sulfate flotation technique. Samples in which *Giardia* ssp cysts were present were considered positive. PCR of the β-giardin gene was performed on 40 positive samples each of humans and dogs, obtaining 24 and 20 amplified of each origin respectively. RFLPs were obtained by digesting the amplified with *Hae III* nuclease in two percent agarose gels. Regarding the obtained RFLPs of each species, it was possible to demonstrate the existence of two different strains of *Giardia* ssp., of which one of them had the same pattern for both humans and dogs. It is concluded that, in Culiacan, Sinaloa, Mexico, humans and dogs share at least one causative diarrhea strain of *Giardia* ssp., suggesting the possibility of cross-transmission.

Key Words: *Giardia*, PCR technique, β-giardin gene

T39 Presence of eggs Toxocara spp. in soil of public parks in Culican, Sinaloa. M. C. Rubio Robles*, S. M. Gaxiola camacho, N. Castro del Campo, B. N. Verduzco, and M. N. López, *Universidad Autonoma de Sinaloa*, *Culiacan, Sinaloa, México*.

Toxocariasis is a zoonotic infection caused by the parasitic Toxocara spp. commonly found in the intestine of dogs and cats. Disseminated for defecate in parks and public areas. Toxocariasis can result in a condition known as visceral and or ocular larval migrans, which is associated with inflammation of body organs and or the central nervous system. Symptoms of which are caused by the movement of the worms through the body, include fever, coughing, asthma, or pneumonia. Toxocariasis most often occurs in children, who often play or eat (pica)dirt that potentially is contaminated with Toxocara spp. eggs. The eggs of Toxocara spp. are extremely resistant to adverse environmental conditions, capable of surviving in soil for many months. The objective of the work was to determine the presence of eggs of Toxocara spp. in the soil of the children play area in public parks of the city of Culiacan, Sinaloa. Egg presence was determined by means 291 soil samplings in 23 public parks of the city. Established by random stratified samplings, and collecting soil samples using the technique of double W; We took surface soil scraping of 100 grams of earth for each sample and deposited it in previously identified plastic bags. The samples were later transferred to the laboratory of parasitology of the FMVZ-UAS to be analyzed by means of the sedimentation and flotation techniques. The samples indicate that of the 23 parks analyzed 13 (54%) contained eggs of Toxocara spp.; We conclude that the contamination with eggs of Toxocara spp.; shows up in a percentage in the area of children play areas in the public parks of the city of Culiacan, Sinaloa. Knowing the danger this parasite represents for the public health, indicates to us the risk to which are exposing especially our children, mainly if they frequently use these recreation places. Therefore the necessity arises of implementing control strategies and education for the prevention of the infections by fecal origin in public parks.

Key Words: Toxocara, Public parks, Parasites

Dairy Foods: Chemistry and Microbiology

T40 Partition coefficients for toxic agents in multiple phase foods: Separation of raw whole milk. J. E. Schlesser*[1], J. E Jablonski[1], and P. Mariappagoudar[2], [1]*FDA, National Center for Food Safety*, *Summit, IL*, [2]*Illinois Institute of Technology, National Center for Food Safety*, *Summit, IL*.

Contamination of milk with toxic compounds may be accidental or intentional. Since milk consists of skim milk and cream phases, it is of interest to determine into which phase the toxin will partition. Aconitine, nicotine and strychnine were chosen as model toxin contaminants. An HPLC method for analysis of aconitine, nicotine, strychnine from milk products was developed. Sample clean up techniques consisted of liquid-liquid partitioning (hexane/water-acetonitrile), solid phase extraction (OASIS HLB), and manipulation of pH of sample to avoid volatility and hydrolysis losses. Analysis was conducted with an HPLC with dual band UV detector. Nicotine and strychnine levels were measured at 260nm and aconitine at 232nm. Centrifugation of whole milk was used to simulate commercial separation. Whole milk was placed into 50 ml centrifuge tubes, spun for 30 minutes at 2000 x g and 5°C. Skim milk from tubes were decanted and mixed. Cream layer adhering to the side of bottles was dissolved and mixed together. The mixed samples were used for fat testing or if spiked, for testing of toxins. Centrifugations were conducted at 30 minutes and 5 days after spiking to simulate contamination in the plant and on the farm, respectively. Mean recoveries for the three analytes in skim milk, whole milk and cream ranged from 72.1% to 89.2%. Centrifugation of 3.25% whole milk resulted in a fat content of 39.5% and 0.07% for cream and skim milk respectively. Whole milk was spiked with 1 ppm of each of the three toxins. Aconitine, nicotine and strychnine were found in both cream and skim milk streams. Initial partition coefficient for aconitine was 0.769 in cream, and increased to 1.121 by day 5. Initial partition coefficient for nicotine was 0.49 in cream, and increased to 0.761 by day 5. Initial partition coefficient for strychnine was 1.064 in cream, and increased to 1.135 by day 5. Between day 0 and day 5, partition coefficients for the toxic compounds in skim milk decreased.

Key Words: Aconitine, Nicotine, Strychnine

T41 Modelling of the high-pressure and temperature induced pH change in whey protein isolate solutions. H. Hernández-Sánchez*[1], J. O. Rodiles-López[1], M. E. Jaramillo-Flores[1], and G. V. Barbosa-Cánovas[2], [1]*Depto. Grads. Alimentos, Escuela Nacional de Ciencias Biologicas, Instituto Politecnico Nacional*, *Mexico, DF, Mexico*, [2]*Washington State University*, *Pullman*.

High hydrostatic pressures (HHP) can lead to greater ionization in certain biological systems resulting in a temporary decrease in pH while under pressure. It has been reported that in some cases, the HHP-induced denaturation of whey proteins is decreased when the pH during the treatment is less than 7. The objective of this study was to evaluate and model the effect of high hydrostatic pressure and temperature on the final pH of whey protein isolate (WPI) solutions. WPI solutions at a concentration of 5% (w/v) were treated with HHP of 200, 400 and 600 MPa at different temperatures (25, 40 and 55°C)

for holding times of 0, 0.1, 5, 10 and 15 min in a warm isostatic press with a cylindrical pressure chamber (height = 0.25 m, diameter = 0.10 m). The pH of the samples was measured 2 h after the pressure was released. A Box-Behnken experimental design for the three experimental variables was used for the study The WPI solution had an initial pH value of 6.9 and decreased to average values of 6.74 ± 0.12, 6.67 ± 0.12 and 6.65 ± 0.15 for 200, 400 and 600 MPa, respectively. The regression analysis produced the following equation: pH = 3.73 exp (0.000152 t) / ($P^{0.00676}$ $T^{0.31449}$) where t is the holding time in min, P is the pressure in MPa and T is the temperature in K. The model implicates an increase in pH with the holding time but a predominant decrease of this parameter with both pressure and temperature. It can be concluded that the HHP treatment at the temperatures tested in this study create favorable pH conditions that could decrease the denaturation of the whey proteins. This conclusion only involves the effect of HHP and temperature on the pH and does not include the effect of these parameters *per se* on the denaturation process of the whey proteins.

