic bacteria counts were on average one log higher for reduced fat cheeses than for full fat control cheese with counts decreasing with aging. Free amino acids (FAA) in cheeses increased with aging. FAA was higher in reduced fat cheeses than in the full fat control cheese. Reduced fat cheeses containing *L. helveticus* exhibited the highest FAA ($p<0.05$) content. The relative aminopeptidase activity of *L. helveticus* and *L. lactis subsp. diacetylactis* was higher than that of *L. reuteri* and *B. linens*.
Descriptive sensory panelists (n=9) did not detect differences among cheese treatments after 3 and 6 mo. ripening but age-developed flavors (fruity, nutty, brothy, free fatty acid) increased between 3 and 6 mo. ($p<0.05$). Also, sweetness increased in cheese with aging. Expert panelists (n=6) detected differences in textural quality among the cheeses ($p<0.05$). Textural quality of reduced fat cheeses increased with aging. Reduced fat control cheeses and reduced fat cheeses with *L. helveticus* and *L. reuteri* received the highest textural quality scores. The reduced fat control cheeses and reduced fat cheeses with *L. helveticus* and *L. lactis subsp. diacetylactis* received the highest flavor quality scores from the expert panelists. The addition of *L. helveticus* or *L. lactis subsp. diacetylactis* adjunct cultures to reduced fat Edam cheeses increased proteolysis and textural quality.

**Key Words:** Adjunct cultures, Proteolysis

**414 Effect of fat composition and milk treatment on development of cheese texture.** M. Almena-Aliste* and Y. Noel, *National Agronomic Research Institute, INRA, Dairy Technology and Analysis Research Unit, Jura, France*.

Effects of fat composition and milk treatment (raw/pasteurized) on changes in textural properties during ripening were evaluated on a semi-hard model cheese. Fat composition was changed by using milk fat produced by cows fed either with pasture or hay. The experimental cheeses were made in a pilot plant following the technological scheme of a pressed uncooked type cheese and then ripened at 13°C for 5 weeks. An experimental design with 4 randomized complete blocks and a $2^2$ factorial treatment structure was used. Texture was evaluated by rheological analyses on cheeses, at 1-day and then each week, by using a Texture Analyzer fitted with a cylindrical probe of 10-mm diameter. The test combines penetrometry (to a depth of 10 mm) and relaxation (over 3 min). The following rheological parameters were calculated from the force-time curve: *slope, force and work to the maximum penetration depth, residual modulus, relaxation time and the constant terms K1 and K2*. Texture of 5-wk-old cheese was also evaluated by sensory analyses. Gross composition, calcium and chloride content were measured on 1-day and 5wk-old cheeses. The pH was measured directly into 1-day old cheese. The repeated measurement data from cheeses were analyzed by using an ante-dependence model. Fat composition strongly modified the rheological properties of the 1-day-old cheeses. Cheeses manufactured with milk fat from cows fed with pasture showed more elasticity and less mechanical resistance. Effects of cream type on cheese texture could be explained by differences between fatty composition induced by cow feeding. Effect of milk treatment was significant on parameters associated with the mechanical resistance and the viscous component of cheese, at the latest stages of ripening. The pasteurized cheeses were firmer and less viscous. Effects of fat composition and milk treatment on cheese texture were less significant at intermediate ages of ripening (1-3 weeks). An interaction between the 2 factors was identified with the force to the maximum penetration depth on 1-day and 5wk-old cheeses.

**Key Words:** Cheese Texture, Pasteurization, Fat composition

**415 Texture evaluation of cheese with soft consistency: effect of testing conditions on penetrometric parameters.** M. Almena-Aliste*[1,2], Y. Noel[1], and A. Cepeda Suez[2], [1]*INRA, Dairy Technology and Analysis Research Unit, Poligny (France)*, [2]*Hygiene and Inspection of Foods, Faculty of Veterinary-University of Santiago de Compostela, Spain*.

Rheological tests are used to evaluate objectively the textural properties of cheeses. Testing conditions have a critical role for this evaluation. The uniaxial compression test is currently being standardized within the IDF, while penetrometry has been less studied. The study aimed to select the testing conditions for penetrometry on cheese with soft consistency, considering both tool shape and penetration depth. Three penetrometric tool shapes were tested (ball, round or flat-end cylinders) at 3 different penetration depths (5, 7 and 10 mm). A mold ripened soft cheese was used as an experimental model. Cheeses were selected at 6 different ages from 0 to 5 ripening weeks in order to obtain a variability of textural properties. Rheological measurements were carried out with a Texture Analyzer at a constant displacement rate of 0.8 $mm.s^{-1}$, directly on whole cheeses at the ripening temperature (17°C). The different measurements were randomly distributed on the cheese at each age. Three variables were calculated from the force-displacement curve: the slope of the initial linear part, the maximum force and the work to maximum force (area under the curve until the maximum penetration depth). Data were analyzed by Analysis of Variance and Principal Component Analysis. Results showed that tool shapes influenced significantly penetrometric parameters. Both tool shape and penetration depth had lower effects at the beginning and the end of ripening time. The round-end cylinder tool was suitable for studying the change of mechanical properties of cheese during ripening but penetration depth should be adapted to cheese height. More the ball was the most appropriate tool shape allowing quasi non-destructive control on young and mature cheeses.

