broilers and pigs, nitrate adaptation did not further enhance the bactericidal effects of ECP. Rapid reduction of ruminal nitrate may account for specie differences. Discovery of nitro-compounds that are more resistant to ruminal degradation may enhance the efficacy of ECP against enteropathogens in cattle.

Key Words: *E. coli*, Chlorate, Cattle

W276 Effect of caffeine on inactivation of *Escherichia coli* O157:H7 in laboratory media. S. A. Ibrahim*, *North Carolina A&T State University, Raleigh*.

Escherichia coli O157:H7, a leading cause of bacterial food borne disease outbreaks, is responsible for approximately 73,500 cases of foodborne illness per year. Recent research has shown that caffeine has the ability to inhibit DNA repair in bacteria and therefore could be mutagenic compound. The objective of this research was to determine the effectiveness of caffeine on inactivation of (*E. coli* O157:H7 in Brain Heart Infusion (BHI) broth. Overnight samples of six *E. coli* O157:H7 strains (E 1730, E 4546, E 0019, Cider, 380 and 944) were used in this study. These strains were inoculated individually at an initial inoculum level of 2 log CFU/ml into BHI broth containing caffeine with different concentrations of 0.0, 0.25, 0.5, 0.75, 1.00, 1.25, 1.50, 1.75, and 2.00%. Samples were then incubated at 37 C for 24hrs. Samples were withdrawn at different time intervals to determine turbidity using spectrophotometer at 575nm. Results revealed that the addition of caffeine inhibited the growth of *E. coli*. Significant growth inhibition was observed with concentration levels 0.50% and higher. These results indicate that caffeine has potential as an antimicrobial agent and should be investigated further as a food additive to increase the biosafety of consumable food products.

Key Words: Caffeine, *Escherichia coli* O157:H7

W277 Using origanox in combination with sodium lactate and sodium acetate to inhibit the growth of *Escherichia coli* O157:H7. S. A. Ibrahim* and S. R. K. Dharmavaram, *North Carolina A&T State University, Raleigh*.

Escherichia coli O157:H7, a leading cause of bacterial food borne disease outbreaks, is responsible for approximately 73,500 cases of foodborne illness per year. Origanox, a commercial spice, has been shown to have antimicrobial properties. It is believed that the effectiveness of origanox can be enhanced by the use of organic acids. The objective of this research was to determine the effectiveness of origanox alone and in combination with chemical preservatives; sodium acetate and sodium lactate on inactivation of *E. coli* O157:H7 in Brain Heart Infusion (BHI) broth. Overnight samples of five *E. coli* O157:H7 strains (E 1730, E 4546, E 0019, Cider and 944) and a mixture of the five strains were added to BHI broth at an initial inoculum level of 2 log CFU/ml. Several combinations of sodium lactate (0,1, and 2 % w/v), sodium acetate (0 and 1 % w/v), and origanox (0, 0.05 and 0.1 % w/v) were used as treatments. The samples were stored at 37 °C for 12 hrs and population changes of *E. coli* O157:H7 were followed using optical density (O.D. 610 nm) measurements and CFU techniques every two hours. Our results indicated that origanox was effective in controlling the growth of *E. coli* O157:H7 at concentration of 0.1 % in BHI broth. Sodium lactate alone was found to be effective at 3% concentration. Sodium lactate at 1-2% in combination with 0.05% origanox or sodium acetate at 1% in combination with 0.05% origanox was found to be the most effective in controlling the growth of *E. coli* O157:H7, $>$ 4 log reduction. Treatments containing a combination of 1% sodium lactate, 1% sodium acetate and 0.05% origanox showed significant reduction of *E. coli* O157:H7, $>$ 5 log reductions. Use of origanox at 0.05% could reduce the usage of chemical preservatives such as sodium lactate and sodium acetate to inhibit the growth of *E. coli* O157:H7.

Key Words: Origanox

W278 Selection of anti-bacterial peptides against *E. coli*O157:H7 and UTI from f88-4/15 library. C. J. Fu*, F. J. Schmidt, S. A. Mounter, and M. S. Kerley, *University of Missouri-Columbia*.

Phage display technology was used to select anti-bacterial peptides against pathogenic *E. coli* O157:H7 (isolates PA 1 and PA 2 from human clinical case and ground beef, respectively) and UTI (isolate PA 3 from a urinary tract infection case). After 4 rounds of affinity selection, 40 phage clones (PC 1 to 120) bearing colonies selected against each pathogen were examined. The purified phage clones were used to test their function of inhibition/killing the pathogenic *E. coli*. DNA sequencing indicated that only 2 phage sequences were repeated in 16 colonies from the PA 1 and PA 2 selection. A single clone dominated the PA 3 selection (12/16). No similar peptide sequences were found from published databases by BLAST search. Several PC (PC 5, 16, 41, 42, 46, 84, 94, and 95) inhibited or killed the pathogens (40 to 85% within 2 hours). Phage clones selected against either PA 1 or PA 2 inhibited both strains but not PA 3. However PCs selected against PA 3 inhibited PA 1 and PA 2.

Key Words: Pathogenic *E. coli*, Peptide, Phage Display

Dairy Foods: Microbiology

W279 Lactic acid fermentation by *Lactobacillus reuteri* in laboratory medium supplemented with various nutrients. S. Phetsomphou* and S. A. Ibrahim, *North Carolina A&T State University, Raleigh*.