Key Words: High hydrostatic pressures, Whey protein isolate, pH

T42 The impact of β-glucan on the stability of model dairy protein dispersions. J. E. Bock, K. A. Schmidt*, and G. E. Milliken, *Kansas State University*, *Manhattan*.

β-Glucan (BG) has shown promise as an immunomodulatory agent. Inclusion of BG in dairy-based beverages may benefit immunocompromised consumers, but the effect on beverage stability is uncertain. The objective of this study was to determine the impact of BG on the stability of model dairy protein dispersion systems. Model dispersions were created using ratios of 25:75, 50:50, or 75:25 sodium caseinate to whey protein isolate; 1% or 5% protein content; pH 7, 5, or non-adjusted; and BG at 100 µg/mL. Particle size distributions, apparent viscosity, and dispersion stability were measured within 24 h post-mixing. A four factor factorial experimental design was used with ANOVAs to determine significant factors. LSD mean differentiations were performed with significance at Î±=0.05. Cluster analysis characterized additional relationships among dispersion formulation, stability, particle size distribution, and apparent viscosity. BG inclusion resulted in a smaller mean particle size (121.2 µm vs. 147.2 µm) but did not affect stability. BG affected viscosity (1 sec^{-1}) but only as a pH*protein content*BG interaction. BG increased the viscosity of non-adjusted pH dispersions at both protein contents but decreased viscosity in pH 5 dispersions at 1% protein and pH 7 dispersions at 5% protein. Stability was greatest (>95%) for dispersions at pH 7 with a mean particle size around ~225 µm and a large particle size distribution spread around the mean. Mean particle sizes tended to decrease and form more compact particle size distributions as pH decreased, resulting in less stability. At a 5% protein level, non-adjusted and pH 7 dispersions had similar attributes as casein content increased. The results indicate that BG does not affect dispersion stability but that pH, protein content, and protein ratio are important factors. The evidence suggests that dairy protein beverages with a range of protein contents and viscosities could be feasible vehicles for BG delivery.

Key Words: β-glucan, Proteins, Dispersions

T43 The stability of a functional dairy based beverage. K. A. Schmidt*, *Kansas State University*, *Manhattan*.

Functional beverages are growing in popularity. However the addition of a functional ingredient to a dairy–based beverage may lead to instability, which could decrease consumer acceptance. In the past, β-glucan (BG) has demonstrated immune-modulating effects under certain conditions and has been incorporated in a dairy-based dispersion; however the stability of such of a beverage is unknown. Thus, the objective of this study was to determine the impact of BG on the storage stability of dairy beverages. NDM (11% w/v) beverages were prepared with and without BG (100 µg/mL), heat-treated to 95°C for 5 min, then filled into containers. Products were stored at 4°C for up to 57 d and tested bi-weekly for particle sizes, sedimentation, stability, and total plate counts. Three replications were done. A two-factorial design was used and data were analyzed with ANOVAs to determine significance ($\alpha = 0.05$). All significant means and interactions were differentiated by the LSD test. Results showed that BG addition did not affect the particle size (~0.172 µm) if the sample was well-mixed prior to analysis. However, if analyzing the particle sizes at the bottom of a quiescently-stored container, the particle sizes increased (~0.172–0.22 µm) during the first month of storage, indicating that beverages regardless of BG addition exhibited some association and settling of larger-sized particles. Sedimentation in the BG-containing beverages was observed after 1 d of storage, but by d 29, all beverages had equivalent sedimentation. Beverage stability decreased (~1.2%) from d 1 to d 29, and then remained constant throughout the storage period. At d 57, the pH and total plate counts were still within acceptable ranges for beverages formulated at native pH. These results provide evidence that NDM-based functional beverages can be formulated and stored at 4°C for 2 months with minimal changes in particle sizes, sedimentation, or stability.

Key Words: β-glucan, Dairy beverages, Stability

T44 Electrophoretic characterization of protein aggregates formed by high pressure treatment of whey protein concentrate solutions. H. A. Patel*[1,2], H. Singh[3], and L. K. Creamer[2], [1]*Institute of Food, Nutrition and Human Health, Massey University*, *Palmerston North, New Zealand*, [2]*Fonterra Research Centre*, *Palmerston North, New Zealand*, [3]*Riddet Centre, Massey University*, *Palmerston North, New Zealand*.

The objective of the present investigation was to characterize pressure-induced aggregates of whey proteins using tailor-made combinations of various polyacrylamide gel electrophoresis (PAGE) techniques. Whey protein concentrate (WPC) solutions were pressure treated in the range from 200 to 800 MPa for 30 min. The protein aggregates formed as a result of these pressure treatments were characterized using various one-dimensional and two-dimensional (2D) PAGE techniques in the absence or presence of a disulfide-bond reducing agent. It was found that pressure treatment of WPC solutions generated both hydrophobic and disulfide-linked protein aggregates consisting of immunoglobulin, lactoferrin, bovine serum albumin, β-lactoglobulin (β-LG), and α-lactalbumin (α-LA), as identified by native and then non-reduced sodium dodecyl sulfate (SDS) 2D PAGE and SDS and then reduced SDS 2D PAGE, respectively. Only disulfide-linked β-LG dimers were observed at 200 MPa, whereas more severe pressure treatment generated dimers, trimers, tetramers, and higher polymers and 1:1 complexes of β-LG and α-LA. The amount of high molecular weight aggregates not dissociated by SDS increased with the severity of the pressure treatment. It was found that in addition to disulfide-linked aggregates, hydrophobic-bonded dimers, trimers, tetramers, etc. of the whey proteins were also observed on native and then non-reduced SDS 2D PAGE.

Key Words: Whey protein concetrates, 1D and 2D PAGE, Disulphide-bonded aggregates

T45 Comparison of whey protein nitrogen index and aggregation of proteins in low-, medium-, and high-heat skim milk powders. H. A. Patel*[1,2], L. K. Creamer[2], and H. Singh[3], [1]*Institute of Food, Nutrition and Human Health, Massey University*, *Palmerston North, New Zealand*, [2]*Fonterra Research Centre*, *Palmerston North, New Zealand*, [3]*Riddet Centre, Massey University*, *Palmerston North, New Zealand*.

Various types of skim milk powders are used in the recombined milk industry. These skim milk powders are broadly classified as low-, medium-, and high-heat powders. This classification is usually based on the whey protein nitrogen index (WPNI), which is the amount of undenatured whey protein present in the powder and is related to the heat treatments that are used during the manufacture of milk powders, particularly during evaporation and the so-called pre-heat treatments. In the present study, we compared the WPNI measured in a series of powders with the aggregation of proteins as analyzed by various polyacrylamide gel electrophoresis (PAGE) and capillary electrophoresis techniques. Non-reduced sodium dodecyl sulfate (SDS) and then reduced SDS two-dimensional PAGE gave an indication of the type, composition, and amount of whey proteins involved in the disulfide-linked protein aggregation in three different skim milk powders. It was found that the resultant loss of native protein content (as analyzed by PAGE and capillary electrophoresis) correlated well with the WPNI. The results of this preliminary study suggest that the WPNI gives a method that could be used to predict the extent of protein aggregation. Conversely, protein aggregation could be used to estimate the WPNI values.

