3.35 (SD 1.98), 80.91 lbs (SD29.2), 207 days (SD 139.59), and 24,321 lbs (SD 5022) respectively. Herd, Parity, DIM and DIM were all significant effects in the model (P < .01). Of the hygiene score traits Tail head, Flank and Belly were not significant. However, as Udder, Rear legs, and Udder - Rear legs composition scores increased SCS also increased. For each 1 standard deviation increase in Udder, Rear legs or Udder - Rear legs composition score, SCS increased by 0.13, 0.17 and 0.17, respectively. Similar herds with predominance of environmental mastitis infections and similar somatic cell count levels may expect to see a 40-50,000 change in herd SCC for each 1-unit change in cow hygiene scores.

Key Words: SCC, Cow hygiene score

W260 Implementation of a pilot Dairy Quality Management Program in Maryland. R. R. Peters*[1], R. A. Kohn[1], J. W. Simms[1], D. M. Schwartz[1], S. W. Fultz[1], M. R. Bell[1], J. E. Hall[1], J. Fearer[2], D. Booth[2], M. Clarke[2], K. Hendricks[2], and D. Shinham[2], [1]*University of Maryland, College Park, MD,* [2]*Maryland Department of Agriculture, Annapolis, MD.*

A one-year pilot Dairy Quality Management Program (DQMP) was launched starting with a one-day training program for five Maryland dairy producers and their advisors on July 3, 2001. The training program focused on three programmatic areas: biosecurity, animal health, and animal nutrient management. As a pilot program, it was emphasized that a major objective for everyone was experiential learning. The team approach to problem solving was implemented to enhance learning. Dairy advisory teams usually included six professionals. The initial team meeting with the producer started with a survey of farm and herd health information, herd goals and concerns, employee management, a farmstead map, detailed maternity and heifer-calf management practices, and ranking of current herd health concerns. Subsequently, a walk-through progressing from youngest to oldest animals was conducted with the advisory team using risk assessment forms. At the completion of risk assessment, the team convened with the farm family. Areas in need of improvement were discussed from two perspectives: most important for animal health risk and most practical for producer to improve. As assessments were completed, the advisory team outlined a herd plan with three to five goals supporting the overall herd goals initially discussed with the producer. The herd plan included the person responsible for task implementation, deadline for implementing the practice and the frequency to conduct task. Rations were examined and milk urea nitrogen was measured monthly to evaluate herd nutrition. The producer and team met at least quarterly to monitor progress. A personal interview was completed for each herd using producer attitude and herd plan as criteria for evaluation. All producers expressed a positive experience with DQMP. Farms changed 1 to 7 (median = 3) management or facility changes per farm. It is concluded that producers will adopt and implement DQMP on their farms.

Key Words: Dairy, Quality, Management

Dairy Foods: Microbiology and Cheese

W261 EPS and lactic acid production by *S. thermophilus* 1275: influence of pH, temperature, nutrients and co-culturing with non-EPS starter. B. Zisu*[1], G. Harvey[2], and N. P. Shah[1], [1]*Victoria University, Melbourne, Australia,* [2]*Dairy Farmers, Tingalpa, Queensland, Australa.*

Lactic acid bacteria that synthesise exopolysaccharides (EPS) are used increasingly in the dairy industry to improve rheological behaviour, mouthfeel and texture of fermented milks. We have identified a strain of *Streptococcus thermophilus* 1275 which produces both capsular and extracellular EPS.

The objective of this study was to examine EPS and lactic acid production by *S. thermophilus* 1275 in skim milk under various pH and temperatures, supplementation with whey protein concentrate (WPC) and co-culturing with non-EPS *S. thermophilus*.

S. thermophilus 1275 was grown in skim milk in a Biostat B fermenter and samples were taken at 0, 6, 12, 18 and 24 h to determine the amount of EPS, and levels of lactic acid, lactose, glucose and galactose. The bacterial count was also enumerated. EPS was isolated by protein removal and precipitation with ethanol and quantified using the phenol-sulphuric method. Lactic acid, lactose, glucose and galactose were determined using HPLC.

S. thermophilus 1275 produced 406 mg/L of EPS and 3.09 g/L of lactic acid at 37^{o}C. High temperatures and low pH reduced the EPS production, which ultimately ceased at pH 4.5. Maximal growth of the organism and lactic acid production occurred at conditions different to those for EPS production. The pH, temperature, WPC and co-culturing played an important role in the rate and the amount of EPS and lactic acid produced. EPS production peaked at pH 5.5 and at 37-40^{o}C reaching at 458mg/L. The EPS production was further stimulated by co-culturing with non-EPS *S. thermophilus* reaching at 832mg/L and the highest lactic acid concentration of 31.41 g/L. EPS production was highest at 1029 mg/L with WPC supplementation at pH 5.5, however, lactic acid concentrations were lower with WPC supplementation. Significantly less lactic acid was produced when the pH was not controlled during fermentation with or without WPC supplementation.

EPS production can be increased by supplementation with WPC. WPC also reduced the concentration of lactic acid. Co-culturing with non-EPS *S. thermophilus* significantly increased EPS production and may provide a more attractive means of increasing EPS production, thereby improving textural and functional characteristics of dairy foods without the use of additives.

Key Words: Exopolysaccharides, Co-culturing, Nutrient supplementation

W262 selection of prebiotics utilization from *Lactobacillus acidophilus* ATCC 43121 for synbiotics. E. Y. An*[1], S. Oh[2], and S. H. Kim[1], [1]*Korea University,* [2]*Hnkuk Yakult Institute.*

The number of food and other dietary products containing live Bifidobacterium and Lactobacillus bacteria has increased in recent years. In the large intestine, prebiotics, in addition to their selective effects on bifidobacteria and lactobacilli, have influenced many aspects of bowel function through fermentation. The selected synbiotic pairs of stimulated lactobacillus strains and oligosaccharide enhancing their growth were studied to determine the effect of probiotics, prebiotics and synbiotics. This research was investigated effective ability of *L. acidophilus* ATCC 43121 bacteria on minimal media by ratio of adding prebiotics which was used as substrates. Viable cell count of *L. acidophilus* ATCC 43121 and pH of media were measured during twelve, twenty four hours incubation at 37 with seven prebiotics which were of different concentrations to increase the growth of *L. acidophilus* ATCC 43121 selectively. From this experiment results, the effect of prebiotics was signicantly($P<0.05$) higher in control media compared to media adding ratio of fructooligosaccharide, lactulose, raffinose of incubation for twenty four hours. The addition ratio expansion of this three prebiotics was increased consequently by strains growth but pH was decreased. For this experiment response surface methodology to create the right mix ratio which will maximize the bacteria's vital energy by using mix of selected three prebiotics and from this, the right mixture ratio was 36.5%, 0.00% and 63.5%.

Key Words: *Lactobacillus acidophilus*, Prebiotics, Synbiotics

W263 Factors affecting autoaggregation behavior of bifidobacteria. S. A. Ibrahim*, O. A. Hassan, C. W. Seo, Y. Murad, M. Worku, and G. Shahbazi, *North Carolina A&T State University.*

Recent evidence suggests that the addition of bifidobacteria as a dietary adjunct or probiotic may have important health benefits. However, in order for these bacteria to manifest beneficial effects, they need to achieve an essential mass through aggregation. Consequently, the ability of bifidobacteria to aggregate is a desirable property sought for use in commercial food preparations. The objective of this research was to determine the effect of media composition and incubation temperatures on autoaggregation behavior of bifidobacteria. Another objective of this work was to determine the cell surface characteristics of bifidobacteria as related to autoaggregation. Autoaggregation behavior of bifidobacteria was determined using different media (TPY, Wilkins-Chalgren and

mMRS) and incubation temperatures (34, 37, and 42 C). Autoaggregation ability was measured as autoaggregation percentage. In this procedure, overnight culture was shaken at different times (30, 60, 90, 120, and 150 min). After shaking, 2 ml of the upper suspension of the culture was transferred to anther tube and the optical density (O.D.610nm) was measured. Three types of autoaggregation behavior characterized the strains: (1) autoaggregation sensitive (S) for strains that formed a precipitate resulting in a clear solution, (2) autoaggregation resistant (R) for strains that produced consistent turbidity, and (3) autoaggregation moderate (M) for strains that showed slight turbidity. Results on the media composition showed that TPY broth increased the autoaggregation behavior of the tested strains, whereas Wilkins-Chalgren and MRS reduced autoaggregation behavior. Calcium ions induced the autoaggregation. Tween 20 and Tween 80 reduced autoaggregation behavior. Higher incubation temperature (42 C compared to 34 C) increased the ability of strains to autoaggregate. Hydrophilic and electrostatic surface properties influence the autoaggregation behavior of bifidobacteria. Our data indicated that media selection; incubation temperature, and calcium ions are important factors affecting autoaggregation behavior of bifidobacteria. Autoaggregation should be considered when selection of bifidobacteria for their specific use in commercial preparations.

Key Words: Bifidobacteria, Autoaggregation

W264 Screening and selection of acid and bile resistant *Lactobacillus reuteri*. S. A. Ibrahim*, S. Ahmad, C. W. Seo, G. Shahbazi, M. M. Salameh, and M. Worku, *North Carolina A&T State University*.