**Key Words:** Texture of Soft Cheese, Penetrometry, Testing conditions

**416 Effect of late blowing inhibitors on bacteriological and chemical changes in Swiss cheese.** S. M. El-Gindy*, *Assiut University, Egypt*.

In Swiss cheese made from milk infected with 100 spores of *Clolstridium tyrobutyricum* and containing 0.02% potassium nitrate the bacterial content was markedly lower than that of cheese made from milk containing 2% of a nisin producing *Str. lactis* and that of the control cheese, after 6 weeks. Microorganisms present in all cheeses belonging to streptococci, lactobacilli, and micrococci. Streptococci were detected until the end of 6 weeks, micrococci predominated after 24 weeks. Addition of nisin-producing *Str. lactis* resulted in a higher pH value, reduced the decomposition of protein, did not improve cheese consistency and retarded flavor development. Potassium nitrated caused no appreciable change in the acidity of cheese, and improved the consistency. Both the nisin-producing *Str. lactis* and potassium nitrate prevented late blowing cheese. However, the latter may be preferred as the former was not beneficial to acid development and ripening of cheese.

**417 Modification of buttermilk bunctionality with biosilicates.** B.G. Fryksdale* and B. Jimenez-Flores, *California Polytechnic State University, San Luis Obsipo, CA*.

Previous research has shown that biosilicates (BS) can be used to modify the functionality of dairy cream. This research was to determine if buttermilk (BM), can also be modified by this process. Four types of BS were evaluated; Calcium silicate (Ca), magnesium silicate (Mg), Hyflo (Hy), and R648. Treatment consisted of addition of BS to BM (0.5% w/w), agitation at 160F for 5 min., followed by removal of the BS by filtration. Treated BM was then used to prepare emulsions of 10% and 40% fat by homogenization at 1500/500psi, 155F. A spectrum of analysis was conducted, which included, small and large deformation rheological characterization, foaming and foam stability, particle size analysis, emulsion stability, and SDS-PAGE. Rheological characterization revealed G' of the control to be 1025 Pa. Treatment with Ca and Mg increased G' to 1350 Pa and 1200 Pa, respectively, while treatment with R648 and Hy had no effect. Steady stress sweeps of the 10% fat emulsions were used to compare complex viscosity. Viscosity of control was 0.0018 Pa.s while the Ca and Mg samples had higher viscosities of 0.003 Pa.s and 0.005 Pa.s, respectively. Differences in foaming characteristics of the 10% fat emulsions were also found. Samples treated with Ca and R648 showed greater foaming over others (a= 0.05). Additionally, the Ca sample produced a lasting stable foam, while others collapsed within 15 minutes. Emulsion stability evaluated by creaming rate monitored by a dynamic light scattering instrument showed decreased emulsion stability for all treated BMs. Particle Size Analysis showed that the Control and Ca 10% fat emulsions had a mean particle size of 1.68 while the Hy, Mg, and R648, all showed increased particle sizes of 1.78, 2.03, and 1.82, respectively. SDS PAGE showed that the BS that induced that greatest changes in functionality also had adsorbed a larger amount of certain proteins, particularly the high MW proteins commonly associated with the MFGM. In conclusion, certain BS, particularly Ca and Mg have tremendous potential to alter the functionality of the buttermilk. Ultimately, this novel process can be as a tool to help develop traditional or low fat dairy products with improved or unique texture, using exclusively dairy ingredients.

**Key Words:** Buttermilk, Biosilicates, Functionality

**418 Effect of glycomacropeptide and high pressure homogenization on the stability of milk protein emulsions.** S Bhatia* and R.L. Richter, *Texas A&M University, College Station*.

The objective of the study was to determine the effect of the presence of the glycomacropeptide in whey protein concentrate on the stability of emulsions prepared from milk proteins. Emulsions were made in solutions that contained 3% protein from a source of whey protein concentrate that contained the glycomacropeptide and a whey protein concentrate that did not contain the glycomacropeptide. Sunflower oil was added at 3 and 7% and the solution homogenized at 30 and 90MPa. The particle size distribution, creaming rate, and the viscosity of the emulsions was measured. The creaming rate was faster for samples homogenized at 30 MPa than for samples homogenized at 90MPa and results after homogenization at 30MPa were inconsistent. The mean particle size of particles in samples homogenized at 90 MPa was 0.322 and 0.3427 $\mu$m. For samples with and without the glycomacropeptide, respectively. These values were 50% smaller than the size of particles

after homogenization at 90 MPa. The particle size in samples prepared from whey which contained the glycomacropeptide was smaller than the particle size in the emulsions prepared from whey which did not contain the glycomacropeptide when the samples were homogenized at 90MPa. There was no difference in the particle size of samples homogenized at 30MPa. Emulsions prepared from whey protein concentrate that did not contain the glycomacropeptide had the greatest viscosity after homogenization at both homogenization pressures. The viscosity of these emulsions was greater after homogenization at 30MPa than after homogenization at 90MPa.. However, these emulsions were unstable and the results were variable. These results show that emulsions of this composition are more stable if they prepared by homogenization at 90 rather than 30MPa.