Key Words: Protein aggregation, Disulfide-linked aggregation, WPNI

T46 Comparison of the effects of heat and high hydrostatic pressure treatments on the aggregation of proteins in fresh skim milk. H. A. Patel*[1,2], H. Singh[3], S. G. Anema[2,3], and L. K. Creamer[2], [1]*Institute of Food, Nutrition and Human Health, Massey University*, *Palmerston North, New Zealand*, [2]*Fonterra Research Centre*, *Palmerston North, New Zealand*, [3]*Riddet Centre, Massey University*, *Palmerston North, New Zealand*.

Various heat treatments (e.g. pasteurization and sterilization) have been used for the preservation of milk and milk products. These heat treatments were compared with a range of high pressure treatments up to 800 MPa using various polyacrylamide gel electrophoresis techniques developed for the purpose. It was found that each of the whey proteins responded differently to heat and pressure treatments. Pasteurization aggregated some of the whey proteins and incorporated some of the κ-casein. Sterilization aggregated most of the whey proteins. Both hydrophobic and disulfide-linked protein aggregates of various sizes were formed by heat and pressure treatments. Heat (or pressure) exposed a Cys residue of β-lactoglobulin, which catalyzed the formation of new disulfide bonds, leading to aggregation of the whey proteins. These aggregates then reacted with κ-casein and possibly with αs_2-casein to give cross-linking of casein and whey proteins. These changes were not completely reversed when the environment reverted to the native environment. Comparatively larger protein aggregates were formed by heat treatment than by pressure treatment. The stability of the native structure is also explainable in structural terms. From the results, it is concluded that pressure and heat affect the individual proteins differently so that there are benefits in each of the treatments when applied to mixtures of proteins. This poster will demonstrate that there are some important differences and many similarities in the protein aggregation in heat- and pressure-treated samples, which may have implications on the quality of the final product.

Key Words: Skim milk, Protein interactions, Heat treatments

T47 Effects of protein concentration and pH on the pressure-induced aggregation of whey proteins in whey protein concentrate solutions. H. A. Patel*[1,2], H. Singh[3], and L. K. Creamer[2], [1]*Institute of Food, Nutrition and Human Health, Massey University*, *Palmerston North, New Zealand*, [2]*Fonterra Research Centre*, *Palmerston North, New Zealand*, [3]*Riddet Centre, Massey University*, *Palmerston North, New Zealand*.

The objective of the present investigation was to study the effects of protein concentrations on pressure-induced denaturation and aggregation of whey proteins. Various one-dimensional and two-dimensional polyacrylamide gel electrophoresis techniques showed that several intermediate species, such as non-native monomers of β-lactoglobulin (β-LG) and α lactalbumin (α-LA), β-LG dimers, α-LA dimers etc., were formed when dilute whey protein concentrate (WPC) solutions (0.5 and 2% w/v) were pressure treated. Very high molecular weight aggregates and particulates that could not penetrate the gel were formed when concentrated WPC solutions (12 % w/v) were subjected to the same pressure treatments. The effect of pH on the solubility of the whey protein aggregates was also determined by adjusting aliquots of the high-pressure-treated WPC solutions to different pHs between pH 3.5 and pH 7.2 and centrifuging them. Very little polymeric protein was found in the supernatant at pHs between 4.9 and 5.3, whereas significant amounts of polymeric proteins were present in the supernatant at higher and lower pHs. This behaviour was similar to that of heat-treated WPC solutions, reported in the literature. After high pressure treatment, the relative loss of the native structure of β-LG was greater than that of bovine serum albumin at all concentrations used in present study, whereas α-LA was the most pressure resistant among all the whey proteins. This was found to be in converse to the response of these proteins to heat treatment. It may be possible that the hydrophobic cavity in the structure of β-LG was responsible for its apparently anomalous behaviour as a result of pressure treatment.

Key Words: Whey protein concentrates, α-Lactalbumin, β-Lactoglobulin

T48 Impact of trisodium citrate on rheology and microstructure of yogurt. T. Ozcan Yilsay*[1,2], W. J. Lee[2], and J. A. Lucey[2], [1]*Uludag University*, *Bursa, Turkey*, [2]*University of Wisconsin*, *Madison*.

Casein micelles are disaggregated by removal of colloidal calcium phosphate (CCP) with Ca-chelating agents, such as, trisodium citrate (TSC). Objectives of this research were to investigate the effect of TSC on the physical properties of yogurt. Reconstituted skim milk was heated at 85°C for 30 min and various concentrations (5 to 40 mM) of TSC were added to milk. pH was re-adjusted to 6.50. Milk was inoculated with 2% yogurt culture and incubated at 42°C, until pH was 4.6. Acid-base titration was used to determine changes in the state of CCP of milk. Total and soluble Ca contents of milk were determined. Gel formation was monitored by dynamic low amplitude oscillatory rheology. Microstructure of yogurt was observed using confocal scanning laser microscopy and whey separation was also determined. Addition of TSC reduced casein-bound Ca while increasing solubilization of CCP. The G′ value of gels increased with addition of low levels of TSC and highest G′ values were observed in samples with 10-20 mM TSC; higher (>20 mM) TSC concentrations resulted in a

large decrease in G′ values. At low TSC levels, the removal of CCP crosslinks may have facilitated greater rearrangements and partial disruption of the micelle structure; both of which may help to increase G′ and loss tangent (LT) values. At high TSC concentrations, micelles were completely disrupted and CCP crosslinks were dissolved, both of which resulted in weak yogurt gels with large pores. Gelation pH and yield stress significantly decreased with high TSC levels. No maximum in LT at pH ~5.1 was observed during gel formation in yogurts made with high TSC levels as CCP was completely dissolved prior to gelation. Lowest whey separation levels were observed in yogurt made with 20 mM TSC and whey separation greatly increased at > 25 mM TSC. In conclusion, low concentrations of TSC improved several important yogurt characteristics whereas the use of levels that disrupted casein micelles resulted in poor gel properties.

Key Words: Yogurt, Trisodium citrate, Rheology

T49 Identification of off-flavor compounds in Whey protein concentrate using head space solid phase microextraction-gas chromatography-olfactometry -mass spectrometry. I. Javidipour and M. Qian*, *Oregon State University*, *Corvallis*.

Whey protein concentrate (WPC) has become a popular ingredient in food industry, both in sports nutrition as well as traditional products such as dairy, bakery, meat, beverage and infant formula due to its excellent nutritional value and unique functional properties. Whey protein concentrate typically has a shelf life of 9-12 months when stored at normal conditions. However, WPC can develop off-flavor during the storage, and those unpleasant off-flavors limit its application in some delicate formulations. To investigate the chemical nature of the off-flavor, two WPC samples (one was regular 80% WPC, one was instantized 80% WPC) were stored at room temperature for 12 months to develop the off-flavor. Three g of samples were dissolved in 7 mL of deodorized water, and the off-flavor compounds were extracted using a DVB/Carboxen/PDMS fiber (2 cm, 50/30 µm) at 45°C for 3 h. The off-flavor compounds were analyzed using gas chromatography-olfactometry and GC-mass spectrometry on a DB-Wax column. Based on the aroma intensity, the most important off-flavor compounds were identified as dimethyldisulfide (sulfur, rubbery), dimethyltrisulfide (gas, cabbage), pentanal (green), hexanal (grass), heptanal (grass, rancid), octanal (green, fatty), nonanal (fatty), t-2-octenal (fatty, rancid), t-2-decenal (tallowy), t-2-nonenal (old book), t,t,2,4-nonadienal (fatty, earthy), 1-octanol (fatty, waxy, green) and 1-hexanol (green, herbaceous). The results demonstrated that the off-flavor compounds in WPC were mostly lipid and protein oxidation products.