Lactic acid is a product that has numerous applications in the chemical, pharmaceutical, and food industries. Lactic acid bacteria have been used widely for the production of lactic acid. However, certain nutrients are needed for the maximum production of lactic acid. Therefore, objective of this research was to investigate the effect of nutrient supplements and carbohydrate substrates on lactic acid production using free and calcium alginate immobilized *Lactobacillus reuteri*. *L. reuteri* MM 2-3 in a free cell form and calcium alginate beads (immobilized) was used to determine lactic acid production in laboratory medium supplemented with different nutrients: yeast extract, beef extract, tryptone peptone, and proteose peptone at 0, 10 and 20% concentrations or carbohydrate substrates: maltose, lactose, glucose, sorbitol and sucrose at 10% concentration. Fermentation experiments were conducted in 500 ml flasks with 300 ml final volume at 37 °C for 24 hrs. At different time intervals (2 hrs), samples were withdrawn, and analyzed for pH values and lactic acid concentrations. Fermentations of immobilized *L. reuteri* in samples containing yeast extract, phytone and proteose peptone at 20% produced the highest concentrations of lactic acid after 24 hrs with pH measurements (3.20, 3.41, and 3.61, respectively) as compared to the control (4.70). Lactic acid concentration ranged between 9.00 and 12.50. Regarding carbohydrate substrate (sugar) fermentation, maltose produced the lowest pH (3.32) followed by glucose, lactose and sorbitol (3.47, 4.00, and 6.11 respectively). Results indicated that immobilized cells of *L. reuteri* MM2-3 produced higher lactic acid and in a shorter time when compared to free cells. The results show that immobilized *L. reuteri* could be used for the production of high lactic acid concentrations in a laboratory media supplemented with both yeast extract and maltose.

Key Words: *L. reuteri*, Lactic Acid

W280 Influence of an *Arthrospira (Spirulina) platensis* biomass on acid production of lactococci. N. Molnár, L. Varga*, J. Szigeti, and B. Gyenis, *Institute of Food Science, Faculty of Agricultural and Food Sciences, University of West Hungary, Mosongyarovar, Hungary*.

Arthrospira (Spirulina) platensis is a planktonic cyanobacterium belonging to prokaryotic algae. Its dried biomass typically contains 3% to 7% moisture, 55% to 60% protein, 6% to 8% lipids, 12% to 20% carbohydrate, 7% to 10% ash, 8% to 10% fiber, 1% to 1.5% chlorophyll *a*, and a wide range of vitamins. *A. platensis* has recently been marketed and consumed as a safe human food and has been approved for human nutrition by many governments, health agencies, and associations of some 80 countries, including the United States. The effect of

a spray-dried *A. platensis* biomass, on the rate of acid development by various strains of major lactic acid producers in mesophilic dairy starter cultures such as *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* was evaluated in cows milk. Acid production of the starter culture strains screened was mostly stimulated significantly ($P < 0.05$), although to varying degrees. The components of the cyanobacterial biomass responsible for the stimulation observed were found to be nitrogenous compounds (peptone, adenine, and hypoxanthine). The *A. platensis* biomass rich in trace elements, vitamins, and other bioactive substrates also had a highly beneficial effect on the nutritional value of milk, thus providing a new opportunity for the manufacture of functional fermented milks, *i.e.*, *Arthrospira*-enriched cultured cream and buttermilk.

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Key Words: *Arthrospira (Spirulina) platensis*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*

W281 Occurrence of Glutathione sulphydryl(GSH) and Antioxidant Activities of Probiotic Lactobacillus spp.

Y. H. Yoon* and J. R. Byun, *Department of Animal Science and Technology, Chung-Ang University*.

The antioxidative ability on the basis of reduced glutathione sulphydryl level, the inhibition activities of linoleic acid peroxidation of cell free extract of Lactobacillus spp and the effects of types of media and growth phase of the cells on the cellular GSH level have been determined. Correlation between reduced glutathione sulphydryl level and antioxidative ability of Lactobacillus spp. was analyzed: Lactobacillus casei HY 2782 contained the highest level of GSH among the probiotic strains with 25.15μmole/g, the cellular GSH level of L. casei HY 2782 reached maximum after 24h of cultivation and tended to decrease on further cultivation up to 72h, it revealed significantly higher level of cellular GSH when grown in de Man Rogosa and Sharpe (MRS) broth than in tryptone phytone yeast extract (TPY) broth or bromcresol pruple destroxe (BCP) broth($p<0.05$).The antioxidant activity of cell free extract of Lactobacillus spp have been shown to be significantly differed among the strains in the inhibition of linoleic acid peroxidation by thiobarbituric acid(TBA) test($P>0.01$). L. casei HY 2782 and L. acidophilus ATCC 4356 revealed a high degree of antioxidative effect in linoleic acid oxidation system. Spearmans rank correlation quotient between inhibitory activity on linoleic acid peroxidation and cellular GSH levels of Lactobacillus spp was 0.65 which means a significant positive correlation (Key words; GSH level, antioxidant effect)

Key Words: GSH Level, Antioxidant Effect

W282 Functionality and survivability of probiotics in carbonated yogurt beverage.

F. Lee and M. Guo*, *University of Vermont, Burlington*.

A prototype carbonated yogurt beverage was developed using a probiotic yogurt as a base and carbonated with 0, 1, 2 or 3 volumes of food-grade carbon dioxide. Inulin (natural prebiotic ingredient) and probiotic bacteria were integrated into the product to create a symbiotic dairy beverage that could benefit the health of consumers. Mean chemical composition of the beverage from all 3 trials consisted of 16.3% total solids, 14.6% carbohydrates, 2.2% protein, 1.7% fat and 0.2% ash. Mean content of Ca, P, K, Mg, Na, Cu and Zn in the beverage were 745, 380, 671, 50, 222, 0.2 and 13 mg/kg, respectively. Mean pH values of the beverage carbonated with 0, 1, 2 and 3 vol of CO2 during the 10-wk study were 3.58, 3.70, 3.59 and 3.56, respectively. Averaged viscosity values of the beverage for the same treatment and period were 26.3, 24.0, 25.3 and 21.1 mPa.s, respectively. Visual inspection and negative results from 3M yeast / mold and coliform films indicated the beverage remained stable up to ten weeks in refrigerated storage. Survivability of probiotic bacteria in the beverage during a 10-wk study was evaluated according to enumeration methods of Chr Hansen, Inc. Initial estimated levels of *L. acidophilus* in the beverage carbonated with 0, 1, 2 and 3 vol of CO2 were 7.4, 6.8, 6.9, and 7.3 log CFU/ml, respectively. Correspondingly, initial levels for *Bifidobacteria* were 7.2, 7.3, 7.3 and 9.0 log CFU/ml; and 7.7, 7.9, 8.0 and 8.1 log CFU/ml for *L. casei*. Levels of *L. acidophilus* in the beverage decreased below 6.0 log cfu/mL shortly after week 4, regardless of carbonation levels. Population of *Bifidobacteria* gradually decreased and remained above 6.0 CFU/ml through week 10 in beverages carbonated with 2 and 3 vol CO2. Levels *L. casei*of also decreased steadily but remained above 6.0 CFU/ml only through week 8 in all carbonated beverages. In summary, carbonated symbiotic yogurt beverage can be developed to deliver probiotic bacteria. Carbon dioxide may have positive impact on the survival of certain probiotics, i.e., *Bifidobacteria*.