**Key Words:** Glycomacropeptide, High Pressure Homogenisation, Milk Protein Emulsions

**419 Influence of pasteurization time/temperature and homgenization/pasteurization sequence on emulsion characteristics and influence of storage time.** C. Bolling*[1], S. E. Duncan[1], T. Keenan[1], W. N. Eigel[1], K. Waterman[1], and K. Kaylegian[2], [1]*Virginia Polytechnic Institute and State University, Blacksburg,* [2]*Wisconsin Center for Dairy Research, Madison.*

Emulsification of modified butteroil in a dairy system would be beneficial for incorporating butteroil in food systems. A low melt fractionated butteroil has a more desirable nutritional profile. Emulsification of this butteroil with milk derived components would yield a 20% reformulated cream which is more desirable to the health conscious consumer.The objective is to determine the effect of HTST (71.7°C for 15 sec.) and UHT (148°C for 2 sec.) pasteurization and 2 stage homogenization (13.6 / 3.4 Mpa) before and after heat processing on emulsion stability and physical properties of reformulated creams throughout a 13 day storage period.Viscosity, creaming stability, feathering, and sensory quality of reformulated 20% cream formulated with fractionated low-melt butteroil (BO) in different milk-derived components were analyzed over a 13 day storage period at 7°C. Raw milk was separated at 55°C into skim milk (SM) and cream. Buttermilk (BM) was obtained from churning cream. Aqueous phase (AP) was obtained from a butter processor. The milk component formulations used for emulsification of the butteroil were 20% BO + 80% SM, 20% BO + 70% BM + 10% AP, and 20% milk fat cream. Creams which underwent UHT heat treatment displayed higher viscosity than creams heat treated at HTST. The 70%BM/10%AP/20%BO formulations which underwent UHT pasteurization were unstable. In addition, the 80%SM/20%BO formulation that underwent homogenization followed by UHT pasteurization was unstable. All creams demonstrated a decrease in creaming stability over a 13 day storage period. Regardless of formulation, storage period, homogenization sequence, and heat treatment, all formulations feathered from pH 4.70 to 5.00. All creams met standard quality expectations based on responses of 10 experienced panelists.

**Key Words:** Reformulated cream, Pasteurization, Viscosity

**420 Rheological properties of microfibrous cellulose and its interaction with milk components.** J.M. Angold* and R. Jimenez-Flores, *Cal Poly State University, DPTC, San Luis Obispo, CA.*

Microfibrous cellulose (MFC) is a stable biopolymer that is produced by the aerobic fermentation of *Acetobacter xylinum* and consists of a small fibrous diameter (0.1mm) as well as a large surface area. PrimaCel, a product made primarily of MFC has been used as a thickening and stabilizing agent in salad dressings and sauces. The commercialized application of PrimaCel in dairy products has not been developed, however, preliminary research shows great potential for PrimaCel to be used to create low fat and fat free dairy products. The focus of this project was to understand how PrimaCel interacts with milk and its components at a macromolecular level. PrimaCel was tested in caseinates and whey protein isolates to determine if interactions were occurring between the proteins present in milk. PrimaCel (0.8%) was homogenized with water, skim, and both 3 and 5 percent of either potassium caseinate, sodium caseinate, calcium sodium caseinate, or whey protein isolate. Analysis was done using small deformation dynamic oscillatory rheology with increasing frequency from 1-100 radians/sec. The PrimaCel was found to have dilatant properties when mixed with caseins or whey proteins with a larger storage modulus (G') than loss modulus (G"). It was also determined that as the percentage of casein or whey protein was increased from 3-5% there was a decrease in G'. Overall the PrimaCel homogenized in whey protein concentrate formed a gel with G' approximately 2.5 times larger than PrimaCel in casein. PrimaCel (0.8%) homogenized in 3% whey protein isolate gave a G' range of 42-50Pa, whereas in casein the G' ranged from 11-19Pa. It was also determined that with a higher pressure of homogenization PrimaCel forms a stronger gel in all of the components tested. Differential Scanning Calorimetry was used to correlate these results with enthalpy changes in cellulose and protein interactions.

**Key Words:** rheology, milk proteins, microfibrous cellulose

**421 Effect of inulin on some rheological and physical properties of acid milk gels with inulin.** G. Perez-Hernandez* and R.L. Richter, *Texas A&M University, College Station.*

The objective of this study was to evaluate the effect of inulin on some rheological and physical properties of acid milk gels. NFDM was reconstituted to 12% MSNF with 0 and 2% fat and 0, 2, 5 and 10% inulin. The milk was heated at 90°C for 10 min and homogenized (20MPa). Glucono-$\delta$-lactone (2% w/v) was added and the milk stored for six hours at 20°C. Then samples were stored overnight at 7°C and the rheological properties of stirred gels were then measured using a Brookfield rotational viscometer. Static and Dynamic yield stress were measured using the vane method. Syneresis was measured by centrifugation and the slope of the linear regression was determined as a coefficient of syneresis. The gel structure was observed by confocal scanning laser microscopy. Samples inoculated with yogurt culture were used for pH and acidity experiments. A Herschel-Bulkley model described the upward shear-rate flow behavior of the samples, while the downward shear rate curves were nearly linear. The hysteresis loop was used as a thixotropy coefficient. Increased fat and inulin concentration increased static and dynamic yield stress. With the increase in inulin and fat content, K and $\sigma_o$ value increased whereas n decreased affecting the Herschel-Bulkley models in the upward shear rate curve. The thixotropy coefficient increased as fat and inulin concentration was increased. Syneresis coefficient decreased with fat and inulin concentration. No differences were seen in the structure of the gels. Kinetics of pH and acidity were not affected by the concentration of fat and inulin. Inulin can be incorporated into yogurt to increase the gel strength and decreased syneresis and it will not affect the protein matrix.