Key Words: Whey protein concentrate, SPME, Off-flavor compounds

T50 Off-flavor development of whey protein concentrate during storage investigated by headspace solid-phase microextraction-gas chromatography. I. Javidipour and M. Qian*, *Oregon State University*, *Corvallis*.

Whey protein concentrate (WPC) is a nutritious and functional protein ingredient and has gained popularity to be used in many traditional as well as novel food products. It typically has a 9-12 months of room temperature storage shelf life. Fresh manufactured WPC has a bland or slightly dairy, utensil, whey flavor. However, inadequate flavor stability during storage is generally recognized and the off-flavor becomes one of the major factors limiting the usage of WPC. To understand the flavor stability of WPC, off-flavor formation in WPC 80 and instantized WPC 80 was investigated in this study. Both samples were stored at 35, 45 and 55 °C while another instantized WPC80 sample was filled with argon and stored at the same temperatures. Samples were taken weekly for a period of three months. A headspace solid-phase microextraction-gas chromatography technique was used to study the off-flavor formation. Multiple internal standards were used to build the calibration curves and the off-flavor compounds were quantified. The result showed that lipids oxidation products such as aldhydes, ketones and free fatty acids were the main source of off-flavor. Protein breakdown and Maillard reaction also played a role in off-flavor development of WPC. Storage temperature seemed to be very important in the formation of those off-flavor compounds. The results could be used to build a model to estimate shelflife of WPC at extreme conditions.

Key Words: Whey protein concentrate, SPME, Off-flavor

T51 Differentiation of cheese sauces made with different starches and evaluation of the effect of starch type on flavor loss using FTIR spectroscopy. M. C. M. Soledad*, C. J. Kuo, L. E. Rodriguez-Saona, and W. J. Harper, *The Ohio State University*, *Columbus*.

A growth in the utilization of cheese sauces in institution and foodservice markets has been observed. Starches are added in the formulation to reduce texture problems in cheese sauces but contribute to flavor loss. This study was aimed to develop a simple and fast protocol to differentiate cheese sauces made with different starch types and to evaluate probable contribution of starch to flavor loss by using Fourier-Transform infrared (FTIR) spectroscopy combined with multivariate analysis. Sauces were prepared using the Stephan processed cheese maker. Natural Swiss cheese was used as source of cheese flavor. The native starches compared included: waxy corn (NWC), waxy rice (NWR), sago (NS), and tapioca (NT). Freshly prepared samples were placed on contact with a three-reflection diamond crystal plate for attenuated total reflectance infrared (ATR-IR) measurements. Classification models (Soft Independent Modeling of Class Analogy, SIMCA and Hierarchical Cluster Analysis, HCA) generated from derivatized infrared spectra (4000-800 cm^{-1}) were created and evaluated based on class distances and clustering. Specific infrared spectral information was obtained that allowed for the generation of classification (SIMCA) models that exhibited tight and well-separated clusters and discriminated among samples containing different native starches. Most of the discrimination among cheese sauces was in the 900-1150 cm^{-1} dominated by C-O and C-C stretching modes, the 1500-1200 cm^{-1} region associated to O-C-H, C-C-H, and C-O-H bending vibrational modes and the 1800-1700 cm^{-1} region associated with carbonyl group of various R(CO)OH or R(CO)OR. The highest absorbance peak at the carbonyl region was observed from the sauce made with NS suggesting least effect on flavor loss. This technique could contribute to the development of simple and rapid protocols for monitoring the presence of native starches in cheese sauces and elucidate the effect of starch addition on flavor loss of cheese sauces.

Key Words: Cheese sauce, FTIR, Starch

T52 Validation of ED-XRF as a reliable method for determining the mineral composition of skim milk powders. S. Uson*, C. Immoos, and R. Jiménez-Flores, *California Polytechnic State University*, *San Luis Obispo*.

Milk powders are an efficient method of delivering such nutrients to areas of the world where fresh milk is not readily available or cost-efficient. Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

is a well-established method for measuring mineral compositions. However, it is a more difficult and time-consuming process. Non-destructive Energy Dispersive X-ray fluoresence (ED-XRF) also measures mineral compositions, but requires very little preparation of the sample and results are obtained in a matter of minutes. This is an advantage in the analysis of large numbers of samples. The objective of this study was to validate the use of ED-XRF for the determination of minerals in skim milk powders (SMP). One hundred SMP samples were obtained from various sources. Standard values of mineral content and concentration were obtained using ICP-MS. Samples, placed in acid cleaned tubes, were allowed to dry at 60°C for 48 hours. They were treated with 70% HNO_3 and digested on a hotplate. Samples were brought to 5ml total volume with 2% HNO_3, I, S, Ga, Ti, In, and Y. They were analyzed for Al, K, Ca, Fe, Ti, Co, Ni, Sr, Cu, Zn, Mn, Cr, and Ba. Although unnecessary, samples for ED-XRF were formed into pellets of 4 g of powder under 20 tons of pressure. Triplicates were made for each sample. Spectra were recorded using a pin diode detector with a collection time of 92 s, tube current 26 μA, and tube voltage of 15kV. Results of ED-XRF, for the most part, proved to be comparable with those obtained by ICP-MS. While it was found that Pb is not detectable in the samples by ED-XRF, there was very good correlation between most of the elements in both methods (Variance ≤ 1%) ; with Al, Cr, and Cu having the greatest variation among samples and the lowest correlation coefficients. Thus, ED-XRF proved to be an acceptable method for the determination of mineral content and composition of SMP.

Key Words: X-ray fluorescence, Minerals, Skim milk powder

T53 Impact of storage on sensory profiles and volatile components of skim milk powder. R. E. Miracle, A. E. Croissant*, M. A. Lloyd, S. E. Zevchek, and M. A. Drake, *North Carolina State University*, *Raleigh*.

Fresh low heat skim milk powder (SMP) should ideally exhibit a mild and bland flavor reminiscent of fluid skim milk. A shelf life of anywhere between 6-36 months for non-instantized unfortified SMP under optimal storage conditions has been proposed by various sources. Characterizing SMP flavor variability, flavor stability and its role in consumer acceptance is crucial. Further, identification of key compounds that are associated with flavor degradation is important in order to identify rapid instrumental methods for evaluation of SMP flavor quality. Previous studies have evaluated sensory properties concurrently with labor-intensive solvent extraction instrumental approaches. The objective of the current study was to evaluate the efficacy of a more rapid instrumental volatile analysis to assess changes in SMP across storage time. Low heat SMP commercially packaged in 3-ply 22 kg bags were received from six commercial facilities on the west coast of the United States within 3 weeks of production. SMP were stored in the dark at 21C, 40% RH and sampled every 3 mo for sensory and instrumental analysis through 30 mo. SMP were rehydrated at 10% solids (w/w) for analysis. A trained descriptive sensory analysis panel conducted flavor profiling of the SMP. Instrumental volatiles were extracted by solid phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS). Compounds were identified by comparison of retention indices and GC-MS data against reference standards. Selected compounds were quantified by standard addition. Univariate and multivariate statistical analyses were applied to analyze the collected data. The SMP were differentiated by both sensory and instrumental volatile analyses. As SMP aged, cardboard flavor and astringency increased (P<0.05). Concurrently, the concentration of many aldehydes increased while the abundance of maltol decreased (P<0.05). Instrumental analysis differentiated the aged SMP from the fresh control, similar to sensory analysis. SPME of volatile components may provide a rapid way to assess SMP quality.