Key Words: Yogurt, Beverage, Probiotic

W283 Influence of bile salts on growth, antimicrobial activity and β-galactosidase activity of *Lactobacillus reuteri*.

S. A. Ibrahim* and S. A. Ahmed, *North Carolina A&T State University, Raleigh*.

It is well known that the presence of lactobacilli is important for the maintenance of the intestinal microbial ecosystem. They have been shown to possess inhibitory activity toward the growth of pathogenic bacteria such as *Escherichia coli* O157:H7 and *Salmonella*spp. This inhibition could be due to the production of inhibitory compounds such as organic acids, hydrogen peroxide, bacteriocins, or reuterin or to competitive adhesion to the epithelium. In order to survive in and colonize the gastrointestinal tract, lactobacilli should express high tolerance to acid and bile. They should have the ability to adhere to intestinal surfaces and produce large quantity of β-galactosidase . The purpose of this work was to investigate the influence of bile salts on growth, antimicrobial activity and β-galactosidase activity of *Lactobacillus reuteri*. Six strains of *Lactobacillus reuteri* (CF 2F, DSM 20016, MF 14C, MM 7, MM 2-3, and SD 2112) were used in this study. These strains were grown in modified Trypticase-protease peptone-yeast extract (TPY) broth containing 0.0 or 0.4% bile salt at 37 C for 48 hrs. The extent of bacterial growth was monitored by measuring the optical density of the samples at 610nm after various time intervals. The effect of bile salt on the production of antimicrobial compounds was tested using the difusion assay. The β-galactosidase activity was determined during the growth in the presence of bile salt. Results showed that growth and antimicrobial activity decreased in the presence of 0.4% bile salt ($P< 0.05$). The β-galactosidase activity was varied among the tested strains. MM 2-3 showed higher β-galactosidase in the presence of bile salt. Activity ranged between 800 and 1400 Miller units. Bile salt does not affect β-galactosidase activity of MF 14C strain. Our results demonstrate that bile salt has an influence on the biochemical properties of *Lactobacillus reuteri*. Bile salt should be considered when probiotic strains are selected for useful industrial applications.

W284 Incidence of *Escherichia coli* O157:H7 in raw milk and survival of a five strain cocktail of *E. coli* O157:H7 during the 60 days aging period of hard cheese made from unpasteurized milk.

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The incidence and concentrations of *E. coli* O157:H7 present in raw milk as delivered to Midwest milk processors were determined. For incidence, raw milk were inoculated in pre-enrichment and enrichment broths and incubated before plating. One ml samples of raw milk was pipetted onto 2 BCM plates and incubated for determination of concentration of the pathogen. All 237 samples tested were less than the lower limits of detection for incidence and concentration. Hard cheese was made from unpasteurized milk inoculated with 10^{1} cells/ml of a five-strain cocktail of acid-tolerant *E. coli* O157:H7. Samples of unpasteurized milk, curd and whey were collected during the cheese manufacturing process. After pressing, the blocks of hard cheese were packaged into plastic bags, and sealed with a vacuum-packaging machine, and aged at 7 ^{o} C. After 1 week, the cheese blocks were cut into smaller uniform-sized pieces, and vacuum sealed in clear plastic pouches for ease of sampling at the various aging intervals. Samples were plated and enumerated for *E. coli* O157:H7 using BCM for *E. coli* O157:H7 (+) Plating Medium. Populations increased to 10^{2} in the drained curd and to 10^{3} at milling and pressing. Population of *E. coli* O157:H7 in cheese aged for 60 and 120 days at 7 ^{o} C, decreased less than 1 log and 2 logs, respectively. After 150 to 180 days, levels declined to <1/ml. Cheese samples in storage were inoculated in pre-enrichment and enrichment broths and incubated before plating. After approximately 240 days, no growth of

E. coli O157:H7 was seen on the plates after either pre-enrichment or enrichment.

Key Words: Raw Milk Cheese, *Escherichia coli* O157:H7, *E. coli* O157:H7 in Raw Milk

W285 Incorporation and survival of probiotic bacteria in yogurt for use as a functional food. S. Hekmat*[1], S. Royal[2,3], and G. Reid[2,3], [1]*Brescia University College at The University of Western Ontario, London, Canada,* [2]*The University of Western Ontario, London, Canada,* [3]*Lawson Health Research Institute, London, Ontario, Canada.*

Both *Lactobacillus fermentum* RC-14 and *Lactobacillus rhamnosus* GR-1 have been shown to be probiotic agents with intestinal and urogenital therapeutic properties, and both colonize the intestine when ingested in skim milk. Low-fat (1%) probiotic yogurt was made by fermenting standard yogurt starter cultures, *L. delbruekii var bulgaricus* and *S. thermophilus*, mixes with *L. fermentum* RC-14 and *L. rhamnosus* GR-1. Survival of *L. fermentum* RC-14 and *L. rhamnosus* GR-1 was monitored using selective MRS agar containing 50 μg/mL tetracycline or 15 μg/mL fusidic acid, respectively, after 1, 7, 14, 21, and 28 days of storage at 4°C. In all treatments, *L. rhamnosus* GR-1 survived better than *L. fermentum* RC-14. After one day of storage, mean colony counts of *L. fermentum* RC-14 and *L. rhamnosus* GR-1 were 7 x 10^5 and 4 x 10^7 CFU/mL respectively. After one month of refrigerated storage, these counts had decreased to 4 x 10^3 for RC-14 but remained stable at 2 x 10^7 CFU/mL for strain GR-1. This study provides a method to derive a new probiotic yogurt as a vehicle to deliver beneficial bacteria to consumers. Such a yogurt, with high counts of probiotic bacteria, would be the first of its type in Canada.