**Key Words:** Inulin, Acid Milk Gels, Rheological Properties

**422 Rheological and physical characterization of derivitized whey protein solutions.** H.M. Hudson*, C.R. Daubert, and E.A. Foegeding, *North Carolina State University, Raleigh.*

Dairy ingredients mimicking the thickening functionality of gelatin, hydrocolloids, and starches have been a focus of current research. Pregelatinized starch is often employed in food applications because of the instantaneous nature of thickening and stability imparted by modification. Proteins have traditionally been excluded as an instantaneous viscosifying agent due to the requisite thermal treatments to create structure. Whey protein isolate (WPI) gels were prepared while manipulating heating time, pH, and mineral type/content, producing a variety of gel types/networks. Gels were subsequently frozen, freeze-dried, and ground into a powder. Once reconstituted in deionized water, gel powders were evaluated based on solubility, electrophoresis, and rotational viscometry. The protein powder exhibiting the largest apparent viscosity, highest degree of hydrolysis, and greatest solubility was selected for further analysis. Rotational viscometry was performed on solutions prepared from the selected derivitized powder at pH values between 3 and 8, temperatures of 5-90 C, and shear rates between 1-100 s-1. Small amplitude oscillatory rheology was conducted at a constant stress of 1.0 Pa; frequency was ramped from 0.1 to 20.0 Hz, while temperatures were alternated between 25 and 90 C. Rheological analyses reveal a processing technique able to derivitize WPI into a product capable of forming cold-set weak gel structures suitable for thickening over a wide range of temperature and pH food systems. This cold-set feature may impart a wide range of potential applications such as malted-milk beverages, protein drinks, and nutritious liquid formulations for athletes, infants or the elderly.

**Key Words:** Whey Protein Isolate, Acid Hydrolysis, Cold-Set Gels

**423 Tryptic hydrolysis of β-lactoglobulin A, B, and C.** H. C. Nilsson[2], M. A. Paulsson[2], C. J. Coker[1], J. P. Hill[1], and L. K. Creamer*[1], [1]*New Zealand Dairy Research Institute, Palmerston North, New Zealand,* [2]*Univesity of Lund, Lund, Sweden.*

The A variant of β-lactoglobulin (BLG) is hydrolyzed more rapidly than the B variant by several different proteolytic enzymes. The rate of loss of BLG A, B, and C with trypsin hydrolysis was studied as a function of pH, temperature, BLG concentration, added urea, and added palmitate using RP-HPLC (reversed-phase high-performance liquid chromatography) and SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Peptide identity was confirmed by mass spectrometry. The major finding is that a number of the trypsin-sensitive peptide bonds are broken more rapidly in BLG A than in BLG B or C but that many others are cleaved at the same rate for all variants. Higher temperatures, addition of urea, and higher pH all increased the rates of reaction but palmitate decreased the reaction rate markedly. All of these treatments (except increased protein concentration) decreased the rate of BLG A hydrolysis relative to BLG B, but did not change the relative rates of BLG B and BLG C hydrolysis. It is not obvious whether BLG A is inherently less stable than BLG B or BLG C, as the thermal unfolding and the urea-induced equilibrium unfolding of BLG A is intermediate between that of BLG B and that of BLG C.

**Key Words:** Tryptic hydrolysis, Genetic variants

**424 Binding of small amphipathic molecules to β-lactoglobulin.** L. K. Creamer*[1], M. Blair[1], R. Korte[2], and G. B. Jameson[2], [1]*New Zealand Dairy Research Institute, Palmerston North, New Zealand,* [2]*Massey University, Palmerston North, New Zealand.*

There has been much speculation about the role of bovine β-lactoglobulin (BLG) as a carrier of vitamins and fatty acids. We have innovated the technique of induced-circular dichroism (CD) to measure the association of reporter molecules with bovine BLG. One interesting example is 2:5-dinitrobenzoic acid covalently bonded to BLG. This probe is not chiral itself, but when it is bound to a chiral site it shows strong CD bands. Similarly retinol shows weak CD bands in the absence of BLG but strong signals in the presence of BLG at pH 8.0. This signal is weakened by addition of palmitic acid to the mixture showing partial displacement of retinol by the fatty acid. This indicates that the major site for retinol binding is the hydrophobic cavity that has been identified crystallographically as the binding site of fatty acids. BLG binds retinol strongly above pH 7.5, whereas it binds parinaric acids strongly above pH 5. These and other results indicate that the Tanford transition affects retinol binding but not parinaric acid binding, which is, however, affected by the protonation of the parinaric acid carboxyl group. Comparable experiments with retinoic acid suggest that its binding mode to BLG changes over the pH range 3-10, probably as a consequence of movement within the hydrophobic cavity. Experiments with retinyl acetate and retinyl palmitate suggest that the palmitate end of retinyl palmitate is in the cavity but the acetate end of retinyl acetate is in the solvent.