Key Words: Skim milk powder, Flavor, Storage stability

T54 Evaluation of chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems. A. E. Croissant*[1], L. Dean[2], S. Washburn[1], and M. A. Drake[1], [1]*North Carolina State University*, *Raleigh*, [2]*USDA-ARS*, *Raleigh, NC*.

Consumer interest in the organic and natural food sector is growing. Sales of organic products are projected to continue double-digit growth through 2010. Flavor remains a vital concern for the consumer. Fluid milk composition and flavor variations have been attributed to feed, seasonal variation, and breed. The objectives of this study were to compare chemical properties and consumer perception of fluid milk from cows fed pasture-based (PB) or total mixed ration (TMR) diets. Fluid milk was collected from two herds; one fed on a PB diet and one fed on a TMR diet. Milk from Holstein and Jersey cows was collected separately and milkfat was standardized by breed. Volatile compounds were extracted by solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS). Compounds were identified by comparison of retention indices and GC-MS data against reference standards. Fatty acid profiling was also conducted. A trained descriptive sensory analysis panel documented the flavor profiles of the milks. Triangle tests and acceptance testing were conducted with consumers in separate sessions. Instrumental and sensory analysis differentiated the PB and TMR milks (P<0.05). Concentrations of many volatile compounds were different between PB and TMR milks including 3-methyl indole, indole, toluene, limonene, octanoic and decanoic acids (P<0.05). PB milks contained higher percentages of unsaturated fatty acids, including conjugated linoleic acid (CLA) (P<0.05). Trained panelists documented higher intensities of sweet aromatic and grassy flavors in PB milks compared to TMR milks (P<0.05). Consumers were able to detect differences between pasture-based and TMR milks, and consumer acceptance scores were higher for TMR milk compared to PB milk (P<0.05). These results indicate distinct flavor and compositional differences between TMR and PB milks which are crucial issues to consider and optimize for the growing organic dairy market.

Key Words: Fluid milk, Flavor, Feed

T55 Heat stability of skim milk powder. M. Faka*[1], M. J. Lewis[1], A. S. Grandison[1], and H. Deeth[2], [1]*University of Reading*, *Reading, United Kingdom*, [2]*University of Queensland*, *Brisbane, Qld, Australia*.

The heat stability of skim milk powder depends principally on the stability of casein micelles. This project studies the changes in some factors influencing casein micelle stability during the production of skim milk powder, such as ionic calcium, pH, casein micelle size and ζ-potential. An in - can sterlisation procedure was used to measure heat stability, followed by viscosity measurement. Skim milk powder was considered to be heat stable when the reconstituted milk did not coagulate after sterilisation at 115°C for 15 minutes. Two types of powder were produced from pasteurised skim milk, namely a low-heat and high-heat powder, the difference being in the preheating of skim milk at 92°C for 10 minutes prior to evaporation for high-heat powder. The high-heat powder was heat stable when reconstituted to both 9 and

25% total solids and was found to have a lower ionic calcium, higher ζ-potential and larger casein micelle size in comparison to low-heat powder. The heat stability of low-heat powder was statisfactory when reconstituted to 9% total solids but decreased when total solids increased to 25%. The effect of water quality on these properties and on heat stability was evaluated, using soft water (no calcium) and hard water (250 ppm total hardness). Although water quality affected some of the measured properties, it did not have a marked effect on the heat stability. The heat stability of a low-heat powder was considerably improved by two preocedures which reduced ionic calcium by about 30%, prior to evaporation. This reduction in ionic calcium was accompanied by associated changes in some of the other measured attributes of milk. Hence, the heat stability of skim milk powder could not only be improved by the preheating of milk but also by alterations in the calcium equilibrium in milk.

Key Words: Heat stability, Skim milk powder, Ionic calcium

T56 Quantification of fructooligosaccharides in infant formula. S. Gokavi*, M. S. Alam, and M. Guo, *University of Vermont*, *Burlington*.

Oligosaccharides are the third most abundant solid constituents in human milk in which these are believed to play two major roles i.e., defense agents by acting as receptor analogues to inhibit the binding of enteropathogens to the host cell receptors and bifidogenic factors. Cow's milk is in lack of oligosaccharides and infant formulas made with cow's milk may be deficient in oligosaccharides. It is believed that supplementing infant formula with certain oligosaccharides could improve the nutritional value of formula and help mimic some of physiological functions of mother's milk. The objective of the present study was to quantify fructooligosaccharides in infant formula. Infant formulas, seven from China and three from the United States of America, were collected and analyzed for fructooligosaccharide content using a colorimetric method. The principle of the method is that sucrose, starch and maltosacchrides (if present in the sample) are hydrolyzed to D-glucose by sucrase, amylase and maltase, respectively. Fructooligosaccharides are hydrolyzed to fructose and glucose by fructanase enzyme and the reducing sugars are reacted with p-hydroxybenzoic acid hydrazide resulting in color which is measured at 410 nm using a spectrophotometer. Fructooligosaccharide content of most infant formulas analyzed ranged from 0.10±0.01% to 0.52±0.14% and only one formula had 1.39±0.20%.

Key Words: Infant formula, Fructooligosaccharides, Colorimetry

T57 Effect of individual cow variation and interaction with diet on the content of health-promoting fatty acids in milk fat from dairy cows. C. Tyburczy*, A. L. Lock, J. A. Kelsey, D. G. Peterson, B. A. Corl, and D. E. Bauman, *Cornell University*, *Ithaca, NY*.

Many fatty acids have been associated with a postive effect on biomarkers of coronary heart disease risk, and the enhancement of these fatty acids in milk fat may be desirable to improve the overall healthfulness of dairy products. Health-promoting fatty acids (HPFA) include oleic acid, linoleic acid, linolenic acid, rumenic acid (conjugated linoleic acid), vaccenic acid, eicosapentaenoic acid, and docosahexaenoic acid. The objective of the current analysis was to examine the extent to which individual cow variation, and interaction with diet, affects the milk fat content of HPFA. Data from over 250 cows were collected from previous studies that examined effects of diet and individual phenotypic variation on milk fat composition (J.Dairy Sci. 85:2164; 86:2588; 88:489). Milk fat content of HPFA ranged from 23.3 to 38.3% with individual HPFA varying 3-fold. Milk production variables (days in milk, milk yield, milk fat content, and milk fat yield) were not correlated with total HPFA content ($R^2 < 0.05$ for all variables). Intake of the experimental diets (polyunsaturated oils or extruded soybeans) resulted in a 13 to 46% increase in HPFA content in milk fat, when compared with the respective control diet. In the series of diets examined, the greatest increase resulted from supplementation with 2% soybean oil plus 1% fish oil. Dietary supplementation was effective for maintaining an elevated HPFA content in milk fat over a period of 12 weeks. Individual differences were apparent, and the hierarchy among individuals was maintained even when cows switched between diets. These data suggest that individual variation may offer an opportunity to select for cows naturally producing a high level of HPFA in milk fat. An additional opportunity exists to formulate diets that result in a substantial increase in the HPFA content in milk fat. A potential outcome of producing modified milk is the development of niche markets, but over time it may be possible to more broadly apply these changes, thereby improving the overall healthfulness and public perception of dairy products.