Key Words: Functional Foods, Probiotic Yogurt

W286 Development of probiotic concentrated yogurt using direct reconstitution method. S. Hekmat*, V. W. Y. Ng, and A. J. Holman, *Brescia University College at The University of Western Ontario, London.*

Concentrated yogurt is traditionally made by draining the yogurt in a double layer cheese cloth bag. This process is time consuming and may introduce unwanted microorganisms into the yogurt through contamination. The objective of this study was to produce probiotic concentrated yogurt by direct reconstitution method. Full-fat milk powder and non-fat milk powder were reconstituted with either water or milk to 12, 16, 18, 20, 23, and 26% total solids (TS). Some of the samples contained 0.3% or 0.6% gelatin. The mixtures were heat treated at 85°C for 30 min., cooled to 41°C, and then inoculated with 4% of the starter yogurt cultures containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. The mixtures were stirred well and fermented for approximately 5 h at 42°C until the desired pH was reached. The samples that were reconstituted with milk did not ferment at all. The samples with 20 and 23% TS containing full-fat milk powder and 0.3% gelatin resulted in the best quality in terms of appearance, flavour, texture, and overall quality.

Key Words: Concentrated Yogurt, Probiotic Yogurt

W287 Suitability of *Kluyveromyces* spp. for use in single-cell protein production from sweet cheese whey. B. Åsványi, J. Szigeti, and L. Varga*, *Institute of Food Science, Faculty of Agricultural and Food Sciences, University of West Hungary, Mosonmagyarovar, Hungary.*

Only 51.2% of liquid whey produced in the United States is further processed into food or feed ingredients. An alternative to the traditional uses of whey is the production of single-cell protein (SCP). In this study, the suitability of the strains of two *Kluyveromyces* species (*i.e.*, *K. marxianus* var. *marxianus* LAF4 and *K. lactis* KE 231) for use in SCP production was examined. The major parameter measured was the dry weight of the yeast biomass. The *Kluyveromyces* strains tested were grown in unfractionated, heat-treated sweet cheese whey. Fermentations were run batchwise for 48 h in an automated BIOFLO III® batch/continuous fermenter under identical conditions with respect to pH, aeration, and agitation rate. The parameters set were computer-controlled using Advanced Fermentation Software® version 3.42. Samples were taken every 4 h with an MX3 Biosampler®. After proper centrifugation and drying, the dry weight of yeast biomass was determined. The lactose, glucose, galactose, and ethanol contents of samples were also measured. *K. marxianus* var. *marxianus* LAF4 was shown to be superior in terms of suitability for production of SCP from sweet cheese whey to *K. lactis* KE 231 under commercial conditions.

Key Words: Single-Cell Protein, *Kluyveromyces* spp., Whey

W288 Ethanol metabolism by probiotic lactic acid bacteria *in vivo*. W. Y. Yang*[1], Y. T. Ahn[2], K. S. Lim[2], C. S. Huh[2], Y. J. Baek[2], and H. S. Kim[1], [1]*Culture Systems, Inc., Mishawaka, IN,* [2]*Korea Yakult Co., Ltd, Kyunggi do, South Korea.*

Recent studies have shown that probiotic lactic acid bacteria have alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) activities. However, little is known about the metabolism of ethanol and acetaldehyde *in vivo*. The aim of this study is to test the possibility that probiotic lactobacillus strains are able to metabolize ethanol and acetaldehyde *in vivo*. Four *Lactobacillus* spp. and one *Bifidobacterium* spp. were used for this experiment. Male Swiss Webster mice weighing between 19-21 g were used for *in vivo* experiments. The lactic acid bacteria of 10^9 cfu/ml were delivered orally once a day to the mice for 30 days. Ethanol, diluted in water (25:75; v/v), was administered once a day by gastric intubations for 30 consecutive days. The average daily consumption of ethanol was 12 g/kg body weight. Concentrations of ethanol and acetate *in vivo* were measured with HPLC. The acetaldehyde concentration was analysed by head-space gas chromatography. All lactic acid bacteria were able to metabolize ethanol *in vitro*, with *Lactobacillus fermentum* CS332 exhibiting the highest degradation of ethanol. There was an increase of ethanol breakdown in the jejunum and colon of the mice treated with *L. fermentum* CS332 . Ethanol content was 18.2% less in the jejunum and 32.9% less in the colon. Its ability of acetaldehyde conversion into acetate was also significantly higher than the control ($P<0.05$). The breakdown of ethanol and the conversion of acetaldehyde into acetate were observed in mice intestines by lactic acid bacteria after ethanol intake. Based on these data, we suggest that lactic acid bacteria have a beneficial impact on degradation of ethanol and acetaldehyde following heavy drinking.

Key Words: Lactic Acid Bacteria, Ethanol Metabolism

W289 Effects of proteolytic starter cultures on melt characteristics of low moisture part skim (LMPS) Mozzarella cheese. S. Das* and R. I. Dave, *South Dakota State University, Dairy Science Department, Brookings.*

The study assessed the feasibility of using selected starter cultures and different types and levels of coagulating enzyme for making LMPS Mozzarella cheese with desired functionality. LMPS Mozzarella cheeses were made from cows milk standardized to 1.8% fat made with four different types of starter cultures comprising *Streptococcus thermophilus* (ST), *Lactobacillus helveticus* (strain SH-Z or L-11), and *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB-12). Cheeses were made with ST and combination of ST and SH-Z, ST and L-11 or ST and LB-12. Rennet and *Cryphonecteria parasitica* (CP) were used at two different levels (1X or 6X) as coagulating enzymes. Cheeses were analyzed for fat, protein, moisture, total solids, calcium, salt, and ash on day 1. Changes in melt characteristics and proteolysis during storage (4°C) were monitored on 1, 7, 15, and 30 days (d). Meltability of cheese as measured by modified Schreiber test showed differences for cheeses made with different types of starter cultures, and also for different types and level of coagulating enzymes. Cheeses made with ST + SH-Z with coagulant CP at 6X level of enzyme resulted in highest melt area. Softening time and temperature, and melting time and temperature as measured by melt profile analysis were also significantly effected by the type of starter cultures and storage period. Extent of flow and flow rate were higher for ST + SH-Z cheeses and increased further upon 4°C storage of 30 d. Soluble protein as measured by 12% TCA also increased during storage and was highest for cheeses made with ST + SH-Z with coagulant CP at 6X level of enzyme. As the aging progressed, faster breakdown of intact caseins by CP and proteolytic lactobacilli SH-Z took place resulting in a faster weakening of the protein matrix of the cheese, which in turn translated into favorable changes in functional characteristics, especially meltability. LMPS Mozzarella cheese made with proteolytic

culture along with CP has a clear advantage to cheese manufacturers and end users to achieve the desired melt properties in cheese.