Probiotic supplements are becoming increasingly popular in the United States and Europe. Although there are many different types of probiotics, the most common live cultures found in yogurt products are *L. bulgaricus*, *S. thermophilus*, *L. acidophilus*, and bifidobacteria. In addition to these beneficial cultures, some dairy industries are beginning to add *Lactobacillus reuteri* to their products as a beneficial culture. *L. reuteri* helps prevent and treat both viral and bacterial diarrhea enhancing the body's resistance to gastrointestinal disease. However, in order to survive and colonize in the gastrointestinal tract, *L. reuteri*needs to show high tolerance to acid and bile salt. The purpose of this work was to investigate the effect of acid and bile salt on the survival and growth of *L. reuteri*. Five strains (CF 2F, DSM 20016, MM 7, MM 2-3, and SD 2112) of *L. reuteri* were used in this study. Cultures were inoculated into fresh MRS broth with various concentrations of bile salt (0.0, 0.1, 0.2, 0.3, and 0.4%) and pH values (pH 2.0, 3.0 and 6.5). Samples were then mixed well and incubated at 37 C for 48 hrs. Bacterial growth was monitored by measuring turbidity at 610 nm in a spectrophotometer at different time intervals during the incubation period. Results showed that a 0.3% bile salt concentration caused a significant reduction in the growth of all tested strains ($P < 0.05$). The survival of *L. reuteri* differed significantly among tested strains; MM 2-3 showed significantly higher growth rates than the other tested strains over the 48 hr incubation period. At a 0.2% bile salt concentration, a significant growth reduction ($P < 0.01$) was observed for strains CF2 F and MM7. None of the tested strains survived low pH (2.00 and 3.00). The results suggest that acid and bile tolerance is an important selection characteristic for the use of *L. reuteri* cultures as a dietary additive.

Key Words: *Lactobacillus reuteri*, Acid and bile resistant

W265 Fourier transform infrared (FTIR) spectroscopy for rapid detection, identification, and enumeration of bacteria in foods. H. Yang, C. W. Seo, and S. A. Ibrahim*, *North Carolina A&T State University*.

The presence of microorganisms in food products has important ramifications for safety, quality, regulations, and public health. Rapid and reliable methods are required for the detection of microorganism, especially foodborne pathogens. The use of Fourier transform infrared (FTIR) spectroscopy and chemometrics (partial least square (PLS) regression and hierarchical cluster analysis (HCA)) for the rapid detection, identification, and enumeration of bacterial in cultures was investigated. In this study, gram-negative (*Escherichia coli* O157:H7 (H1730, F4546, Cider, E0019) and *Salmonella typhimurium* (ATCC 14208)) and gram-positive (*Lactobacillus reuteri*(SD2112, MM2-3, MM7, CF2-7F, MF14-C) were used. Pathogens were grown in brain heart infusion agar (BHI) whereas lactobacillus strains were grown in MRS broth. All strains were incubated at 37 C for 24hr. FTIR spectrometer with attenuated total reflectance (ATR) was used to measure aqueous microbial samples. Fresh broth without microorganism was used as background. The spectral data collection was just taken about 3 minutes. Different spectral regions (3700 - 2800 cm^{-1} and 1800 - 1000 cm^{-1}) were used to identify and classify. Bacteria were clustered into negative ((*E. coli* O157:H7, *S. typhimurium*) and positive (*L. reuteri*) groups while the rate of correct classifications is 100%. HCA even demonstrated the differences among H1730, F4546, Cider, E0019 strains of *E. coli* and SD2112, MM2-3, MM7, CF2-7F, MF14-C strains of *L. reuteri*, separately. A dendrogam indicated that CF2-7F was different from the rest of *L. reuteri* because it was found in the infant sample while the other were from adults. PLS regression was used for enumeration of bacteria. A R-square value was 0.999 from PLS model based on spectral data and cell numbers. Our results indicated that FTIR spectroscopy could be used as a rapid method for the identification and enumeration of bacteria in foods.

Key Words: Fourier transform infrared (FTIR), Dairy foods, Pathogens

W266 Encapsulation of *Lactobacillus reuteri* with sodium alginate for continuous production of lactic acid. S. A. Ibrahim*, C. W. Seo, S. Phetsomphou, and G. Shahbazi, *North Carolina A&T State University*.

Lactic acid fermentation is a well-known process used to preserve food products. The most common approach in lactic acid fermentation is the use of batch system. However with this process several factors limit efficiency of the production of lactic acid. For example, the end products may cause an inhibitory effect on the lactic acid bacteria (LAB). Consequently an alternative method, one that does not have inhibitory effects on LAB is needed. A possible method that meets these challenges involves immobilization of LAB with sodium alginate. The objective of this research was to determine the ability of encapsulated *Lactobacillus reuteri* (*L. reuteri*) in sodium alginate to produce lactic acid. In this study, the production of lactic acid was compared using two types of fermentation methods: Batch and batch bead fermentation. Six strains of *L. reuteri*, CF 2-F, DSM 20016, SD2112, MM 7, MF 14-C and MM 2-3 were used. These strains were grown in lactobacillus MRS at 37C for 24 hrs. The cells were then washed and suspended in 10-ml peptone water. Sodium alginate beads were prepared by resuspending the 10-ml culture in 7% sodium alginate solution. Beads were manufactured by dropping sodium alginate culture into ice-cold (2 C) 0.4M calcium chloride using a separatory funnel. Under comparable conditions the sodium alginate encapsulated cells were allowed to ferment in 500-ml lactobacillus MRS and whey based medium at 37C for 24 hrs. Samples were withdrawn at two -hour intervals during storage period and analyzed for pH value, lactose, glucose, and lactic acid. Results showed that the pH reached 4.00 within 15 hrs with beads fermentation and reached 5.40 using conventional batch. This indicates that higher acid yields can be produced using bead fermentation. Strain MM 2-3 produced the highest lactic acid yield as measured by pH value (pH 3.70) and lactic acid (8.0%) while strain SD2112 produced the lowest acid yield as measured by pH value (pH 4.18) and lactic acid (2.0%) levels. Our results suggest that using immobilized cells of *L. reuteri* could have potential use to produce lactic acid for commercial applications in food and pharmaceutical industries.

Key Words: *Lactobacillus reuteri*, immobilization

W267 Antimicrobial activity of *Lactobacillus reuteri* against *Escherichia coli* O157:H7. S. A. Ibrahim*, M. M. Salameh, W. M. Brown, G. Shahbazi, and C. W. Seo, *North Carolina A&T State University*.

Lactobacillus reuteri (*L. reuteri*) is known to produce a broad-spectrum of antimicrobial compound, reuterin. The antimicrobial spectrum of reuterin includes Gram-positive and Gram-negative bacteria. The purpose of this study was to determine the antimicrobial activity of *L. reuteri* against the foodborne pathogen, *Escherichia coli* O157:H7 (*E. coli O157:H7*). Six different strains of *L. reuteri* (CF2-F, DSM 20016, MF14-6, MM2-3, MM-7) were incubated at 37C for 24 hrs in two different growth media (MRS without glycerol, and MRS with 0.2 M glycerol solution). Samples were centrifuged (5,000g/15 min) to obtain supernatants (200μl) which were then tested against five strains of *E. coli*

O157:H7 (944, 1730, Cider, E0019, F4546). *E. coli* O157:H7 growth was monitored by measuring turbidity at 610 nm in a spectrophotometer at different time intervals during incubation at 37 C for 8 hrs. Results show that *L. reuteri* has the ability to produce antimicrobial compound against *E.coli* O157:H7 only in the presence of glycerol. Such inhibition could not be observed when *L. reuteri* was grown in MRS without glycerol. Two strains of *L. reuteri* showed total growth inhibition against the foodborne pathogen. The growth inhibition could be observed within 4 hrs in LB broth. The growth inhibition was observed before the end of the exponential phase. The activity of *L. reuteri* against *E. coli* O157:H7 was confirmed using agar diffusion assay. These results suggest potential application of *L. reuteri* as a natural biopreservative to control growth of *E.coli* O157:H7 and ensure the safety of our foods.

Key Words: *Lactobacillus reuteri*, *Escherichia coli* O157:H7

W268 Development of endospore-specific primers for the analysis of microbial populations in milk powder. M. Arendts*[1], C. Kitts[2], and R. Jimenez-Flores[1], [1]*Cal Poly DPTC,* [2]*Cal Poly Biological Sciences*.

A comprehensive risk assessment of the microbial quality of milk powder should include information about endospores as well as viable bacteria. *Bacillus* endospores are present in raw milk, used in milk powder production, in numbers ranging from less than 10 to greater than 100 per g of solid. However, in the finished product they range from less than 1000 to over $5x10^5$ per gram, meaning that endospore-forming bacteria will have the most significant effect on the microbial quality of the powder. Molecular methods offer a unique and sensitive tool for rapid microbial detection. Our focus is to apply polymerase chain reaction (PCR) methods to detect early germination of endospores in milk products. We have studied the germination gene, GerC3, from endospore-forming members of the family *Bacillus*. This led to the development of specific primers for PCR detection. In the Dairy Products Technology Center (DPTC) endospore library, we have been able to detect five specific strains that contribute to the lipolysis, casein hydrolysis, starch hydrolysis, and acid production of milk products using our primers. The primers designed in this work identified either a 100bp or a 500bp in a conserved region of the GerC3 gene found in the five DPTC target strains. These bands have been detected during germination activity in all five of these *Bacillus* strains. Spore germination has been difficult to study because it involves extremely rapid physiological responses in a spore whose structure is biochemically intractable. We have evaluated the developed primers in Reverse Transcriptase- PCR (RT-PCR) in the early detection of specific endospores present in skim milk powder resulting in the ability to document the presence or absence of endospore forming bacteria. Results indicate that the rapid growth of endospore forming bacteria can be monitored using RT-PCR.