Key Words: Milk fat, Functional foods, Coronary heart disease

T58 Conjugated linoleic acid from butter fat is absorbed and incorporated into tissue lipids to a greater extent than when consumed as a dietary free fatty acid supplement. A. L. Lock*[1], D. E. Bauman[1], and A. M. Salter[2], [1]*Cornell University*, *Ithaca, NY*, [2]*University of Nottingham*, *LEICS., UK*.

Dairy products are the major source of conjugated linoleic acid (CLA) in the human diet, with the *cis*-9, *trans*-11 isomer (rumenic acid, RA) representing 75-90% of total CLA. Dairy products also contain vaccenic acid (VA, *trans*-11 C18:1) which can be converted to RA through via Δ9-desaturase. An alternative source of RA is dietary supplements which are normally available as free fatty acid (FFA) preparations. The aim of the current study was to compare the absorption and tissue deposition of RA when consumed from butter, specifically enriched in RA and VA, with that consumed as a FFA supplement. Golden Syrian Hamsters were fed chow-based diets supplemented with 0.2% cholesterol and containing 20% added fat, for 14 d. This fat consisted of either 20% standard butter (containing 0.4 and 1.4% of total fatty acids as RA and VA, respectively) supplemented with 5 g/kg RA-FFA, or 20% RA/VA-enriched butter (containing 3.6 and 15.4% RA and VA, respectively). The total RA content of the two diets was 5.5 g/kg of diet. Prior to sacrifice, animals were injected with Triton to prevent the breakdown of chylomicrons by lipoprotein lipase. Plasma chylomicrons were isolated and chylomicron, liver and adipose tissue fatty acid composition determined. Compared to the RA-FFA supplement, chylomicrons from animals fed RA/VA-enriched butter contained 74% more RA ($P < 0.001$). RA concentrations were also higher in adipose tissue and liver (104% and 146%, respectively). In a second experiment hamsters were fed RA/VA-enriched butter in the presence or absence of sterculic acid (a known inhibitor of Δ9-desaturase). Inhibition of Δ9-desaturase reduced the accumulation of RA in adipose tissue and liver by 36% and 37%, respectively ($P < 0.001$). Thus tissue concentrations of RA were significantly higher in animals fed equivalent amounts of RA as milk triacylglycerol than as a FFA supplement. This appears to be due to a combination of increased absorption and de novo synthesis through the action of Δ9-desaturase on VA.

Key Words: CLA, Milk fat, Desaturase

T59 Production of the bacteriocin thermophilin 110 in whey-based media. G. A. Somkuti*, S. E. Gilbreth, and D. H. Steinberg, *Eastern Regional Research Center, USDA-ARS*, *Wyndmoor, PA*.

Thermophilin 110 is a bacteriocin of the dairy bacterium *Streptococcus thermophilus* with a high level of activity against pediococci that cause spoilage in wine and beer fermentations. Whey-based media were evaluated to establish optimum conditions for producing thermophilin 110 on a larger scale. Whey permeate solids were reconstituted to 3% lactose concentration, and the effects of yeast extract and the nontoxic organic buffer salts MOPS, MES and PIPES were tested on the growth of *S. thermophilus* ST110, changes in medium pH and thermophilin 110 accumulation over a period of 24 h. The presence of 0.5% yeast extract was essential for thermophilin 110 production which paralleled growth of *S. thermophilus* ST110 at 37°C and reached the highest level after 10-12 h, followed by a gradual decline, as shown by bioassays with *Pediococcus acidilactici* as the target organism. The presence of organic buffer salts decreased the rate of decline in medium pH and generally resulted in increased dry cell mass (µg/ml) and higher levels of thermophilin 110 (units/µg of cells) produced. The greatest effect was shown by the addition of 1% MES to the medium which reduced pH drop to 1.8 units after 10 h (compared to 2.3 pH units in the control), and resulted in a 1.5-fold increase in cell mass (495 µg/ml) and a 7-fold increase in thermophilin 110 production (77 units/µg of dry cells) over the control. Further, the productivity of *S. thermophilus* ST110 after 10 h in the whey permeate medium was 10-fold higher than in conventional tryptone-yeast extract-lactose medium (7.8 thermophilin 110 units/µg of dry cells). The results showed that the use of whey-permeate based media may offer an economical approach to producing larger quantities of thermophilin 110 that could find applications in controlling spoilage pediococci in wine and beer fermentations.

Key Words: Thermophilin 110, *Streptococcus thermophilus*, Whey permeate

T60 Characterization of the indigenous microflora present in commercial Queso Fresco from Mexico. J. A. Renye Jr.*[1], G. A. Somkuti[1], B. Vallejo-Cordoba[2], D. L. Van Hekken[1], and A. F. Gonzalez-Cordova[2], [1]*USDA-ARS-NAA-ERRC*, *Wyndmoor, PA*, [2]*CIAD, A.C.*, *Hermosillo, Sonora, Mexico*.

Queso Fresco is the most popular variety of Hispanic-style cheese consumed in the United States and Mexico. It is traditionally made from raw milk without specific starter cultures and the functional and flavor properties of the cheese are determined by the native microflora present in the raw milk. In the U.S., Queso Fresco is made from pasteurized milk with non-specific starter cultures resulting in a product with different organoleptic qualities from the traditional cheese. In this ARS-CIAD study, the indigenous microflora from six commercial Queso Fresco cheeses, obtained from Sonora, Mexico, were analyzed to identify bacterial species which may function as specific starter cultures. Four of the cheeses analyzed were made from raw milk and two from pasteurized milk. Classification of bacterial species was based on growth on selective media and 16S rDNA sequencing. The highest colony counts were obtained on M17 and MRS agar used for the selection of streptococci and lactobacilli respectively. Growth on MRS agar supplemented with vancomycin suggested that *Leuconostoc* species were present in all six samples. A high number of coliforms, enterococci and coagulase-positive staphylococci were identified in all raw milk samples. The number of enterococci remained high in cheese samples made from pasteurized milk but a 2 $\log_{10}$ reduction of coliforms was observed. Coagulase-positive staphylococci were reduced by 3 $\log_{10}$ in one pasteurized milk sample but remained high in the other (log 6.26 CFU/g). 16S rDNA sequence analysis revealed that lactobacilli were not present in any sample. *Lactococcus lactis* ssp. *lactis* was identified in four samples and *Leuconostoc mesenteriodes* and *Entercoccus faecium* were identified in all six samples. The results suggest that these species need further study to explore their potential as starter cultures for the production of cheese made from pasteurized milk while preserving the organoleptic qualities of traditional Queso Fresco.