Key Words: Mozzarella Cheese, Melt Characteristics, Strarter Cultures

W290 Exo-polysaccharides production in whey mineral concentrate. N. Pandya*[1], R. Dave[1], A. Hassan[1], and L. Metzger[2], [1]*Dairy Science Department, South Dakota State University,* [2]*Food Science and Nutrition Department, University of Minnesota, St. Paul.*

The use of whey mineral supplements in neutral pH beverages such as tea and coffee has been limited due to its poor solubility and gritty mouthfeel. Neutral and phosphorylated exopolysaccharides (EPS) produced by Lactic acid bacteria (LAB) have the potential to improve the solubility and reduce the gritty mouthfeel of whey mineral concentrate since they can function as a nucleation site for the formation of calcium phosphate micro-granules. The growth of two EPS-producing bacterial cultures (*Lactobacillus helveticus* or *Lactococcus lactis* subsp. *cremoris*) in whey mineral concentrate (WMC) with 5, 7.5 and 10% total solids was studied during a 24 h incubation period. Both cultures propagated in 11% reconstituted skim milk were inoculated in to WMC at 2% (v/v) rate and incubated at 37°C for *Lactobacillus helveticus* and at 25°C for *Lactococcus lactis* subsp. *cremoris*. The samples were analyzed for titratable acidity, pH and microbial counts at 4 h interval for 24 h. Viscosity of the fermented WMC at 20°C was measured after 24 h of incubation and overnight cooling (7°C). Both cultures produced EPS at all the 3 solids level, which was evident from fluorescence microscopy observations, the ropiness produced and increase in apparent viscosity of WMC. The viscosity increase was approximately 3 fold with *Lactobacillus helveticus* and approximately 2.4 fold with *Lactococcus lactis* subsp. *cremoris*. The growth pattern, rate of drop in pH and rate of increase in acidity were almost similar at all total solids level. Also, for both cultures, there was no significant ($P<0.05$) difference in the viscosities of WMC at all solids level studied. It was concluded that WMC could support the growth of both EPS-producing cultures and their EPS production was higher in WMC at low solids level. *Lactobacillus helveticus* is recommended due to its ability to produce higher viscosity.

Key Words: Exopolysaccharides, Whey Mineral Concentrate, Lactic Acid Bacteria

W291 Modification of sweet whey for the growth of *Bifidobacterium bifidum*. H. Hernandez-Sanchez*, M. L. Mier-Espinosa, and M. T. Cruz y Victoria, *Escuela Nacional de Ciencias Biologicas - Instituto Politecnico Nacional.*

Sweet whey has been used on a large scale for different biotechnological processes including the manufacture of food and beverages. The purpose of this work was to study the modification of this cheese byproduct for the growth of the probiotic microorganism *Bifidobacterium bifidum*. The modification was done in two stages: first, hydrolysis with two plant proteases and second, thermal treatment at pH 11. For the whey protein hydrolysis, the raw extracts of two proteases from Mexican plants were assayed using the sweet whey (pH 6) as substrate: mexicain from the cuaguayote fruit (*Pileus mexicanus*) latex and hemisphericin from the timbirichi fruit (*Bromelia hemisphaerica*) juice. The thermal treatment was done with whey whose pH had been adjusted to 11. Temperatures of 60, 70 and 80°C for 15 and 20 min were used in this experiment and the resultant wheys analyzed to detect the formation of lactulose, which is known to stimulate the growth of bifidobacteria. The effect of the modification treatments was followed by inoculating the wheys with *Bifidobacterium bifidum* and registering the growth after 18 h of incubation under anaerobic conditions at 37°C. The activity of hemisphericin was higher on the whey proteins than that of mexicain. When 1% protein sweet whey was used as substrate, 82 530 activity units were obtained with hemisphericin at 35°C after a 4 h incubation period. The highest lactose to lactulose conversion was obtained after 15 min at 80°C with a final concentration of 1.6 g/L of lactulose in the whey. These two conditions were used for the whey modification. After the fermentation processes, the following final counts were obtained: 1×10^{14}, 1.7×10^{14} and 6.4×10^{14} CFU/ml for the untreated whey, hydrolyzed whey and hydrolyzed and heated whey respectively. The results show that when both modifications (enzymatic and thermal) were applied to the sweet whey, the counts of the bifidobacteria were up to 6.4 times higher than those of the unmodified whey. This indicates that these modifications could be useful in the production of probiotics or functional beverages.

Key Words: Whey, Probiotic, Bifidobacteria

W292 Optimization of fermentation conditions for development of environmentally friendly deicer. L. Zhang, S. Gokavi*, J. Li, and M. Guo, *University of Vermont, Burlington.*

Sodium chloride is the commonly used deicer for road management and safety during winter in Vermont and other Northern states. Studies showed that environment is adversely affected by the salt. Whey containing lactose accounting for 90% of its total solids is a byproduct of cheese making and its disposal poses a negative impact on the environment. The objective of this study was to optimize the fermentation conditions to develop an environmentally friendly deicer from whey. Lactic acid bacteria (LAB) from our culture collection were studied for production of lactate from lactose. Among them Lactococcus lactis produced lactate at pH 7.0-7.6. Clostridium formicaceticum (27076) was used to produce acetate from lactate at pH 7.3-8.0. Combinations of different LAB and acetogen were studied for production of acetate from lactose in whey permeate (WP). Combination of Lactococcus lactis and Clostridium formicaceticum produced lactate and acetate and the concentration of acetic acid (AA) after 60-72h was 1.6-2.1% which was increased to 1.8-2.5% by supplementing selected nutrients. The cultures were made lactate and acetate tolerant by growing the cultures in WP having high salt concentration (4%) and isolating and subculturing them. The substrate was optimized by adding 5% tomato juice, 0.1% malt extract, 0.2% ammonium phosphate, 0.2% yeast extract, 0.2% peptone and 0.35% vitamin solution to 5% WP powder as it did not support growth in its pure form. The optimum conditions to ferment this substrate by inoculating 10% of culture were temperature 370C-390C, pH 7.3-7.6 maintained using 4M potassium hydroxide, anaerobic condition maintained using nitrogen gas supplied at 1-2 psi, agitation speed 100 rpm and time 84-96h using continuous flow cell-recycle fermentation in a bioreactor. The AA and potassium acetate production was 3.5-4.2% and 5.7-6.9%, respectively and the culture population reached OD 1.6-1.8 at 660 nm. In conclusion, continuous flow cell-recycle fermentation with optimum conditions could be used to increase the yield of AA and/or potassium acetate.