Key Words: Endospore detection, PCR, Milk powder

W269 The effect of the incorporation of lactobacilli and whey protein isolate on the level of cell glutathion and immunoglobulin M(ig M). Y. H. Yoon*[1] and J. R. Byun, [1]*Department of Animal Science and Technology, Chung-Ang University*.

The effect of the incorporation of whey protein isolates and Lactobacillus spp.in the mouse diet on the level of cell glutathion and Immuniglobulin M(Ig M) in the germ free ICR mouse feeding system.The study was conducted to find out the effect of incorporation of Lactobacillus spp. on the cell gluthatione level and Ig M level in the spleen ,liver and erythrocyte cells. The highest and statistically significant level of glutathione in spleen cell has been shown in L.casei YIT 9018 cell fed group by feeding the diet containing 20% whey protein isolates among the 6 lactobacilli($p>0.05$). which was determined by the method utilyzing glutathione assay kit and the level of cell glutathione revealed to be strain dependent. Providing with the L. casei YIT 9018 or L. acidophilus NCFM increased the level of liver glutathione level significantly. And the level of glutathione in erythrocite increased significantly by feeding the diet containing 20% whey protein isolate and with L. casei YIT 9018 or L. casei CU 001($p>0.05$). Feeding L. casei YIT 9018 with whey protein isolate or L. acidophilus NCFM increased the Ig M level in the splenocyte significantly which was determined by the method of plaque forming unit counting.

Key Words: Glutathione, Imminoglobulin M, Lactobacillus spp.

W270 Evaluation of modified Elliker agar as an enumeration medium for selected Lactic acid bacteria. D. Patel*, L. Goddik, K. Kido, and P. Elliker, *Food Science and Technology, Oregon State University*.

The objective of the project was to evaluate efficacy of Elliker agar medium as a general purpose enumeration medium for lactic acid bacteria. International Dairy Federation (IDF) recommends M17 agar for starter lactococci and streptococci and MRS agar (DeMan Rogosa Sharpe) for starter lactobacilli enumeration. Current IDF protocol requires specific pH, incubation temperature and incubation conditions (e.g. anaerobic incubation) typical for specific starter bacteria. In light of this the Elliker agar medium with specific modifications was utilized to enumerate selected lactic acid bacteria as a convenient medium that can be used easily by the industry in a routine fashion.
Lactic acid bacteria, namely *S*treptococcus thermophilus (ST), *L*actobacillus delbrueckii subsp. bulgaricus Y (LB), *L*actococcus lactis sub sp. lactis ATCC 11454 (LL) and *L*actobacillus acidophilus NCK 1070 (LA) were utilized. All lactic acid bacteria were subcultured in sterile skim milk. Experiments were repeated 3 times. Appropriate dilutions of skim milk cultures were pour plated as per IDF scheme. Additionally, Elliker medium was used in pour plating at comparable pH and a general pH 6.8 for all the comparisons. Elliker agar modifications utilized alternative nitrogen sources such as casein hydrolysate and 3 per cent sterile skim milk. Based on the statistical analysis of data we found that modified Elliker medium gave similar recovery with regards to LA, LL, LB and ST when compared to M17 and MRS agar. It was also found that LB can be enumerated without anaerobic incubation when the purpose is general enumeration. pH had no significant influence in Elliker medium in regard to enumeration. Modified Elliker medium appears to be a good candidate for general purpose enumeration media for lactic acid bacteria.

Key Words: Lactic acid bacteria, International Dairy Federation, Fermented dairy foods

W271 Effects of co-culturing EPS and non-EPS starter cultures and supplementation with WPC on syneresis, textural and rheological properties of set yoghurt. T. Amatayakul*[1], B. Zisu[1], F. Sherkat[2], and N. P. Shah[1], [1]*Victoria University, Melbourne, Australia,* [2]*RMIT University, Melbourne, Australia*.

Exopolysaccharide (EPS) producing starter cultures are becoming increasingly popular for use in the dairy industry. In our earlier study, EPS producing *Streptococcus thermophilus* 1275 when co-cultured with non-EPS *S. thermophilus* produced higher levels of EPS. Supplementation with WPC increased EPS production and reduced the rate of lactic acid production.
The objective was to assess if these approaches could improve syneresis, textural and rheological properties of yoghurt.
Six batches of yoghurts were made in triplicate using 12% reconstituted skim milk (RSM) with or without replacement of RSM with 0.5% WPC and co-culturing with EPS and non-EPS starter culture (75:25). Syneresis was determined as a percentage of whey expelled after centrifugation. A TA-XT2 texture analyser was used to measure textural properties and gel firmness, and rheological properties were determined by using a Haake Rheostress 50 rheometer. HPLC was used to measure the amount of lactic acid produced. EPS was quantified using the phenol-sulphuric method.
Yoghurts made using EPS starters cultures showed reduced syneresis. Control yoghurts made with non-EPS starter and without WPC showed 65.20% syneresis, and those made using co-cultures 60.26%. Co-culturing and partial replacement with 0.5% WPC showed the highest reduction in syneresis at 52.37%. Control yoghurts had the hardest viscosity and hardness. Hardness and viscosity reduced in yoghurts containing EPS starter cultures, whereas WPC increased hardness and the viscosity was unaffected. In addition, yoghurts supplemented with WPC did not show shear thinning behaviour. Yoghurts made with non-EPS *S. thermophilus* had the lowest shear stress regardless of supplementation with WPC. Yield stress was lowest in control yoghurts at 248.70 Pa. Co-culturing and WPC showed the highest yield stress at 363.367 Pa.

Supplementation with WPC and co-culturing with EPS starters has a significant effect ($p<0.05$) on reduction of syneresis, textural and rheological properties of set yoghurt, and may provide an alternative means of improving functional characteristics of yoghurts without incorporating the use of stabilizers.

Key Words: Exopolysaccharides, Rheological properties, Yoghurt

W272 Thermophilin 110: a broad spectrum bacteriocin of *Streptococcus thermophilus*. G. A. Somkuti* and D. H. Steinberg, *Eastern Regional Research Center, ARS-USDA*.

A survey of thermophilic lactic starter cultures for bacteriocin production identified the broad spectrum antimicrobial peptide thermophilin 110 of *Streptococcus thermophilus*ST110, a strain used in yogurt and cheese fermentations. The range of bacteria inhibited by the bacteriocin included lactococci, lactobacilli and pediococci, in addition to related thermophilic streptococci. Production of thermophilin 110 at 37°C paralleled growth of *S. thermophilus* ST110 in a tryptone-yeast extract-lactose medium. After 16 h of growth, bacteriocin titers reached 320 units/ml by an agar well diffusion assay with *Pediococcus acidilactici* as the indicator strain. Thermophilin 110 was sensitive to digestion by proteolytic enzymes and lost its activity after a 60 min exposure to pepsin, pronase and papain. It was also inactivated by amylase treatment indicating glycosylation as a prerequisite for activity. Antimicrobial activity was fully retained after heating crude thermophilin 110 preparations at 80°C for 60 min. Thermophilin 110 was acid resistant and remained stable between pH 3 and 7 but lost its activity after exposure to pH 10. Plasmid analysis of *S. thermophilus* ST110 indicated the absence of plasmids, suggesting that the genetic determinant for thermophilin 110 production is probably located on the chromosome. Inhibition of several species of pediococci is a unique feature of thermophilin 110, implying a potential for applications in controlling the growth of spoilage bacteria in wine and beer fermentations.

Key Words: Bacteriocin, Thermophilin 110, *Streptococcus thermophilus*

W273 The influence of cold adaptation on cryotolerance of *Bifidobacterium infantis*. A. Gevorgyan* and R. F. Roberts, [1] *The Pennsylvania State University*.

The purpose of this study was to determine the influence of cold adaptation on cryotolerance in *Bifidobacterium infantis* strain ATCC 15697 and commercial strain BI-4. Growth of ATCC 15697 and BI-4 in Reinforced Clostridial Broth (RCB) was determined at 20°C, 25°C, and 37°C by measuring OD_{600}. Overall BI-4 grew faster than ATCC 15697 in RCB incubated at 37°C. Neither strain grew in RCB when incubated at 20°C or 25°C for up to 7 days. For cold shock experiments and freeze-thaw challenge, cells were grown in 100 ml RCB at 37°C until mid-log phase (OD_{600}= 0.5) then 25 ml of culture was harvested and resuspended in the same volume of tempered medium (20°C, 25°C, 37°C). Ten ml of resuspended inoculum was transferred into two sterile tubes and incubated at the designated temperatures for 240 min. One ml samples were taken at 0, 30, 60, 120 and 240 min and frozen at -20°C for 24 hours, thawed for 10 min at 30°C, sampled for viable count and then re-frozen. The population of survivors was determined before freezing and after 1, 3, 6 and 9 freeze-thaw cycles by spread plating decimal dilutions on RCA plates and incubating anaerobically at 37°C for 72h. Survivor data were normalized to the initial population (before freezing). Experiments were replicated three times. When BI-4 was incubated at 20°C or 25°C prior to freezing there was no change in population after 9 freeze-thaw cycles. However when BI-4 was incubated at 37°C for 60, 120, and 240 min the strain exhibited 0.5 log decrease in population after 9 freeze-thaw cycles. Overall, ATCC 15697 was more sensitive to freeze-thaw challenge than BI-4. However, the loss of viability was reduced by incubating at 20°C or 25°C when compared to 37°C, especially at incubation time of 120 and 240 min. Viable cells of ATCC 15697 could not be recovered after 6 freeze thaw cycles following incubation at 37°C for 240 min. Viability of both strains after 9 freeze-thaw cycles decreased when incubated at 37°C for longer time (120 and 240 min) suggesting cells in stationary phase are less cryotolerant. Incubation at suboptimal temperatures did not increase cryotolerance of *B. infantis* and the effect of freeze-thaw challenge was strain dependent.