Key Words: Queso Fresco, Starter culture, Raw milk

T61 Production of potassium acetate from cheese whey using immobilized cell fermentation. M. Alam*, J. Li, and M. Guo, *University of Vermont*, *Burlington*.

Conventional deicing salts such as sodium chloride and calcium chloride are causing serious corrosion and major environmental problems. The objective of this study was to explore the use of immobilized cell technology in the production of environment-friendly substitute deicer potassium acetate (PA) from cheese whey. A fibrous-bed bioreactor was constructed using a jacketed glass column (5-cm I.D., 1-L capacity) packed with fibrous matrix (cotton cloth) and connected to a 5-L stirred-tank fermentor through a recirculation line. The fermentation was carried out anaerobically with agitation (100 rpm) after single inoculation of lactate/acetate tolerant *Lactococcus lactis* (10%) and *Clostridium formicoaceticum* (10%) into a nutrient-supplemented whey permeate (5%) in the 5-L vessel and maintained the temperature at 37°C and pH at 7.5 by adding 5 M potassium hydroxide. The circulation started through the fibrous-bed bioreactor after 40 h of fermentation in the vessel and continued for another 40-50 h. Aliquots (10 ml) of the fermentation broth were collected periodically and analyzed by HPLC. The PA concentration in the broth was 3.2-5.0%. Application of encapsulated cultures in the production of PA was also investigated. *L. lactis* and *C. formicoaceticum* were independently immobilized in sodium alginate beads after harvesting the bacterial cells by centrifugation (1,000 X g) at 10°C for 15 min. Batch fermentation of whey permeate using a coculture of the alginate-encapsulated cells produced 2.9-4.8% of PA in the fermentation broth. In comparison, when using free-cell cocultured batch fermentation, the PA production ranged from 2.6 to 3.4% after 60-72 h, and increased to 2.9 to 4.1% with nutrient supplementation. Results of this study suggest that fibrous-bed cell immobilization technology has potential to produce high yield acetate from cheese whey. Successful development and application of the alternative deicer for road maintenance will be beneficial to the dairy industry, transportation systems, and the environment.

Key Words: Potassium acetate, Cheese whey, Immobilized cell fermentation

T62 Effect of *Lactobacillus spp.* and whey protein isolates on Intracellular glutathione and antioxidative activities. J. R. Byun and Y. H. Yoon*, *Chung-Ang University*, *Ansung-Si, Kyunggi-Do, S. Korea*.

Bovine whey protein is rich in cysteine, which is the rate limiting amino acid for synthesis of the antioxidant glutathione(GSH). Some strains of *Lactobacillus spp.* have been reported to contain high levels of GSH in cell extracts. This study is an attempt to find out the effect of dietary whey protein isolates and probiotic lactic acid bacteria on

the intracellular glutathione concentration and antioxidative activities. Treatment of RWPE-1 cells and PC-3M-MM2 cells with hydrolyzed WPI for 48h at concentration of 500µg/ml elevated glutathione sulphydryl (GSH) by 28.2% and 38.4%, respectively compared with control cells receiving no hydrolyzed WPI($P<0.05$). Supplementation with *Lactobacillus casei* HY2782 cell extracts elevated intracellular GSH in both types prostate cells and the degree of elevation was dependent on the type of cells PC-3M-MM2 cell revealed significant elevation($P<0.05$) and RWPE-1 cell revealed an elevation with no statistical significance. The effects of hydrolyzed WPI and *Lactobacillus casei* cell extract on the oxidant induced cell death and DNA damage was Oxidant t-butyl hydroperoxide (TBHP) treatment of PC-3M-MM2 cells with 500µM induced viability by 62.0%. Pretreatment of PC-3M-MM2 cells with buthionine sulfoximine(BSO) prior to the TBHP reduced the viability by 33.7%. The effect of *Lactobacillus casei* cell extract on the oxidant induced cell death was Oxidant t-butyl hydroperoxide (TBHP) treatment of PC-3M-MM2 cells with 500µM induced viability by 33.7%. The effect of *Lactobacillus casei* cell extract on the oxidant induced cell death was Oxidant t-butyl hydroperoxide (TBHP) treatment of PC-3M-MM2 cells with 500 µM induced viability by 82.4%. The effect of *Lactobacillus casei* cell extract on the oxidant induced cell death was pretreatment of PC-3M-MM2 cell with *Lactobacillus casei* cell extract elevated cell viability by 82.4%, a similar degree of protective effect with that of hydrolyzed whey protein isolates against oxidant induced cell death has been revealed.

Key Words: Gluatathione, Whey protein isolates, *Lactobacillus casei*

T63 Characterization of a two-component regulatory system implicated in the bile tolerance of *Lactobacillus acidophilus* NCFM. E. A. Pfeiler*[1], M. A. Azcarate-Peril[1,2], and T. R. Klaenhammer[1,2], [1]*North Carolina State University*, *Raleigh*, [2]*Southeast Dairy Foods Research Center*, *Raleigh, NC*.

Lactobacillus acidophilus NCFM is used industrially as a probiotic culture in yogurt formulation and dietary adjuncts. Tolerance to bile is one characteristic necessary for microbial survival and competition within the intestinal environment. Microarray analysis has shown that several genes are induced upon exposure of NCFM to bile. Among them, the genes in a putative operon (LBA1427 to LBA1432) composed of a two-component regulatory system (2CRS) and four poorly characterized genes, were significantly ($P < 0.01$) induced by bile. Inactivation of the LBA1430 (a predicted histidine kinase, HPK) was accomplished by targeted plasmid insertion via homologous recombination and replacement with a deleted version of the gene. The growth rates of the HPK mutant and a control strain were compared in MRS supplemented with 0%, 0.3%, 0.5% and 1.0% Oxgall. The growth rate for the HPK mutant was reduced significantly in the presence of 0.3% and 0.5% Oxgall. The HPK mutant also showed decreased survival on MRS agar plates as the bile concentration increased from 0.7 to 1%. Reverse transcriptase PCR on intergenic regions confirmed that LBA 1427-1432 are co-transcribed. Mutation of the HPK gene eliminated the co-induction of these genes by Oxgall. The results indicate that *L. acidophilus* relies on this two component regulatory system to regulate the expression of genes that contribute to bile tolerance.

Key Words: *Lactobacillus acidophilus*, Bile, Two-component regulatory system

T64 Characterization of a Gal$^+$ *Streptococcus thermophilus* MR-1C recombinant strain. G. Robitaille*[1], S. Moineau[2], D. St-Gelais[1], C. Vadeboncoeur[2], and M. Britten[1], [1]*Food Research and Development Centre, Agriculture and Agri-Food Canada*, *Saint Hyacinthe, Quebec, Canada*, [2]*Laval University*, *Quebec City, Quebec, Canada*.