Key Words: Deicer, Potassium Acetate, Whey

W293 Extraction of acetic acid from fermented whey permeate broth. L. Zhang, S. Gokavi, J. Li*, and M. Guo, *University of Vermont, Burlington.*

A combined anaerobic fermentation process was developed to produce potassium acetate (PA) from cheese whey. PA can be used as an organic and environmentally friendly deicer. A coculture consisting of homolactic and heterolactic bacteria was used to convert whey lactose to lactic acid and then to acetic acid (AA) in a bioreactor. The AA is extracted using liquid-liquid reactive extraction to produce potassium acetate. A series of extraction tests was carried out to determine the best solvent and conditions for AA extraction from fermented broth. The broth was adjusted to have pH 3.5, 4.7, 5.9, 7.1 and 8.3 and treated with an equal volume of best extraction solvent Alamine 336 and 2-octanol (1:1). The amount of AA extracted at pH 3.5, 4.7, 5.9, 7.1 and 8.3 was 63.5%, 59.7%, 60.9%, 53.0% and 12.0% respectively. The extraction efficiency (EE) was higher when broth contained K+ (66.0%) and Na+ (61.0%). NH4+ lowered the EE (7.8%). There was no significant difference between EE in presence of anions SO42- or Cl-. So it is recommended to use sodium hydroxide or potassium hydroxide to neutralize the pH and hydrochloric acid to decrease pH. EE was 62.8% when the ratio of extractant to broth was 1:1, 46.44% when 2:1 and 42.18% when 1:2. EE was 62.8% when the ratio of extractant to diluent was 50:50, 50.2% when 70:30 and 47.6% when 30:70. The results showed that extraction of acetic acid from whey permeate fermented broth was highest when ratio of Alamine 336 to 2-Octanol was 1:1 and pH 3.5.

Key Words: Acetic Acid, Liquid-Liquid Dxtraction, Potassium Acetate

W294 Evaluation of modified M17 broth for growth of *Lactobacillus reuteri* and *Bifidobacterium* sp. S. A. Ahmed* and S. A. Ibrahim, *North Carolina A&T State University, Raleigh*.

International dairy federation (IDF) recommends M17 broth for starter lactococci and streptococci and MRS broth (DeMan Rogosa sharpe) for starter Lactobacilli growth. M17 broth medium with specific modifications could be utilized for growth of selected *Lactobacillus reuteri* and *Bifidobacterium* sp. as a convenient medium that can be used easily by the industry in a routine fashion. The objective of this study was to evaluate the ability of modified M17 to promote the growth of *Lactobacillus reuteri* and Bifidobacteria. Six strains of *Lactobacillus reuteri* (DSM20016, MM2-3, MM7, SD2112, CF2-7F, and MF14-C) and four strains of *Bifidobacterium* sp. [*B. infantis*(ATCC 15697, ATCC 15702, ATCC 25962), and *B. longum* 79] were used in this study. The modified M17 broth was prepared by adding M 17 37.25 g/L, Beef extract 5.0g/L, yeast extract 2.5 g/L, and peptone from casein 5.0g/L. Glucose solution (20.0 g/100 ml) was autoclaved separately and added to the autoclaved modified M17 broth. Overnight cultures were centrifuged and washed twice with peptone water. Strains were inoculated into fresh M17 and modified M17 broth, then mixed well and incubated at 37 C for 24 hrs during incubated period the bacterial growth was monitored using spectrophotometer at 610 nm. At (0.0, 12, and 24hrs). After 24 hrs all tested strains were plated on MRS agar to obtain microbial population. Results showed that higher microbial growth was observed in all tested strains using the modified M 17. The optical density in the modified M 17 reached over 1.30 while it reached only 0.70 in the original M 17. The bacterial population increases by at least 1 logcfu/ml. Modified M17 could be a good growth medium in quality control laboratories for general purpose of bacterial growth of lactic acid bacteria and probiotics.

Key Words: M17 Broth, *Lactobacillus reuteri*, *Bifidobacterium*

W295 Effect of dietary whey protein and *Lactobacillus Casei* ATCC 393 on the change of lymphocytic cell population in rats. H. J. Lim*, J. G. Kim, H. Y. Oh, S. H. Kim, and K. Y. Whang, *Korea University, Seoul Korea*.

Seventy-five male Sprague Dawley rats (195.26 ± 3.45 g) raised according to the guideline of NRC (1996) were divided into five groups. A commercial murine diet (NIH-31M) was used as a basal diet. Experimental diets used in this experiment were: 1) basal diet (Cont), 2) basal + 1% whey protein concentrates (WPC), 3) basal + 1% live *L. casei* 393 (LLAB), 4) basal + 1% dead *L. casei* 393 (DLAB), 5) basal + 0.5% whey protein concentrates and 0.5% dead *L. casei* 393 (W+D). Both live and dead *L. casei* 393 cultures contained 10^{12} cfu/g. After feeding each experimental diet for two weeks, rats of each group were subjected to inoculating with 0.2 mL of influenza hemagglutinin peptide (H1N1, 60 μg/mL) via intramuscular injection. Blood samples were collected prior-inoculation (day 0) or at days 10 and 15 of post-inoculation. Red blood cells of samples were lysed and each sample was incubated with specific antibodies against surface antigens of lymphocyte (CD3, CD4, CD8 and CD45R). Three different types of lymphocyte (CD3+/CD4+ Th-cell, CD3+/CD8+ Tc-cell and CD3-/CD45R+ B-cell) were sorted by FACS analysis. After inoculation, T-cell population was found to significantly increase in all groups, with highest level in WPC and lowest level in LLAB at day 10 ($P < .05$). The B-cell populations of WPC, DLAB and W+D groups were shown to increase at day 15 compared with days 0 and 10. In contrast, the B-cell population of LLAB group showed highest at day 10, and then decreased at day 15. In conclusion, it is plausible that supplementation of whey protein and *L. casei* 393 increases humoral immunity and dietary live *L. casei* 393 stimulates immune response more rapidly than others.