Key Words: Probiotics, Bifidobacterium, Cryotolerance

W274 Effect of c2 phage peptide on acid development in milk inoculated with *Lactococcus lactis* spp *lactis* C2 with and without c2 phage infection. I. Surjawan and C. L. Hicks*, *University of Kentucky, Lexington, KY 40546*.

Peptides from c2 phage were prepare by hydrolyzing c2 bacteriophage(Φ) with ficin (0.2% at 26°C for 6 h). Inhibition of phage proliferation tests were conducted in milk following a rennet cheese schedule (1 h ripening at 31°C, rennet, cutting, cooking to 37°C, and holding at 37°C) by measuring change in pH during 4.5h of fermentation. Six sterilized pint jars were filled with 96 ml of pasteurized milk. Milks were inoculated (4%) with C2 culture that was grown in medium with (2 jars) and without (3 jars) phage peptides (2%) added. One jar was inoculated with culture grown in medium containing 1% c2 peptide. The milk in this jar also contained 1 % added c2 peptide. One of the milks that was inoculated with culture grown in medium without c2 peptide contained 2% added c2 peptide. Four of the milks were infected with c2 phage (10^3 pfu/ml). The pH decreased fastest in milk inoculated with C2 culture grown in medium without added c2 peptide then in milk inoculated with culture grown in medium containing c2 peptide. These 2 milks had significantly better acid production (pH 5.63 and 5.71, respectively after 4.5 h of fermentation) than the other 4 milks. However, milks that were inoculated with culture grown in c2 peptide (both the 2% peptide medium and 1 % peptide medium with 1% peptide added to the milk) and infected with (Φ)c2 did continue to produce acid (pH 6.01) throughout the fermentation period. When 2% c2 peptide was added to the milk and inoculated with culture grown in medium without peptide, acid development stopped (pH 6.23) after 200 min. Acid production in milk inoculated with culture grown in medium without peptide and infected with (Φ)c2 stopped (pH 6.25) after 120 min of fermentation. These results suggest the culture grown in media containing c2 peptide were protected from c2 phage proliferation and lysis during the fermentation period better than when the peptide was added to the milk, or when no peptide was present.

Key Words: *Lactococcus lactis*, c2 Bacteriophage inhibition, pH Milk

W275 Inhibition of *Salmonella* and *Escherichia coli* phage with c2 phage peptide. C. L. Hicks, J. Tang, and I. Surjawan, *University of Kentucky, Lexington, KY 40546*.

Peptides from *Lactococcus lactis* Φc2 (phage) were prepared by hydrolyzing Φc2 (10^9 pfu/ml) with ficin (0.2% at 26°C for 6 h). Hydrolyzed peptide were partially purified by ultrafiltration (3000 mwco). Ultrafiltration permeate was dialyzed (500 mwco) and freeze dried, then used to formulate growth media. *Salmonella choleraesuis* ssp. *choleraesuis* (Smith) Weldin serotype Typhimurium deposited as *Salmonella typhimurium* (Loeffler) Castellani and Chalmers ATCC 14028 and *Escherichia coli* (Migula) Castellani and Chalmers ATCC 47076 were grown in 1558 medium and 1065LB medium, respectively, with and without Φc2 peptide present (various concentrations) and, with and without their respective phage (*S. choleraesuis* ssp. *choleraesuis* serotype Typhimurium phage ATCC 40282 and *E. coli* lamda 97538). *S. choleraesuis* ssp. *choleraesuis* grew faster when c2 peptide (1.5 and 2.5% concentrations) was added to the 1588 growth medium (incubated at 37°C for 6 h). However, when ATCC 40282 phage was added to the growth medium (infected after 130 min incubation) with and without peptides the media that contained 1.5 and 2.5% peptide had an extended growth period of 21 and 28 min, respectively, before lysis occurred suggesting that c2 peptide had a minor inhibition effect on ATCC 40282 phage proliferation. *E. coli* also grew faster when the c2 peptide (2 and 4% concentration) was added to the 1065LB growth medium. However the most rapid growth was present in the medium containing 2% peptide suggesting that peptide in the 4% medium was starting to block metabolic transport. When the lambda 97538 phage was added to the growth medium (infected after 90 min of incubation) only a slight inhibition of phage proliferation occurred in the 2% c2 peptide medium (20 min) whereas in the 4% peptide medium phage proliferation was suppressed by 120 min suggesting that c2 peptide was an effective inhibitor of lambda 97538 phage proliferation.

Key Words: *Salmonella*, Bacteriophage inhibition, c2 phage-peptide

W276 Correlation between the USU stretch test and the pizza fork test. B. L. Moyes*[1], D. J. McMahon[1], and C. J. Oberg[2], [1]*Utah State University, Department of Nutrition and Food Sciences,* [2]*Weber State University, Department of Microbiology.*

A correlation between the USU stretch test and the pizza fork test would allow the stretch properties of Mozzarella cheese to be measured in an objective manner. The USU Stretch Test uses a modified texture-profile analyzer to pull strands of cheese from a melted reservoir, measuring the load exerted on the probe during stretching. Fifty grams of shredded cheese was melted for 45 min at 65, 70, 75, 80, and 85°C and a three-pronged hook was used to lift the strands of cheese for 30 cm at a rate of 100 cm per min. The load exerted on the probe was recorded and the following parameters were used to search for a correlation with values obtained from the pizza fork test. Pizza fork test values were provided by an industrial partner. Melt Strength was defined as the maximum load obtained during stretching, and the probe extension at Melt Strength was termed the Stretch Extension (SE). Stretch Load (SL) was defined as the load exerted on the probe at any point following Melt Strength. These SL values were also used to calculate the slope of the curve formed as the load decreased after Melt Strength was obtained. In general, greater correlation was found at higher temperatures. At 85°C, the correlation coefficient (r) between the fork test distance and Melt Strength, slope from 10 to 15 cm, SL from 5 to 10 cm, SL from 15 to 20 cm, and SE obtained from the USU Stretch Test were 0.71, -0.80, 0.84, 0.69, and -0.36 respectively. The correlation coefficient for the same parameters were 0.61, -0.68, 0.71, 0.83, and -0.84 at 80°C; 0.61, -0.41, 0.43, 0.60, and -0.54 and 75°C; 0.73, -0.46, 0.61, 0.69, and -0.85 at 70°C; and 0.73, -0.66, 0.72, 0.67, and -0.29 and 65°C.

Key Words: Mozzarella, Stretch testing, Functionality

W277 Impact of cheese defects on U.S. graded cheeses. M Smukowski*[1], W. L. Wendorff[2], Y. Ping[1], and R. D. Rao[2], [1]*WI Center for Dairy Research, Madison, WI, USA,* [2]*University of Wisconsin-Madison, Madison, WI, USA.*

Grading records for over 40,000 metric tonne of Cheddar, Colby, Monterey Jack, and Swiss cheese were obtained from ten national cheese manufacturers or processors. Licensed graders recorded defects and established grades for each lot of cheese. Major defects identified in Cheddar cheese were acid flavor, curdy, short and weak body and open texture. Major defects for Colby and Monterey Jack cheeses were weak body and acid and whey flavors. Over 16% of the Swiss cheese was downgraded due to defective eye formation or utensil flavor. Potential economic impacts of the major cheese defects are reported. Trained panelists evaluated Cheddar cheese obtained from the retail market and found less than 10% of the cheese would have been graded as Grade A. Major defects noted were acid, flat, whey, and bitter flavors. Other defects included short, pasty, and weak body and open texture. It is suggested that cheesemakers must continuously evaluate cheeses throughout the aging process, distribution and marketing of cheeses to effectively assess their cheesemaking procedures and practices.

Key Words: Cheese, Defects

W278 Microencapsulated Iron fortification and flavor development in Cheddar cheese. H. S. Kwak, H. J. Ahn, J. Ahn, and J. S. Seok, *Sejong University, Seoul, Korea.*

This study was designed to examine the effect of microencapsulated iron-fortified Cheddar cheese and vit C as a bioavailable helper of iron on chemical and sensory aspects. Coating material was PGMS, and ferric ammonium sulfate and vit C were selected as core materials. The highest efficiency of microencapsulation of iron and vit C were 72 and 94%, respectively, with 5:1:50 ratio (w/w/v) as coating to core material to distilled water. TBA absorbance was significantly lower in microencapsulated treatments than those in uncapsulated treatments during ripening. The productions of short-chain free fatty acid and neutral volatile compound were not significantly different between microencapsulated and uncapsulated Cheddar cheese during ripening periods. In sensory aspects, bitterness, astrigency and sourness were higher in Cheddar cheese fortified with microencapsulated iron and uncapsulated vit C than others. The present study indicated that fortification of iron as well as vit C did not show any defect problem to Cheddar cheese, and suggested the possibility of iron fortification of Cheddar cheese.