**Key Words:** β-Lactoglobulin binding site, Retinol binding, Retinyl palmitate binding

**425 Effects of genetic variants on the rates of interaction of β-lactoglobulin and κ-casein.** Y. H. Cho[2], H. Singh[2], and L. K. Creamer*[1], [1]*New Zealand Dairy Research Institute, Palmerston North, New Zealand,* [2]*Massey University, Palmerston North, New Zealand.*

The heat-induced interaction of whey proteins, especially β-lactoglobulin (BLG), with κ-casein (KCN) is fundamental to all heat treatments that affect the functionality of milk products. As a starting point we have examined the effect of KCN on the established reaction pathway of heat-induced BLG unfolding and aggregation. KCN was purified directly from milk by centrifugation and size-exclusion chromatographic procedures to give a native polymeric product. KCN was added to either native BLG or to previously heated BLG and the mixtures heated. The rate of loss of BLG and the distributions of intermediate products were determined using polyacrylamide gel electrophoresis (PAGE). It was found that KCN reacted more rapidly with BLG that had been unfolded by prior heat treatment than with native BLG. Some monomeric KCN was also present in the reaction mixtures, no doubt as a result of disulfide bond interchanges between KCN and BLG. In all cases KCN B was more reactive than KCN A and the BLG B was more reactive than BLG A or BLG C.

**Key Words:** κ-Casein, β-Lactoglobulin, Heat-induced interaction

**426 The enzyme activities and milk performance in German Holsteins.** L. Panicke*[1], M. Schmidt[2], J. Citek[3], G. Erhardt[4], V. Rehout[3], and R. Staufenbiel[5], [1]*Research Institute for the Biology of Farm Animals, 18196 Dummerstorf, Germany,* [2]*PH Kielce, Poland,* [3]*Southbohemian University Ceske Budejovice, Dep.of Animal Breeding, Czech Republic,* [4]*University Giessen, Institut of Animal Breeding and Genetics, Germany,* [5]*Free University Berlin, Institute of Veterinary Physiology, Germany.*

A high milk performance connected with a stabile health regarding metabolism and a sufficient fertility is been influenced from a well balanced distribution of energy. The anabolic and catabolic balance between protein synthesis and proteolysis in relation to lipogenesis and lipolysis are influenced by degradative enzymes. Aminopeptidases Arginyl- (ARG E.C.3.4.11.6.), Alanyl- (ALA E.C.3.4.11.14.) and Leucyl- (LEU E.C.3.4.11.1.) were analysed in the serum of 357 German-Holstein cows using Ala- and Arg-Naphtylderivate substrate. The sum of the animal effects of their enzyme activity SAAL = ARG + ALA + LEU increases not linear with the milk performance in the lactation in three different years. Genetic variants of growth hormone gene (GHG) and the milk protein genes ($\alpha_{S1}$-, $\alpha_{S2}$-, β-, κ-Casein and β-lactoglobulin) were determined in contemporary cows. The SAAL activity increased with the milk performance in different GHG genotypes. Under inclusion of β-casein-variants (β-CN) the enzyme activities for SAAL of β-CN A2A2 genotypes decreases to 8486 nMol/l/h under similar performances between β-CN A1A1 and β-CN A2A2, which is almost a half of a standard unit (S-% nearly 10 %). Cows with β-CN A2A2 genotype are associated with higher performance and around -2.5 days better intermediate gestation (serviceperiode) at the actual estimation on other animals with + 208 kg milk and + 11.5 fat+protein-kg (Panicke et.al.1998). Conclusions: A high milk performance could be combined with a high fertility on a genetic basis. Lower enzyme activities together with high milk performance presume, that the protein synthesis is increasing with a reduced proteolysis rate. Lower physiological load contributes to the better fertility. Genetic determined physiological values should not be maximized or minimized, but optimized. This optimization should be quantified.

| **Group** | **Genotype** | | **Number** | **Fat+Protein** | **SAAL** |
|---|---|---|---|---|---|
| | GHG*** | β-Casein | n | kg | nMol/l/h |
| 405 | all | all | 30 | 513 | 6637 |
| 406 | all | all | 141 | 506 | 8213 |
| 407 | all | all | 186 | 593 | 8780 |
| 407 | VV* | all | 9* | 563 | 8394 |
| 407 | VL | all | 44* | 584 | 8536 |
| 407 | LL | all | 133* | 597 | 8888 |
| 407 | LL | A2A2 | 23** | 617 | 8486 |
| 407 | LL | A1A1 | 23** | 613 | 9020 |
| 407 | LL | A1A2 | 48** | 582 | 8897 |

Performance and enzyme activities of different genotypes
* total =186 cows
** the remaining to 133 are other genotypes
*** This part of the research was supported by CEZ J06/98/122200004

**Key Words:** cattle, milk, enzyme

**427 Heat coagulation of camel milk.** A. Metwalli, F. Ibrahim*, and K. Hassanein, *Minia University, Minia, Egypt.*

Heat coagulation time pH curve (HCT/pH) of camel milk in the range of (6.4-7.2) was measured at 120 C. The effect of urea, formaldehyde and mixture of them on heat coagulation time was studied. The results showed that, camel milk is not heat stable. However, the heat stability significantly increased at all pH studied (6.4-7.2) with dialysis of milk against Jennes & Koops buffer. Addition of urea alone in concentration of 10-30mM has no effect on HCT of camel milk. Moreover, addition of formaldehyde till 5mM has almost no effect. While increasing the concentration to 7.5 mM, the HCT greatly increased. It seems that there is a critical concentration of formaldehyde that affects the HCT of camel milk. A mixture of formaldehyde and urea (5mM of each) has a

slight effect on HCT. However, increasing the concentration of urea up to 10mM markedly increased the HCT. It could be concluded that heat coagulation behavior of camel milk is completely different from that of cow milk.