	B-cell			T-cell		
	Day 0	Day 10	Dat 15	Day 0	Day 10	Day 15
Cont	10.57	11.01^{ab}	11.00	23.29^{b}	48.89^{ab}	45.29
WPC	9.50	13.03^{ab}	20.12	16.09^{c}	57.27^{a}	43.94
LLAB	8.85	16.09^{a}	14.15	17.85^{bc}	40.43^{b}	36.90
DLAB	8.71	8.76^{b}	15.22	17.23^{c}	48.88^{ab}	37.09
W+D	10.13	8.87^{b}	14.39	30.20^{a}	46.59^{ab}	40.23
SEM	2.16	1.73	2.30	1.84	2.61	2.76
P-value	NS	0.04	NS	0.01	0.02	NS

Key Words: Whey Protein, Lymphocytes, Rats

W296 Viability of *Bifidobacterium longum* and *Lactobacillus reuteri* in sour cream. S. A. Ibrahim and E. D. Wilson*, *North Carolina A&T State University, Raleigh*.

Over the past two decades the consumption of probiotic products has risen considerably. This is mainly due to the large amount of scientific evidence from human studies, which demonstrate that regular probiotic consumption helps in maintaining a healthy digestive tract. In order for probiotics to be included in dairy food products, they should be in viable quantities for the duration of the shelf life. Therefore, the objective in this research was to determine the viability of probiotic, *Bifidobacterium longum*and *Lactobacillus reuteri* in commercially-available sour cream. Fresh sour cream samples were obtained from a local market and inoculated with one of the following probiotic strains: *B. longum* (ATCC 5708 and NCFB 2254) *L. reuteri* (MM 2-3 and MM 7) and to obtain a final inoculum level of 107cfu/ml. The sour cream samples were then mixed thoroughly and refrigerated at 4 °C for 2 weeks. The samples were analyzed for viable bacterial count using modified BIM 25 agar to enumerate bifidobacteria and MRS agar supplemented with 50 μg/ml vancomycin to enumerate lactobacillus. Our results show that although bacterial counts decreased, the products contained an average 5.0 x 105 cfu/ml of viable probiotics after 15 days of storage. Results also show significant differences ($P<0.05$) among the tested strains during the storage period. Both *B. longum* strains had two log reduction while *L. reuteri* MM 7 had one log reduction over the storage period. *L. reuteri* MM 2-3 shows a slight decline although it was not significant over the storage period. Our results show that the concept of using sour cream as a probiotic carrier is a feasible application for use in the food industry.

W297 Yogurt development from camel milk. I. B. Hashim* and A. H. Khalil, *United Arab Emirates University, Al-Muhandeseen - Giza*.

The camel (*Camelus dromedarius*) has the ability to produce more milk for longer period in arid zones and dry lands. Although camel milk has been used to produce acceptable feta-type cheese, hard cheese and ice-cream, it exhibits antibacterial properties causing problems in fermentation. The rheological and microscopic characteristics of the dromedary milk have shown that its coagulum to be a fragile, heterogeneous curd structure which fails to gel with lactic acid cultures. The objectives of this study were to develop yogurt from camel milk and to determine its sensory characteristics. Yogurt was prepared from cow and camel milk using standard procedure following a commercial yogurt formula [2.5% milk solid nonfat (MSNF), 0.6% commercial stabilizer (CS) and commercial yogurt culture (CYC)]. Yogurt made from camel milk using up to double the amount of the ingredients used for yogurt making (MSNF, CS and CYC) produced viscous yogurt with fragile texture. Addition of carboxy methyl cellulose had no significant effect on yogurt texture while addition of gelatin, sodium alginate (ALG) and calcium chloride (Ca) improved yogurt texture. Sensory profile of yogurts conducted by five trained experienced panelists showed that yogurt containing 1% gelatin or 0.75% ALG + 0.075 % Ca had the best texture. Hedonic ratings by 33 consumers indicated that yogurt made from camel milk using 0.75% ALG + 0.075 % Ca had similar sensory ratings and acceptability (6.0-7.6) as yogurt made from cow milk (6.4-7.9). Fruit yogurt prepared by adding 15% fruit concentrates of various mixtures to camel milk containing (0.75% ALG and 0.075 % Ca) had similar hedonic ratings and acceptability. Production of yogurt from camel milk will enable

many arid regions populations to make use of surplus camel milk with potentials for marketing such products.

Key Words: Camel Milk, Yogurt, Consumer Acceptance

W298 Physico-chemical and sensory properties of liquid-type yogurt with Lactobacillus casei 00692. B. J. Jeon*, J. S. Seok, and H. S. Kwak, *Sejong University, Seoul, Korea*.

This study was carried out to find the physico-chemical and sensory attributes of liquid-type yogurt with Lactobacillus casei 00692 during 72 hr fermentation at 37°. The pH decreased upto 32 hr and plauteaued thereafter, and the titratable acidity increased upto 40 hr. The growth of lactic acid bacteria sharply increased with 9.0 × 109 cfu/ml upto 36 hr of fermentation and slowly increased thereafter. The free amino acids produced during the fermentation reached the maximum value at 40 hr and gradually decreased thereafter. In the result of electrophoresis, the band was the thickest at 44 hr and mostly disappeared at 72 hr fermentation. In a sensory analysis, yogurt flavor was gradually developed during 30 hr, while bitterness score did not significantly changed throughout fermentation periods. The present data showed that the range of optimum fermentation time for liquid-type yogurt using Lactobacillus casei 00692 was from 40 to 44 hr.

Key Words: Fermentation Time, Liquid-Type Yogurt, Lactobacillus casei

International Animal Agriculture

W299 Utilization of *Leucaena leucocephala* as supplement for goats in the semi arid areas of Venezuela. T. Clavero* and R. Razz, *La Universidad del Zulia, Venezuela*.