Key Words: Iron fortification, Microencapsulation, Cheddar cheese

W279 Comparison of microbial populations of unfrozen and frozen control goat cheeses with those of 3 month frozen-stored ones. J. H. Lee*, S. J. Lee, A. Kalantari, and Y. W. Park, *Fort Valley State University, Fort Valley, GA.*

Few scientific studies have been reported on microbial profiles of commercial caprine cheeses in relation to food safety and shelf life of the products. A commercial soft goat cheese was purchased and Monterey Jack (MJ) cheese was manufactured at the pilot plant of Fort Valley State University. Both varieties were prepared in 3 batches, and divided into three equal portions. One portion was stored as unfrozen control (UFC) at 4^oC for 4 weeks (0, 14, 28 days), and the other two subsamples were frozen at -20^{o}C and stored for 0 and 3 months (FZC and 3FZ), then immediately thawed at 4^oC, followed by aging at 4^oC as was done for UFC. Changes in microbial populations were enumerated for total aerobic plate count (TPC), *E. coli* and coliform, yeast and mold, and *Staphylococcus aureus* using 3M Petrifilm techniques. pH and acid degree values (ADV) for all cheeses were determined. The pooled data of the respective TPC (log cfu/g) for UFC, FZC, and 3FZ groups of soft and MJ cheeses were: 6.93, 6.67 and 5.51; 8.44, 8.34 and 8.09, indicating that there were significant ($P<0.05$) reduction in TPC with storage treatments in soft cheeses, whereas no difference in MJ cheeses. The TPCs of 0, 14 and 28 days aging at 4^oC for corresponding cheeses were: 8.01, 5.67 and 5.52; 8.57, 8.17 and 8.15, revealing that there were significant ($P<0.05$) decrease in TPC with aging times in both cheeses. Low levels of coliforms and *E. coli* were found in MJ, but not in soft cheeses, and these cells were significantly declined by freezing and aging. The 2 main effects (storage and aging) were not significant for the pooled data of yeast counts of MJ, whereas those were significant ($P<0.05$) for the soft cheeses. Yeast counts tended to increase with aging in UFC and FZC groups of both cheeses, but significantly decreased in 3FZ group. Mold counts in both soft and MJ cheeses were similar at 3.0 (log cfu/g) for all storage groups. *E. coli*, coliform, and *Staphylococcus aureus* in soft cheeses were non-detectable <1.0 (log cfu/g), suggesting that no food safety hazard was in the cheese.

Key Words: Microbial population, Goat cheese, Frozen-storage

W280 Quantitative analysis of water-soluble volatile free fatty acids in commercial Swiss-type cheeses. T. Ji, W. Harper, and V. Alvarez, *The Ohio State University, Columbus, Ohio.*

Short chain ($\leq C_{12}$) water-soluble volatile free fatty acids (FFAs) contribute to the final flavor characteristics of cheese. Quantification of FFAs in varying Swiss-type cheeses can provide information concerning the ripening processes. The objective of this study was to compare the concentrations of FFAs in varying aged Swiss-type cheeses as an indirect parameter of flavor development. Twelve commercial domestic, imported Gruyere, Emmenthal and Jarlsberg Swiss-type cheeses of varying ages were analyzed in duplicate. A capillary gas chromatograph equipped with a flame ionization detector was used for the analysis of FFAs. Each standard curve of fatty acids was made using authentic fatty acids by diluting in double purified distilled water except higher volatile non-branched fatty acids with even carbon numbers such as octanoic, decanoic and dodecanoic due to low water solubility. Predominant FFAs in all cheeses were ethanoic (99-196 mg/kg cheese), propanoic (81-281 mg) and butanoic (40-131mg) acids. Ethanoic acid (C_2) exceeded the propanoic acid (C_3) in 6 cheeses in which C_3 was less than 100 mg. Butanoic acid (C_4) was greater than C_2 and C_3 in only two cheeses. In four cheeses, C_4 was higher than C_3 and all of these cheeses had more C_2 than C_3. 3-methylbutanoic acid was presented in only 5 of 12 cheeses. Gruyere only showed all of even carbon numbered ($\leq C_{12}$), C_3 and branched fatty acids such as 2-methylpropanoic, 3-methylbutanoic and 4-methylpentanoic acids. Some domestics and Gruyere cheeses containing low C_2 (99 -129 mg) and C_3 (81-98 mg) had high concentrations of decanoic acid. Domestic cheese (3 mo age) and Gruyere (> 6 mo) showed higher volatile non-branched fatty acids with even carbon numbers (C_8, C_{10} and C_{12}). Emmenthal and Jarlsburg had high level of ethanoic (184 and 152 mg) and propanoic (243 and 245 mg) acids. The commercial cheeses generally showed two patterns of lower molecular weight fatty acids: (a) those cheeses where C_2 is greater than 100 mg

and C_3 is greater than 175 mg (6 cheeses) and (b) those that showed less than 100 mg of C_3 (6 cheeses). High concentrations of C_8, C_{10} and C_{12} correlated to low C_3 in most cases.

Key Words: Swiss cheese, Volatile free fatty acid

W281 Compositional differences between whey, salty whey, and press whey from commercial manufacture of cheddar cheese. R. D Rao* and W. L. Wendorff, *University of Wisconsin-Madison, Madison, WI, USA*.

Salty and press whey streams are currently underutilized in the dairy industry because of difficult, costly processing and high salt content. In addition, relatively little information is available on the composition of these whey streams. In Wisconsin alone, over two million gallons of salty whey are produced in a year, most of which is landspread or disposed of into waste treatment systems. This study investigated gross compsitional differences between whey, salty whey, and press whey streams derived from Cheddar cheese. Differences between individual whey protein compsition were also studied. Individual proteins were quantified using SDS-PAGE and digital imaging. Solids, ash, fat, and chloride content were significantly greater in the salty and press whey as compared to standard Cheddar cheese whey. Individual whey proteins analyzed include lactoferrin (LF), bovine serum albumin (BSA), immunoglobulin G (IgG), β-lactoglobulin (β-LG), and α-lactalbumin (α-LA). Salty and press whey showed slightly decreased proportions of IgG compared to that of the standard Cheddar whey. Amounts of BSA (wt. %) were comparable in all samples. The percentage of α-LA in the salty and press whey streams were roughly half of that found in the standard Cheddar whey. β-LG concentrations decreased by about 20% from the standard Cheddar whey to the salty and press whey streams. The percentage of LF increased from less than 1% to greater than 30% in both the salty and press whey. Differences in gross composition between standard Cheddar whey and salty and press whey can be used to determine modifications needed in whey processing. Salty and press whey may be good sources of lactoferrin, making processing of these whey sources a more profitable and viable option for whey processors.

Key Words: Salty whey, Press whey, Lactoferrin

W282 Physico-chemical and microbiological characteristics of Cheddar cheese manufactured with a cholesterol lowering spread and oil high in omega-3 fatty acids. K. J. Aryana* and R. Gough, *Louisiana State University Agricultural Center*.

Milk fat is high in saturated fatty acids. Replacing milk fat in Cheddar cheese with health beneficial lipids could improve consumer appeal and demand for the product. The objective was to study the impact of a gradual replacement of milk fat by a cholesterol lowering spread, Benecol® and oil high in omega-3 fatty acids, Omega Pure™ on the physico-chemical and microbiological characteristics of full and low fat Cheddar cheeses. Cheddar cheese was manufactured by replacing milk fat with Omega Pure™ and Benecol® in the following ratios; milk fat : Omega Pure™ / Benecol® 100:0; 75:25; 50:50; 25:75; 0:100. The attributes studied were color, pH, proteolysis and microbiological profile. Color was measured in L^* a^* and b^* values using a hand held colorimeter; proteolysis was studied by gel electrophoresis; and the microbiological profile was determined by standard plate counts, coliform counts, yeasts and mold counts. The pH was significantly ($P<0.05$) lower when Omega Pure™ / Benecol® was used at 100% fat level in full fat Cheddar cheese compared to the full fat control. Lower usage levels of Omega Pure™ / Benecol® in full and low fat Cheddar cheeses did not result in significant ($P<0.05$) differences when compared to full and low fat Cheddar cheese controls, respectively. The full fat control was lower ($P<0.05$) in b^* (yellowness) values compared to the full fat cheeses with Benecol®. There was a significant ($P<0.05$) and steady decline in b^* values of full fat cheeses made with decreasing amounts of Benecol®. There were slight changes in the gel electrophoretic patterns of the treated cheeses. Coliforms in the controls and the treated samples were estimated at $<$ 10 cfu/ml. The low fat samples appeared to have a higher standard plate count than the full fat samples. Use of the health beneficial lipids altered some characteristics of low fat and full fat Cheddar cheeses.

Key Words: Fermented, Health, Lipids

W283 RAPID method of cheese sample preparation for microstructural studies by electron microscopy. K. J. Aryana*[1] and M. C. Henk[2], [1]*Louisiana State University Agricultural Center*, [2]*Louisiana State University*.

Cheese sample processing for electron microscopy involves several days. A quicker method that processes cheese samples without altering its microstructure would be desirable. The objective was to identify such a suitable, rapid method. The rapid method involved an initial fixation of cheese in a solution of 2% glutaraldehyde and 1% OsO_4 in 0.05M cacodylate buffer for 10 min. This was followed by a second fixation in 2% glutaraldehyde and 2% OsO_4 in 0.05M buffer for an additional 20 min. Both of these solutions were mixed from stock solutions immediately before use, as components would react with each other in the absence of any sample. Sample fixation by the rapid method was attained in a total of 30 minutes compared to 17 hours in the control, i.e., 15 hours (overnight) fixation in 1% glutaraldehyde and 2 hours fixation in 1% OsO_4. After fixation, en block staining with aqueous uranyl acetate was conducted for 30 minutes. This was followed by ethanol dehydration and infiltration in resin. En bloc staining provided uniform staining, ultimately saving time and reducing grid handling and contamination encountered when staining sections with alcoholic uranyl acetate. Additionally, the rapid method was conducted with LR White resin compared to Spurr's epoxy resin in the control. The former is used directly while the latter has to be freshly prepared and involves precise weighing and orderly mixing of four toxic chemicals. The microstructure of cheese processed by the rapid method appeared unaltered when compared to the control. The protein matrices in both the control and the rapid method processed samples picked up the heavy metal stain and were easily seen. The dispersed fat globules were also clearly visible in both the control and treated samples. This rapid method of cheese sample preparation did not alter cheese microstructure and can be recommended for accelerated sample processing for electron microscopy.