**Key Words:** camel milk, heat stability, urea

**428 Reduced fat cheese production by fat removal from aged Cheddar cheese.** B. K. Nelson*, C. C. Nicklas, and D. M. Barbano, *Northeast Dairy Foods Research Center, Cornell University, Ithaca, NY.*

Normally, reduced fat Cheddar cheese is made by removal of fat from milk prior to cheese making. Typical aged flavor does not develop when reduced fat Cheddar cheese is produced by this approach. Previous researchers have demonstrated that aged Cheddar cheese flavor intensity resides in the water-soluble fraction. Therefore, we investigated the feasibility of fat removal after the aging of Cheddar cheese. We hypothesized the typical aged cheese flavor would remain with the cheese following fat removal. Shredding, grinding, and blending were evaluated as methods of cheese preparation. Finely shredded cheese yielded the best fat release and may be the most practical. Efficiency of fat removal at various temperatures, times, and forces was determined. Shredded cheese samples were tempered at specific temperatures between 20°C and 41°C. Centrifugal force was varied from about 2,000 to 23,500×g for times from 5 to 20 minutes. Temperature had the greatest effect ($p<0.05$) on the removal of fat, although force and time were also significant ($p<0.05$). Time and force were less important at higher temperatures. A positive linear relationship between temperature and fat removal was observed from 20 to 33°C. At temperatures above 33°C both fat and water were removed. Fat reduction of 30 to 50% was easily achieved at temperatures ≤30°C and the highest fat reduction achieved was 72%. A 50% fat reduction was attained at 30°C by either 23,500×g for 5 minutes or 6300×g for 10 minutes. A traditional cheese press was used to form the reduced fat cheese into blocks. The fat removed had some aroma but little or no taste. Reduced and full fat cheeses were analyzed for percent fat, protein, moisture, salt, and pH. The full fat aged Cheddar had a composition of 34.0, 25.0, 36.6, 1.74 and 5.15, respectively. The same cheese with approximately 50% fat reduction had a composition of 16.0, 32.0, 45.6, 2.21 and 5.13, respectively. Work is continuing to accumulate sensory data in addition to developing a continuous fat removal process.

**Key Words:** Cheddar, Composition, Reduced fat

**429 Impact of low concentration factor (CF) microfiltration (MF) on fat, protein, and calcium recovery in Cheddar cheese and cheese yield.** M. Neocleous*, D.M. Barbano, and M.A. Rudan, *Northeast Dairy Foods Research Center, Cornell University, Ithaca, NY.*

Raw skim milk was microfiltered twofold (2X) using a 0.1$\mu$m ceramic membrane at 50°C. The average true protein content of the retentate and permeate across the four days of cheese making was 5.31 and 0.53%, respectively. Cream and MF permeate were added back to the 2X skim retentate to achieve a constant casein to fat ratio of 0.68. A vat of cheese was made from each of 1X (control), 1.3, 1.6 and 2X CF cheese milks on four different days using a 4 x 4 randomized block design. Four different batches of raw milk were used. The mean fat content (3.33, 4.21, 5.05, and 6.11%, respectively), casein (2.28, 2.88, 3.45, and 4.15%, respectively), serum protein (0.52, 0.55, 0.56, and 0.59%, respectively), and calcium (0.11, 0.13, 0.15, and 0.17%, respectively) of the standardized microfiltered skim milk increased with increasing concentration factor. Mass balance accounting was done for fat, protein, and calcium in milk, whey, salt whey and cheese. Fat recovery was not influenced by CF. The protein recovery in the cheese increased significantly with increasing CF (72.4, 76.1, 78.9 and 81.4%, respectively), while protein recovery in the whey decreased (27.3, 23.4, 20.5, 17.9%, respectively) with increasing concentration factor. Cheese calcium recovery in the cheese also increased significantly (63.6, 70.6, 74, 78.4%) and whey recovery decreased (33.3, 27.4, 23.2, 19.7%) with increasing CF. These effects were expected because part of the protein and part of the calcium found originally in the milk are removed from the milk by MF prior to cheese making. Theoretical cheese yields were calculated using the Van Slyke formula and the Barbano formula. No significant changes were found in cheese yield efficiency with increasing concentration factor, but there was an increase in actual, composition adjusted, and theoretical cheese yield (9.1, 11.6, 13.9, 16.8 kg per 100kg of milk, respectively) with increasing concentration factor.