A field experiment was conducted in the dry land farming area of northwest Venezuela in order to evaluate three diets in grazing goats (grazing pasture only (buffel grass); grazing pasture + 0.3 kg of commercial concentrate/animal/d; grazing pasture + restricted browsing for two hours daily *Leucaena leucocephala*) on milk production and milk composition. The experiment was laid out randomized block design. The data showed significant difference (P #88040.05) between treatments. Daily milk yield increased in 35 and 52.7% when goats had access to commercial concentrate or browsing Leucaena as well as grass pasture compared with the control treatment. Daily milk yield in goats with access to Leucaena was insignificantly different than goats on concentrate. Treatments did not affect milk composition. The results suggest that Leucaena can supply an adequate amount of nutrients with similar value to commercial concentrate for milk production without adverse effects on tropical grazing goats.

Key Words: Leucaena leucocephala, Goat, Milk Production

W300 Estimation of genetic and phenotypic parameters of total milk production in Suran Holstein dairy farm. S. Zakizadeh, A. Horufi*, and M. Qolipur, *High Education center of Jihad & Agriculture*.

Most traits of economic importance in animal breeding are quantitative in nature. The phenotypes observed are thus the combined results of the action of many genes or quantitative trait loci and environmental effects. As the selection of dairy cattle is focused on the improvement of yield and composition of milk, the object of this research was to estimate genetic parameters and breeding values of total milk production in a Holstein dairy farm in the northeast of Iran. The data used from Animal Breeding Center of Iran and consisted of total milk records from 2247 cows, between 1990 and 2003. Base population was imported from Canada and the Netherlands in 1990. To investigate environmental effects, following model was analyzed in JMP 3.1.2 Software. The model included random effects of sire and dam, lactation and calving year as fixed effects, calving interval, peak of yield, and days in milk as covariates. All factors were significant ($p<0.05$). It was concluded that the highest milk production would be achieved in the 4th lactation. Animal model procedure was used to estimate genetic parameters and breeding values by DF-REML software. Heritability and repeatability coefficients were 0.2 and 0.36 respectively.

Key Words: Total Milk Production, Genetic and Phenotypic Parameter Estimation, Milk Production Heritability

W301 Assessment of microbial colonization and kinetics degradation of Distichlis grass irrigated with fresh or brackish water in dromedary camels. G. Alhadrami*[1], A. El Awad[1], and M. Pessarakli[2], [1]*Department of Aridland Agriculture, College of Food Systems, United Arab Emirates University, Al-Muhandeseen - Giza,* [2]*Department of Plant Sciences, University of Arizona, Tucson*.

An in situ study was conducted to investigate the extent and kinetics degradation of DM, NDF, ADF and microbial colonization of *Distichlis spicata* grass (halophytic grass) irrigated with fresh water (DFW) or with brackish water (DBW) in the rumen environment of dromedary camels. Three camels fitted with rumen cannulas were used in this experiment. Camels were fed concentrate and Rhodes grass hay individually to maintain body weight constant and had free access to fresh water. Distichlis plants were labeled with ^{15}N as an internal marker. Amount of ^{15}N in excess of 0.366 atom% were considered as enrichment. Dilution of enrichment estimated percentage of microbial nitrogen. Labeled DFW and DBW grasses were weighed and placed in nylon bags and incubated in the rumen of the camels for up to 48 h. Differences in DM, NDF and ADF degradation were not significant between DFW and DBW, except at 48 hours the DM ($P\leq0.04$) and NDF ($P\leq0.027$) of DBW were significantly higher. Contamination expressed as percentage of microbial-N to total residual-N increased with incubation time and was less in DBW compared to DFW, differences were significant after 24 h ($P\leq 0.011$) and 48 h ($P\leq 0.001$) of incubation. Microbial colonization (expressed as percentage of microbial crude protein) and microbial cell mass followed the same trend. After 48 h of rumen incubation, microbial nitrogen was 30.3% in DBW and 42.6% in DFW. Microbial crude protein was 4.7% in DBW and 6.2% in DFW. In both DFW and DBW, microbial colonization and microbial contamination increased with incubation time. Microbial contamination affected estimates of in situ ruminal protein degradation of Distichlis grass irrigated with fresh water more than the Distichlis grass irrigated with brackish water.

Key Words: Distichlis Grass, Microbial Contamination, Dromedary Camels

W302 Effect of molasses on nutritional quality of *Pithecellobium dulce* silage. T. Clavero* and R. Razz, *La Universidad del Zulia, Venezuela*.

This study determined the influence of varying levels of molasses and ensiling time on the content of the nitrogenous fractions, chemical composition and fermentation quality during ensiling of *Pithecellobium dulce* in the farming systems of Venezuela. Chopped fresh plant materials of about 1 cm length were ensiled into laboratory silo ans stored at 25°C. Treatments were applied according to a 3x3 factorial arrangements in a completely randomized design. Factors studied were three rates of legumes:molasses, 1:8, 1:10, 1:12 (w/v) and three storage periods 1, 2 and 3 months. After opening the silos, dry matter (DM), pH, total nitrogen content (NT), rumen soluble nitrogen (SN), protein nitrogen (NP), nitrogen in acid detergent fiber (NADF), nitrogen fixed to the cell wall of the total nitrogen (NNDF/NT), in vitro DM digestibility (IVDMD), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined. DM of *Pithecellobium dulce* was not changed during ensiling and the molasses additive had not significant effect on the silage DM. The mean pH values decreased significantly ($P\leq0.05$) with increased level of molasses and storage period, respectively. The lowest pH value (4.06) was obtained with the relation 1:12. No significant differences in NT, NP, NADF, NNDF/NT, pH, ADF and NDF were found between molasses treatments. Content of NS and digestibility increased significantly ($P\leq0.05$) with increased level of molasses. Except for NP and NS, the ensiling time significantly affected ($P\leq0.01$) the loss in digestibility, NT, NADF, NNDF/NT, pH, ADF and NDF. The greatest losses ocurred within 1-2 months of ensiling. The results showed that *Pithecellobium dulce* fodder can be preserved successfully by ensiling with molasses additive.

Key Words: *Pithecellobium dulce*, Silage Quality, Molasses