Key Words: Structure, Fermented, Fixation

W284 Effect of setting pH on the properties of mozzarella cheese made from whole milk and dry milk protein concentrate by direct acidification. S. Rehman, N. Farkye, and Y. Boorus, *California Poly technic State University, San Luis Obispo, CA*.

The pH of milk at setting affects the properties of mozzarella cheese. Milk protein concentrate (MPC) containing 64.0% protein, 20% lactose and 2% calcium was used to standardize whole milk to a protein to fat ratio of 1.4 for Mozzarella cheese manufacture. Our objective was to compare the effect of pre-acidification of whole milk standardized with MPC to different pH values in Mozzarella cheese made by direct acidification. Standardized, pasteurized (72°C 16 s) was divided into three lots, A, B and C and respectively adjusted to pH 5.6, 5.8 and 6.0 with 2% citric acid prior to setting (5 mL chymosin / 100 kg milk). The coagulum was cut and the curds were cooked (36°C) and stretched (82°C). Cheesemaking was repeated thrice. All cheeses were stored at 4°C for 5 weeks. Composition, yield, meltability, baking properties and hardness in the cheeses were determined by standard methods, while primary proteolysis was assessed by urea-polyacrylamide gel electrophoresis and determination of water-soluble N contents of the cheeses. Significant differences ($P<0.05$) in the % moisture (51.54 ± 2.09, 50.87 ± 2.32, 47.94 ± 1.85) and calcium contents (mg/kg cheese, 36.75 ± 1.183, 45.76 ± 4.24, 53.75 ± 2.05) were observed for vats, A, B, C respectively showing that decreasing milk pH caused increase in moisture and decrease in calcium. No significant ($P>0.05$) differences were observed in lactose, protein, fat and yield of the cheeses, % fat or protein recoveries. The % total solids recoveries increased significantly with increase in setting pH of milk. The milks pre-acidified to pH 5.6 gave the cheeses with best meltability, least hardness, minimum browning while baking on pizza and highest levels of proteolysis. The results of this study suggest that if Mozzarella cheese with better functional properties is to be manufactured by using MPC, then the milk should be pre-acidified to pH of 5.6.

Key Words: Milk protein concentrate, Mozarella cheese, Direct acidification

W285 Effect of calcium on functionality of fat free Mozzarella cheese. N. S. Joshi, R. I. Dave, and K. Muthukumarappan, *South Dakota State University, Brookings, SD.*.

Mozzarella cheese consumption has increased steadily for many years. Calcium plays significant role in functional properties of Mozzarella cheese. Fat free Mozzarella cheese has not become popular because it has poor melt properties. Our recent research on part skim Mozzarella cheese indicated that cheeses with reduced calcium possess better melting properties, particularly softening, melting and flow. Therefore a study was planned with an objective to examine effects of altering calcium levels on functionality of fat free Mozzarella cheeses.
Skim milk was preacidified to four pH levels (control = no treatment, T_1 = pH 6.2, T_2 = pH 5.9 and T_3 = pH 5.6) using citric and acetic acids to alter calcium content in cheeses. The cheeses were made by direct acidification method using glucono-delta-lactone and were analyzed for composition (moisture, protein, fat, salt, ash, and calcium), melt area (modified Schreiber test), melt profile (softening and melting time-temperatures, extent and rate of flow), color (L^*), and proteolysis (soluble nitrogen). The data were analyzed using PROC GLM and PROC MIXED procedures of SAS®.
Preacidification of skim milk significantly ($P < 0.05$) reduced the ash and calcium contents, whereas, rest of the components remained at par in all the cheeses. As the calcium in the cheeses reduced from 0.79 % in control to 0.66 % in T_1, 0.59 % in T_2 and 0.50 % in T_3 cheeses flowed faster ($P < 0.001$) with higher flow rate and required significantly ($P < 0.001$) less time to melt. The control cheese had higher ($P < 0.05$) post melt whiteness (L^*) as compared to experimental cheeses (90 vs. 88.9, 88.5 and 88.7 in T_1, T_2 and T_3 respectively). Soluble nitrogen was the highest in T_3 (1.80 %) followed by T_2 (1.13 %), T_1 (0.82 %) and control (0.60 %) on d30. Refrigerated storage of all the cheeses resulted in increase in melt area ($P < 0.01$), flow rate ($P < 0.001$), extent of flow ($P < 0.01$), and soluble nitrogen ($P < 0.001$) along with decrease in melting time ($P < 0.05$) and melting temperature ($P < 0.001$). The post melt whiteness of the cheeses was not affected by refrigerated storage.

Key Words: Fat free Mozzarella, Calcium, Functioanlity

W286 Changes in microstructure of part skim Mozzarella cheese as a function of calcium. N. S. Joshi, K. Muthukumarappan, and R. I. Dave, *South Dakota State University, Brookings, SD.*.

Mozzarella cheese has unique functional characteristics that are not available in other cheese varieties. Casein of the reduced calcium curd better emulsifies the fat and its subsequent distribution within the continuous protein matrix decides rheological and functional properties of Mozzarella cheese. Thus calcium is a key factor in determining the basic structure of Mozzarella cheese. Our objective was to understand the role of calcium in microstructure of part skim Mozzarella cheese.
Calcium content of part skim Mozzarella cheeses was altered by manufacturing cheese from milk preacidified to four pH levels (control =no treatment, T_1 = pH 6.2, T_2 = pH 5.9 and T_3 = pH 5.6) using citric and acetic acid. Direct acidification method using glucono-delta-lactone was followed for cheese making. Cooking and draining time were adjusted to obtain uniform moisture content in all the cheeses. Structure of the cheeses was evaluated by scanning electron microscopy (SEM) as well as confocal laser scanning microscopy (CLSM) techniques. Information obtained from both the microscopic analyses was quantified in terms of numbers, area and size of the fat globules using software HL Image ++. Calcium content of the cheeses was significantly different (control = 0.65, T_1 = 0.48, T_2 = 0.42 and T_3 = 0.35 %), whereas rests of the compositional parameters were similar ($P > 0.05$). The microstructure study using both SEM and CLSM revealed that reduced calcium cheeses had greater number of round fat particles (control = 125, T_1 = 193, T_2 = 184, and T_3 = 215 in SEM and control = 86, T_1 = 87, T_2 = 125, and T_3 = 140 in CLSM), and their distribution in reduced calcium cheeses was also more uniform. The above findings support our hypothesis that casein in the reduced calcium cheese better emulsifies the fat globules and significantly improve the melting of Mozzarella cheese.

Key Words: Mozzarella, Microstructure, Calcium

W287 Effects of stage of lactation and aging on functional properties of Colby and Cheddar cheeses manufactured from goats' milk. D. W. Olson*[1], D. L. Van Hekken[1], M. H. Tunick[1], K. A. Soryal[2], and S. S. Zeng[2], [1]*USDA, ARS, Eastern Regional Research Center, Wyndmoor, PA,* [2]*Garza Institute for Goat Research, Langston University, Langston, OK.*

In the United States, goats in many herds begin their lactation at the same time. In this study, the effects of cheese milk obtained at various stages of lactation (early, peak, and late) and cheese storage (0, 8, and 16 wk for Colby and 0, 8, 16, and 24 wk for Cheddar at 4°C) on sliceability, meltability, and color changes upon heating (232°C for 5 min or 130°C for 75 min) of Colby and Cheddar cheeses manufactured from Alpine goats′ milk were evaluated. The cheeses were manufactured at Langston University, OK. Sliceability (force required to cut through a sample) was measured using a TA.XT2 Texture Analyzer with a wire cutter attachment. Meltability was measured using the Schreiber Melt Test. Color including whiteness was measured using a HunterLab ColorQuest XE colorimetric spectrophotometer. A greater cutting force to slice the cheese was required when measurements were made at 0 wk of storage using peak lactation milk instead of early or late lactation milk to make Cheddar cheese. No consistent effects of stage of lactation were observed on the whiteness and meltability of Colby and Cheddar cheeses. With aging, the whiteness before and after heating of both types of cheese and the force required to cut through the cheeses decreased but their meltability increased. The changes in the color, meltability, and sliceability were greater between 0 and 8 wk than the corresponding changes occurring between 8 and 16 wk of storage. The changes in the functional properties closely follow the proteolysis of the cheese as it ages. The stage of lactation has less impact on the functional properties of the cheeses than aging and indicates that storage and proteolysis are key factors in the functional quality of goats′ milk cheeses.