**Key Words:** Microfiltration, Cheddar cheese, Yield

**430 Automatic data acquisition and analysis of cheese melt profile.** D. Venkatesan[1], C. H. Hwang[1], and S. Gunasekaran*[1], [1]*University of Wisconsin, Madison.*

We have developed a device called the "UW Melt Profiler" to monitor the cheese melt profile (i.e. cheese height vs. time curve) during heating of disk-shaped cheese samples in an oven. This melt profile contains information that may signify several cheese melt/flow events during heating. Currently, the cheese melt profile is analyzed using computer spread sheet programs. This approach is not only tedious but is also very inconsistent. Therefore, our objective was to develop an automatic data acquisition and analysis procedure and software. The LabView (National Instrument, Inc.) was used as the basis for our software development. The software developed, named "CheeseMelt" is designed to interface with user via on-screen prompts. Upon execution, CheeseMelt automatically acquires cheese height and temperature data continuously as a function of heating time. A graphic interface displays the melt profile in real time. The program terminates data collection when the preset end point is reached and simultaneously processes the melt profile data. An output containing a number of useful cheese melt profile parameters such as: cheese softening point, end point, rapid flow time, maximum flow rate, average flow rate, total flow time etc. is generated. The performance of this automatic procedure was successfully verified with different cheese types, experimental protocols, and data analysis procedures. These parameters are relative measures of cheese properties, which can be used as product development and/or quality control tools. Therefore, our automatic analysis method is expected to simplify the cheese melt/flow evaluation procedure.

**Key Words:** Cheese, Melt profile, Software

**431 Rheological characteristics of Monterey Jack hard goat cheese.** R Attaie*[1] and R. L. Richter[2], [1]*Prairie View A&M University, Prairie View,* [2]*Texas A&M University, College Station.*

Pasteurized milk from French Alpine goats was processed to Monterey Jack hard goat cheese. Commercial mesophilic starter culture was activated twice and added to milk at the rate of 2% (v/v). After addition of rennet, cutting of the coagulum started 60 min later. Acidity of curd ranged from 0.21 to 0.23% and pH ranged from 5.60 to 5.70, when salting started at the rate of 2.5% (salt/curd). Starter culture was grown in tryptic soy broth in bulk quantities and cells were harvested by centrifugation at 6500 x g for 10 min at 4°C. The harvested cells were washed with distilled water and disrupted with a sonicator for a total of 10 min. The cellular extract was mixed with curd at the rate of 0.14 and 0.27% (g/g). The objective of this study was to accelerate the ripening process of Monterey Jack goat milk cheese. Cheeses were stored at 4° to 5°C for 30 weeks and rheological properties were determined using an Instron Testing Machine. The force required to measure cheese hardness at 20% compression for the first cycle increased significantly after 12 week of aging. However, the force required to measure hardness at 75% compression during the same cycle did not differ. Similarly, the force required to measure cheese hardness at 20% compression for the second cycle increased significantly during aging while the force for the 75% compression did not differ. The mean elasticity values did not increase significantly at either 20% compression or at 75% compression during aging.

**Key Words:** Goat cheese, Rheology, Ripening

**432 Effect of cheese making conditions on texture of Arza-Ulloa cheese during ripening time.** M. Almena-Aliste*[1,2], Y. Nöel[1], and A. Cepeda Sáez[2], [1]*INRA, Dairy Technology and Analysis Research Unit, Poligny, France,* [2]*Hygiene and Inspection of Foods, Faculty of Veterinary, University of Santiago de Compostela, Spain.*

The effects of 7 technological factors ($CaCl_2$ addition of milk - Starter dose - Coagulant type - Coagulant dose - Salting conditions - Curd washing - Pressing time) on texture of Arza-Ulloa cheese (NW-Spain) were studied by using a fractional design. The effects were evaluated on experimental cheeses at 3 ripening ages (0, 2 and 5 weeks) based on

physico-chemical, proteolysis and rheological analyses. Each technological factor was studied at two levels, except the factor *salting conditions* which had three levels: *milk, curd, brine*, differing by stage of addition and concentration of salt used. Salting conditions followed by curd washing had the most pronounced effects on the formation of textural quality. These factors had a dominant effect on the cheese characteristics at each ripening time through their effect on the formation of cheese basic structure, especially on the interactions between proteins - minerals - water. Starter dose and coagulant type and dose influenced cheese properties but their effects were less intense and more dependent on ripening time. The results suggest that calcium addition and pressing time could modulate the effects of salting, curd washing and coagulant dose, through compensatory effects on basic cheese structure. Finally, the effects of the technological factors on characteristics and variability of product are been modulate by the ripening phenomena. The experimental design allowed to estimate the main linear effect of the technological factors on the characteristics of cheese. The multidisciplinary approach applied in this study and the integrated interpretation of results contribute to improve knowledge of the mechanisms that determine the development of cheese texture.

**Key Words:** Textural Quality, Cheesemaking conditions, Ripening time

**433 An empirical method for cheese yield prediction.** C. Melilli[1], J.M. Lynch[2], S. Carpino*[1], A. Cappa[3], G. Licitra[1], and D.M. Barbano[2], [1]*Consorzio Ricerca Filiera Lattiero-Casearia, Ragusa, Italy* , [2]*Northeast Dairy Foods Research Center, Cornell University, Ithaca, NY,* [3]*Associazione Provinciale Allevatori, Vicenza, Italy.*