Key Words: Goats' milk cheese, Functional properties, Stage of lactation

W288 Effects of milk pasteurization and aging on functional properties of Mexican Mennonite cheese. D. W. Olson*[1], D. L. Van Hekken[1], M. H. Tunick[1], P. M. Tomasula[1], F. J. Molina-Corral[2], and A. A. Gardea[2], [1]*USDA, ARS, Eastern Regional Research Center, Wyndmoor, PA,* [2]*Centro de Investigacion en Alimentacion y Desarrollo, Cuauhtemoc, Chihuahua, Mexico.*

Currently, little is known about the functional properties of commercially available semi-hard cheeses manufactured by the Mennonite community in Chihuahua, Mexico. In this study, sliceability, meltability, and color changes upon heating of Mexican Mennonite cheeses made from raw or pasteurized milk were compared. Two brands of raw milk cheese and two brands of pasteurized milk cheese, obtained from four different manufacturers in Chihuahua, Mexico, were analyzed in triplicate after 0, 4, 8, 12, and 16 wk of storage at 4°C. Sliceability (force required to cut through a sample) was measured using a TA.XT2 Texture Analyzer with a wire cutter attachment. Meltability was measured using the Schreiber Melt Test on samples heated to 232°C for 5 min. Color was measured using a HunterLab ColorQuest XE colorimetric spectrophotometer on samples before and after heating at 232°C for 5 min or 130°C for 75 min. Compared to pasteurized milk cheeses, raw milk cheeses had less browning and total color change after heating at 130°C, melted more at 232°C, and required less cutting force. With aging, all cheeses increased in meltability, decreased in whiteness when measured before heating, and required less cutting force to slice. In addition to proteolytic breakdown occurring in both cheeses as they age, the differences in the functional properties are a result of, in part, the mixed microflora present in the raw milk cheeses compared to the more homogeneous microflora provided by presence of dairy cultures in pasteurized milk cheeses.

Key Words: Mexican Mennonite cheese, Functional properties, Shelf-life

W289 Proteolysis and rheology of soft goat milk cheese after frozen storage. D. L. Van Hekken*[1], M. H. Tunick[1], D. W. Olson[1], and Y. W. Park[2], [1]*USDA, ARS, Eastern Regional Research Center, Wyndmoor, PA,* [2]*Fort Valley State University, Fort Valley, GA.*

Seasonal milking practices for dairy goats in the US limit the availability of domestic fresh soft goat cheese. With the demand for soft goat cheese

increasing, freezing of the fresh curd could allow US goat producers to supply soft cheeses throughout the year. In this study, the effects of freezing and long term frozen storage on the proteolysis and texture of soft goat cheese was evaluated. Plain soft cheeses were obtained from a grade A goat dairy in Georgia and received three storage treatments: fresh control (FC) at 4°C for up to 4 wks, frozen (-20°C) and thawed after 2 d (FTC) or 3 mo (3MF), then stored as FC group. Although all frozen samples showed minute ice crystal formation throughout the body of the cheese, no free liquid was noted when samples were thawed. Proteolysis was monitored using SDS-PAGE and rheological properties were measured using a universal testing machine and a dynamic analyzer. At the start of refrigerated storage, all samples that had been frozen (regardless of length of frozen storage) had 1 to 2% less beta-casein than the fresh cheeses. After 4 wk of refrigerated storage, all cheeses showed 2 to 3% proteolytic breakdown of beta-casein. FC cheese had a fragile texture with values of 10.6 N for hardness, 10.1 mm for springiness, 0.10 for cohesiveness, 9.3 mJ for chewiness, 15.9 kPa for elastic modulus, 5.28 kPa for viscous modulus, and 1.75 kPa.s for complex viscosity. The FTC cheese had slightly lower values for hardness (7.36 N), cohesiveness (0.08), and chewiness (5.2 mJ) and elastic and viscous moduli decreased from d1 to d28 (11.3 kPa and 3.60 kPa, respectively). However, the 3MF cheeses were slightly harder and chewier than the FC cheese and the viscoelastic properties were similar to those of the FC cheese. Frozen storage of soft goat cheese affects its textural quality through the creation and removal of ice crystals in addition to the proteolytic breakdown of caseins in the cheese matrix.

Key Words: Goat milk cheese, Proteolysis, Rheology

W290 Effect of sodium chloride and acid on rennet coagulation and curd firmness of high heat-treated milk. M. R. Acharya* and V. V. Mistry, *MN-SD Dairy Foods Research Center, South Dakota State University.*

Raw whole milk was pasteurized at 62.8, 68.3, 73.9 or 79.4°C for 30 min and divided into ten portions. Five levels of sodium chloride, 0 (S0), 0.5 (S1), 1.0 (S2), 1.5 (S3) or 2.0% (S4) or five levels of 2% lactic acid, 0 (A0), 1.5 (A1), 3.0 (A2), 4.5 (A3) or 6.0 ml/100 ml of milk (A4) were added to formulate a total of 10 treatments. There were three replications. From each treatment, 100 ml sample was inoculated with 1.0 ml of 2:100 diluted rennet solution and incubated in a water bath at 32°C. Curd firmness was judged at intervals using a knife to determine cutting time. A Formagraph was used to measure the curd formation characteristics. Ten ml milk from each treatment was inoculated with 200μL of 1:100 diluted rennet solution at 32°C and the unit was operated for 180 min. Formagraph plots were used to determine rennet coagulation time (r, min), time to reach firmness of 20 mm (k_{20}, min) and firmness (mm) at 30, 60, 90 and 120 min as a_{30}, a_{60}, a_{90} and a_{120}, respectively. Only treatments A2, A3 and A4 could reach cutting strength at 79.4 and 73.9°C. At 68.3°C all treatments except controls reached cutting strength. Rennet coagulation time by both subjective (knife) test and Formagraph reduced from control (S0) to S1 and then increased with increase in sodium chloride content and reduced with increase in level of acidification. Values of k_{20}, a_{30}, a_{60}, a_{90} and a_{120} indicated similar trends as rennet coagulation time. It is concluded that desired cutting time and curd firmness, suitable for cheese making can be obtained from high heat treated milk with added sodium chloride or acid.

Key Words: Sodium chloride, Lactic acid, Rennet

W291 An accelerated cheese ripening in cholesterol-reduced Cheddar cheese by β-cyclodextrin. H. S. Kwak, C. S. Jung, H. J. Ahn, and J. Ahn, *Sejong University, Seoul, Korea.*

This study was carried out to find whether cheese ripening process was accelerated in cholesterol-reduced Cheddar cheese or not, which made by cream separation following by 10% β-CD treatment. The cholesterol removal rate of the cholesterol-reduced cheese was 91.9%. The production of short-chain free fatty acids (FFA) increased with ripening time in both control and experimental cheeses. The short-chain FFA data showed that cholesterol-reduced cheese ripened for 2 and 4 wk released a similar amount of FFA in control cheese ripened for 16 wk (4 mo) and 24 wk (6 mo). With ripening period, the increase of neutral volatile compounds, especially, acetaldehyde, acetone, ethanol and 2-heptanone was more profound in control than in β-CD treated group. In addition, cholesterol-reduced Cheddar cheese produced much higher total free amino acid and bitter amino acids than control during all ripening periods. In sensory analysis, texture score of control Cheddar cheese showed an increasing trend with 32 wk ripening, however, that in β-CD treatment group decreased during a ripening period (8 wk). Above results indicated that the cholesterol-reduced cheese made by β-CD treated cream resulted additionally in an accelerated ripening means.

Key Words: Acceleration of ripening and cholesterol removal, β-cyclodextrin, Cheddar cheese

W292 Influence of feeding strategy (pasture vs TMR) on proteolysis in Ragusano cheese during ripening. V. Fallico*[1], L. Chianese[2], J. Horne[1], S. Carpino[1], and G. Licitra[1], [1]*CoRFiLaC, Regione Siciliana, 97100 Ragusa, Italy,* [2]*Food Science Department, Naples University, Portici, Italy.*

Pasture contributes to aromatic profiles of milk and derived-cheese providing odor compounds that the animal can transfer to milk via the rumen. Aromatic substances were found in the milk and cheese of grazing ewes, but not in those of sheep fed TMR (Total Mix Ration). Proteolysis also contributes to cheese flavour, producing low molecular weight aromatic compounds and amino acids that may act as flavour precursors. The aim of this study was to evaluate the effect of feeding strategy (pasture vs TMR) on proteolysis of Ragusano, a brine-salted pasta filata cheese made from raw cow's milk without starter, during ripening (1, 120 and 210 d). Primary proteolysis was monitored by urea-PAGE, isoelectric focusing (IEF) and immunostaining with polyclonal antibodies against α_{s1} and β-caseins. Reversed phase-HPLC was used to assess secondary proteolysis by fractioning 12% TCA-soluble peptides. Both urea-PAGE and IEF profiles of pasture and TMR cheeses showed similar proteolytic patterns at each level of ripening, indicating that diet had no effect on primary proteolysis. Densitometry of urea-PAGE profiles of cheeses aged 120 and 210 days revealed slightly higher proteolysis levels in TMR cheeses. Similar but not significant ($P>0.05$) trends were found in chemical analyses (15.72 vs 14.23 at 120 d, 15.22 vs 14.16 at 210 d, SN/TN %). Immunoelectrophoretic patterns were useful in identifying the origin of main primary peptides. In vitro hydrolysis reactions with chymosin and plasmin helped to elucidate the potential role of these enzymes in primary proteolysis. Different feeds had a qualitative impact on secondary proteolysis. Peptide patterns resolved better in pasture HPLC profiles suggesting a more defined and balanced action of microbial peptidases involved in oligopeptide and amino acid production. Chemical analyses revealed a nonsignificant ($P>0.05$) trend showing larger 12% TCA-soluble peptide fractions in TMR profiles at each level of ripening.

Key Words: Ragusano cheese, Feed, Proteolysis

W293 Effect of sodium citrate on structure-function relationships of Cheddar cheese. A. J. Pastorino*, C. L. Hansen, and D. J. McMahon, *Western Dairy Center. Nutrition and Food Sciences Dept. Utah State University.*

The objective of this study was to determine the effect of sodium citrate on the structure and functionality of Cheddar cheese. The hypothesis was that citrate (sodium citrate) injection would affect cheese properties mainly through its effect on insoluble calcium (measured as the difference between total calcium and water-soluble calcium of a cheese extract). A 9-kg block of Cheddar was made, vacuum-packaged, and then stored for 2 wk at 4°C. After storage, the cheese was cut into 0.5- to 0.6-kg blocks that were vacuum-packaged and stored for 1 wk at 4°C. Cheese blocks were then high-pressure injected with a buffer solution (pH 5.27) containing 40% (wt/wt) citric acid trisodium dihydrate and 6.25% (wt/wt) anhydrous citric acid, from zero (control) to five times (successive injections performed 24 h apart). Increased citric acid content of cheese from 0.22 (uninjected) to 1.39% (after five injections) caused phosphate solubilization. Thus, the insoluble phosphate content of cheese decreased from 0.54 to 0.45 mmol/g protein. However, unexpectedly, the soluble calcium content decreased from 0.34 (control) to 0.28 mmol/g protein (after five injections), whereas the insoluble calcium content remained unchanged (0.42 mmol/g protein). The decrease in soluble calcium probably resulted from the formation and concentration of crystals in the cheese surface, which was not included in samples for analysis, and from serum expulsion. Higher concentration of solutes in the water phase would increase the volume of serum, but the cheese had limited holding capacity and serum was expelled. Citrate injection increased the sodium content of cheese from 0.63 to 0.93%, but

it had no effect on cheese pH (5.2). After five injections, the protein matrix occupied increased area of cheese matrix (83 versus 78%). Even though citrate injection had no effect on insoluble calcium, and thus the rate and extent of cheese flow were unaffected, increased phosphate solubilization, and possibly decreased ionic calcium content, resulted in expansion of the protein matrix and increased cheese hardness.

Key Words: Calcium, Phosphate, Protein matrix

W294 Continuous manufacture of mozzarella cheese using concentrated microfiltration retentate and recovery of virgin whey proteins. A. V. Ardisson* and S.S.H. Rizvi, *North East Dairy Foods Research Center. Cornell University.*

The objective was to develop a continuous cheese-making process, which utilizes concentration factor (CF) 8-9, pH 6.0 skim milk microfiltration (MF) retentate to produce low-moisture part-skim (LMPS) Mozzarella cheese.
Pasteurized skim milk was microfiltered to a concentration factor of 8-9 at 50C using a 0.1μm nominal pore diameter microfiltration membrane unit with a total area of 0.72 m^2. The system was equipped to maintain a uniform transmembrane pressure (UTMP) in the range of 68.9 KPa to 172.4 KPa. The milk was gradually acidified during microfiltration to pH 6.0 using glucono-d-lactone (GDL) at a concentration of 1.6g/l skim milk to adjust the calcium to protein ratio in the final retentate. Experiments were conducted to test the effect of four different cross flow velocities (CFV): 2.5, 3.5, 4.5 and 5.5 m.s-1on permeate flux, which allowed the determination of fouling of the membrane. Furthermore, flux decay was evaluated at four different transmembrane pressure levels (68.9 KPa, 103.4 KPa, 137.2 KPa and 172.4 KPa). The process was scaled-up to a membrane unit with a total area of 9.1 m^2 for the continuous production of cheese. The obtained retentate was subsequently standardized with heavy cream to a casein to fat ratio of 0.85 and converted into LMPS Mozzarella cheese curd in an Alcurd continuous cheese coagulator using single strength rennet (80μl/Kg retentate). The resulting curd was then cooked and stretched. The analyses performed on skim milk, retentate, permeate and cheeses included total solids, protein (Total N, non-protein N and non-casein N), fat and ash. The fat, moisture and protein contents of the cheese produced by the process as well as its textural characteristics were within the normal ranges for LMPS.

Key Words: Microfiltration mozzarella, Microfiltration retentate cheese, Whey protein depletion of milk

W295 Lexicon development of appearance and texture descriptors for melted cheddar cheese. K. M. Asato*, I. M. Tsai, and M. R. McDaniel, *Oregon State University, Corvallis, OR.*

A lexicon to define the sensory properties of melted cheddar cheese was created using a trained descriptive panel. The lexicon characterizes appearance (surface rupture, meltedness, oiliness, and edge browning) and texture (stringiness, stretchiness, springiness, firmness, toothpull, smoothness, cohesiveness, denseness, and chewiness). The newly developed lexicon was used to evaluate seven samples consisting of three commercial brands of shredded cheddar cheese at different ages (sharp, medium and mild) in order to determine how heat treatment (oven and microwave) affected the sensory perception of melted cheese. Microwave treated cheese was higher than oven treated cheese in all descriptors except edge browning, smoothness and cohesiveness. Under the same heat treatment, melted sharp cheddar was rated higher in oiliness and lower in all texture descriptors than melted medium and mild cheddar.

Key Words: Melted cheese, Cheddar, Sensory

W296 Monitoring spores and spore-forming bacteria populations in commercial skim milk powder production plants using conventional and molecular methods. C. Murillo*[1], C. Kitts[2], and R. Jimenez-Flores[1], [1]*Cal Poly Dairy Products Technology Center,* [2]*Cal Poly Biological Sciences Department.*

The microflora of milk powder consists of a wide array of microorganisms of which special attention is given to *Bacillus spp.* spores and spore formers. *Bacillus spp.* spores survive well in all processing stages and inhabit the milk powder in the dormant state indefinitely. Upon reconstitution, spores may germinate, and through their enzymatic activity become detrimental to quality. The objectives of this study are to 1) enumerate total aerobes, mesophilic, and thermophilic spore populations in commercial, low-heat skim milk powder production plants; 2) characterize the microbial ecology of this process using Terminal Restriction Fragment Patterns (TRFPs); and 3) compare the changes in the ecology during this process. Fluid and powder skim milk was collected from 3 commercial facilities during spring, summer, and fall '01-'02. Sampling points included the raw milk silo, separator, evaporator, and spray dryer. Samples were normalized based on total solids. Every sample was evaluated for total aerobes, mesophilic, and thermophilic spores. For TRFPs community DNA was extracted, amplified by 16S PCR, and digested with *HaeIII* and *DpnII*. Spore formers are predominant in condensed and powdered milk, and tend to increase in the powder with increasing processing time. In raw milk mesophilic and thermophilic spores ranged from ¡25CFU/g to 70CFU/g and ¡25CFU/g to 10^2 CFU/g, respectively. In powder they ranged from ¡25CFU/g to 10^3 CFU/g and ¡25CFU/g to 10^6 CFU/g, respectively. Both spore counts from skim milk showed an increasing trend with run time and rendered the powder out of the 10^3 CFU/g limit. In the ecology TRF patterns successfully described microbial populations, and an overall decrease in microbial diversity between raw and powdered milk was observed. Overall, *Bacillus spp.* were found in 92important organisms included *Clostridium spp.* (57*Staphylococcus spp.* (29*Streptococcus spp.* (9*Bacillus spp.* were present in 100from all 3 plants.

Key Words: Milk powder, Terminal restriction fragment patterns, Quality

W297 Enterotoxigenic *Bacillus* spp. DNA fingerprints revealed in powdered milk products using rep-PCR. R. M. Cooper* and J. L. McKillip, *Ball State University, Muncie, IN.*

As a staple food, milk powders and other dry functional dairy ingredients must reflect strict quality control and a long shelf life. As a means of assessing the microbiological quality of a battery of dry dairy products, the technique of repetitive element palindromic polymerase chain reaction (rep-PCR) was used as a screening tool to detect DNA fingerprinting profiles from potentially enterotoxigenic *Bacillus* spp. in five industrial formulations of lecithin, soy fiber, whey protein concentrate, and nonfat dry milk powder. Following a nonselective enrichment protocol (11-13 h) in tryptone phosphate glucose yeast extract (TPGY) broth to induce spore germination and vegetative cell growth to densities of 10^6 CFU/ml, each dry product was subjected to a commercial DNA extraction procedure and rep-PCR to generate distinct amplicon banding patterns that were analyzed using agarose gel electrophoresis. A distinct 1,230bp diagnostic band consistent with that of previously characterized enterotoxigenic *Bacillus cereus* was demonstrated in rep-PCR from nonfat dry milk, lecithin, and soy powders. The identity of the diagnostic band was confirmed by restriction enzyme analysis, and in each case generated the same digest pattern as the rep-PCR amplicon from the positive control *B. cereus*. These data validate the method of rep-PCR as a viable means of screening powdered dairy ingredients (and perhaps many other foods) for enterotoxigenic *Bacillus* spp. without the need for plating and enumeration using selective and differential media.

Key Words: Enterotoxigenic *Bacillus* spp., rep-PCR, Detection

Food Safety: Food safety; Methods, prevalence and control

W298 Detection of viable Enterobacteriaceae in milk by using real-time broad-range RT-PCR. S. H. Choi* and S. B. Lee, *Sangji University, Wonju, Korea.*

This study was carried out to develop real-time broad-range RT-PCR which could detect viable Enterobacteriaceae in milk. The threshold cycle(Ct) of the RT-PCR was determined by using Multiscribe reverse transcriptase and SYBR Green PCR Master mix(Applied Biosystem) and iCycler iQ(Bio-Rad). Following the RT-PCR , the synthesized DNA was confirmed in agarose gel electrophoresis. The nucleotide sequences of primers were designed based on the ribosomal protein genes, S11