Theoretical cheese yield can be estimated from the milk fat and casein or protein content of milk using classical formulae, such as the Van Slyke formula. These equations are reliable predictors of theoretical or actual yield based on accurately measured milk fat and casein content. Many cheese makers desire to base payment for milk to dairy farmers on the yield of cheese. However, in small factories accurate measurement of fat and casein content of milk by chemical methods or infrared milk analysis are too time consuming and expensive. Therefore, an empirical test to predict cheese yield was developed using simple equipment (i.e., low-speed room-temperature swinging bucket centrifuge, analytical balance, and forced air oven). Weigh 10 ml of milk into a centrifuge tube. Add 0.1 ml of an acetic acid solution to achieve a pH of about 5.9, mix and incubate at 30°C for 10 min. Add 0.1 ml of the diluted chymosin to the tube, stopper, mix immediately for 15 seconds, and incubate at 30°C. At 30 min a firm coagulation should form. Centrifuge for 30 min at 1625 x g. There will be a curd pellet at the bottom and the sides of the tube should be free of curd and the clear whey should be particulate free without free fat at the surface. Decant the whey and quantitatively transfer the curd to a dry, tared, total solids pan. With a metal spatula, cut and spread the curd in the pan. Dry for 4 h in a 100°C forced air oven. Weigh the pan plus residue and calculate the dry curd weight as a percentage of the milk weight. A linear regression of calculated theoretical versus dry weight yields for milks of known fat and casein content was calculated. A regression of equation of $y = 1.26x + 1.58$, where y is theoretical yield and x is measured dry solids yield ($r^2 = 0.974$), for Cheddar cheese was developed using milks with a range of theoretical yield from 7 to 11.3 kg per100 kg. The standard deviation of the difference (SDD) was 0.193 and the coefficient of variation (SDD/mean x 100) was 1.9% for 15 milks.

**Key Words:** Cheese yield

## EXTENSION EDUCATION

**434 Beef Infobase: a new brand of information exchange.** R.M. Kattnig*[1], W.E. Kunkle[2], T. Troxel[3], and B.R. Eastwood[4], [1]*University of Arizona, Tucson,* [2]*University of Florida, Gainesville,* [3]*University of Arkansas, Fayetteville,* [4]*USDA-CSREES, Washington, DC.*

Today's beef industry is faced with the overwhelming task of searching for creditable answers to specific questions. Therefore, a joint effort between the USDA-CSREES, land grant universities, producer commodity groups and the private sector resulted in a virtual library of current, credible information (Beef Infobase). Infobase articles are categorized into general topics including animal welfare/behavior, annual research reports, beef quality assurance, breeding and genetics, business management, carcass and product, facilities and equipment, feeding and nutrition, grazing lands, forages, herd and animal health, herd management, industry facts and figures, marketing, reproduction, and waste and environmental management.

The Infobase consists of peer reviewed articles submitted through five regional editors (Northeast, Southeast, Midwest, Texas/Oklahoma and West). The management of the Infobase is the responsibility of the beef infobase committee, comprised of cooperative extension, producers and representatives of allied industries. ADDS Inc. publishes the Beef Infobase electronically in both CD and web format. ADDS Inc. is a non-profit educational corporation that facilitates cooperation between public and private sectors across various institutions and disciplines. The Beef Infobase has three directors on the board of ADDS Inc.

Keyword search tools are utilized to quickly locate articles. By using the Folio commands, articles can reviewed by searching key words, or the table of contents can be searched for specific articles. Each article lists the title, author and publication origin. Over 1500 articles (from 35 states) from extension publications, university research reports, and industry experts are represented in the info base.

The process of updating, collecting user feedback, and annual product review is the responsibility of the Beef Infobase Committee members. The goal is to keep this product on the leading edge of useful technology. The Infobase is available by purchasing a CD or by subscribing to a web site (www.adds.org). Access to credible information in an easily searchable and retrievable format is an essential tool to provide situation specific answers for today's beef industry. The Beef Infobase can fulfill that need.

**Key Words:** beef, database, beef production

**435 Dairy InfoBase: promoting cooperation, division of responsibilites and national leadership in support of dairy education.** B.R. Eastwood*[1], M.F. Hutjens[2], M.B. Opperman[3], J.M. Mattison[3], and M.J. Joyce[4], [1]*USDA-CSREES,* [2]*University of Illinois, Urbana,* [3]*ADDS Center,* [4]*Wisconsin Milk Marketing Board.*

The dairy infobase is an electronic resource for information, educational programming and support for decision making by dairy producers and the agri-support industry. It is available on CD and the web as a product of the ADDS (Agricultural Databases for Decision Support) program.

The national project which developed the dairy infobase is organized by twenty one subject matter domains. Domain leaders were selected from among individuals recommended by university administrators and dairy project leaders. Domain leaders work with individuals who were recommended for their expertise. Each member of a domain group is considered an editor/developer of the infobase.

The charge to each domain group is to develop a strong and useful section for the infobase. This is done by reviewing the material from the previous iteration of the product, selecting those items to be included in the new version, and recommending additional new materials to incorporate into that domain. The expected result is a section that incorporates a reviewed and selected set of the most useful material to support informed decision making related to that area of the dairy operation.

Domains addressed in the infobase include agricultural safety and health; animal care and behavior; animal health and biosecurity; buildings and facilities; business management; calves and heifers; culling; dry cows and maternity; expansion; feeding and nutrition; forages; genetic improvement; grazing; HAACP and pre-harvest food safety; human resources management; information systems, records and tests; manure, odor, waste management and the environment; marketing and policy; milking management; reproduction; and small-scale and sustainability issues.

The domains facilitate division of responsibilities for developing and updating each section of the infobase. Domain leaders become de facto national leaders for their area of expertise, providing leadership for their domain group in carrying out the development of each section of the infobase product.

**Key Words:** Extension, InfoBase, Database