Streptococcus thermophilus (St) is a lactic acid bacterium extensively used as starter culture in combination with lactobacilli in fermented foods. Capsule-producing strains, secreting expolysaccharides that stay attached to the cell wall, are likely to improve moisture retention, yield, and melting properties of Mozzarella cheese without affecting whey viscosity. However, like the vast majority of strains, the capsule-producing *St* MR-1C is galactose negative. This metabolic property results from the inability of *St* to synthesize galactokinase, an enzyme of the Leloir pathway. Galactose, generated by lactose hydrolysis, is unwanted as it causes excessive browning of Mozzarella cheese during baking and promotes growth of non starter lactic acid bacteria. The aim of this work was to study the fermentation properties of a galactose positive (Gal$^+$) MR-1C recombinant strain. MR-1C was transformed by electroporation with the plasmid pTKRL2TK that carries a functional galactokinase gene from a phylogenetically related strain specie *Streptococcus salivarius*. Unlike MR-1C, the recombinant strain named MR-AAC grew well on M17 broth supplemented with galactose as sole source of carbon. The amount of lactose consumed by MR-1C and MR-AAC during a fermentation process carried out at 40°C in reconstituted skim milk (10%) as well as the acidification rates were similar. The lactic acid/galactose molar ratio for MR-1C and MR-AAC were 1.96 and 2.05, respectively, indicating that both strains excreted the same amount of galactose. In M17 broth containing 0.5% lactose, MR-AAC first expelled galactose faster than it could be metabolized but, unlike MR-1C, excretion rate rapidly decreased as soon as lactose content decreased. These results demonstrate that the transformation of MR-1C with pTKRL2TK conferred a Gal$^+$ phenotype did not prevent galactose excretion during growth in high lactose-containing media such as milk.

Key Words: *Streptococcus thermophilus*, Galactose metabolism, Starter culture

T65 Impacts of Gal$^+$ phenotype on the capsule production by *Streptococcus thermophilus* MR-1C recombinant strain. G. Robitaille*[1], S. Moineau[2], D. St-Gelais[1], C. Vadeboncoeur[2], and M. Britten[1], [1]*Food Research and Development Centre, Agriculture and Agri-Food Canada*, *Saint-Hyacinthe, Quebec, Canada*, [2]*Laval University*, *Quebec city, Quebec, Canada*.

Streptococcus thermophilus (St) is a lactic acid bacterium extensively used as starter culture for the production of fermented dairy products. The strain MR-1C secretes heteroexpolysaccharides that stay attached to the cell wall (CPS) forming a capsule of about 3 µm in thickness. CPS can improve moisture retention, yield and melting properties of the Mozzarella cheese without affecting whey viscosity. Like most *St* strains, MR-1C is unable to metabolize galactose. The aim of this work was to determine (1) how the synthesis of CPS was affected in a galactose-positive *St* strain derived from MR-1C, called MR-AAC, and (2) how this new metabolic trait affected the rheological properties of fermented milk. Reconstituted skim milk (RSM-10%) and M17 broth supplemented with lactose (LM17) were inoculated with MR-1C and MR-AAC and small-static fermentations were carried out at 40°C. To quantify the CPS produced by MR-1C and MR-AAC, an in situ lectin binding assay using fluorescently-labeled peanut agglutinin was developed. The CPS content of the capsule gradually increased

during the exponential growth in both RSM-10% and in LM17. CPS continued to accumulate during the stationary growth phase up to 18 hours of fermentation indicating that CPS production by *St* MR-1C and MR-AAC was not growth dependent. Between the 5th and 18th hour of fermentation, the CPS content per cell increased by a factor of 1.5 and 3 for LM17 and milk, respectively. MR-AAC and MR-1C showed a similar CPS accumulation pattern during fermentation. Milk viscosity at pH 5.3 and rheology of the curd produced with the two strains were also similar. In conclusion, ability to metabolize galactose did not result in a greater CPS production.

Key Words: *Streptococcus thermophilus*, Galactose metabolism, Capsular exopolysaccharide

T66 Pediocin production by *Pediococcus acidilactici* in co-culture with yogurt starter bacteria. G. A. Somkuti* and D. H. Steinberg, *Eastern Regional Research Center, USDA-ARS*, *Wyndmoor, PA*.

The direct production of the antilisterial bacteriocin pediocin in milk is not feasible since *Pediococcus acidilactici* poorly ferments lactose and prefers glucose for growth. Solutions to this problem have included the transfer of a lactose operon to pediococci from lactic bacteria and prehydrolysis of lactose with a suitable β-galactosidase. In a different approach described here, the production of pediocin by *P. acidilactici* (PAF) was evaluated in co-culture with the yogurt starter cultures *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LDB), and the cheese starter culture *Lactococcus lactis* subsp. *lactis* (LLL). Maintenance medium for PAF and LDB was MRS broth, whereas ST and LLL were grown in tryptone-yeast extract-lactose broth. The cultures (50 µl each) were inoculated into milk (2% fat content) samples (10 ml) that were incubated at 37°C for up to 8 h. PAF was tested alone and in combination with ST, LDB and LLL. Samples were taken every 60 min, centrifuged for 10 min and following two-fold serial dilution, cell-free supernatants were tested for bacteriocin activity with *Listeria innocua* as the target organism. Pediocin production was not detectable when PAF was used alone or grown in co-culture with LLL. However, pediocin was apparently produced when PAF was co-cultured with either ST or LDB or in combination with the two yogurt starter cultures, and reached a maximum level estimated at 2,400 units/ml after 6 h of incubation. Since pediococci have been suggested as adjunct dairy starter cultures, the inclusion of pediocin-producing strains in starter mixtures consisting of ST and LDB strains may provide the additional benefit of serving as bioprotective agents to control listerial contamination in fermented dairy products.

Key Words: Pediocin, Lactis starter cultures, *Pediococcus acidilactici*

T67 Selective enumeration of different strains of *Lactobacullus acidophilus* in goat's milk yogurt beverage. S. Li*, S. Gokavi, and M. Guo, *University of Vermont*, *Burlington*.

An important parameter in monitoring viable probiotic cultures in a product is the ability to enumerate probiotic bacteria individually. Differential enumeration of probiotic bacteria is difficult due to the presence of several types of similar microbes in one product. In order to select a strain of *L. acidophilus* that can survive refrigerated conditions during storage of goat's milk yogurt beverage, three strains (ATCC-11975, LA-5 and NCFM) were chosen. To assess their viability, it was important to have a working method suitable for all the three strains. Twelve bacteriological media were evaluated to assess their suitability to selectively enumerate ATCC-11975, LA-5 and NCFM which were used in combination with yogurt cultures (*S. thermophilus* and *L. delbrueckii ssp. bulgaricus* isolated from YC350 obtained from Chr. Hansen). Bacteriological media evaluated included *S. thermophilus* agar, pH modified MRS agar, MRS bile agar and sugar-based (maltose and sorbitol). Incubations were carried out under aerobic and anaerobic conditions at 37°C for 24, 48 and 72 h. M-17 agar with 0.5% lactose and aerobic incubation at 37°C for 24 h were suitable for *S. thermophilus*. The enumeration of *L. delbrueckii ssp. bulgaricus* can be done using MRS agar (pH 5.2) and anaerobic incubation at 37°C for 24 h. MRS agar with 0.2% bile and anaerobic incubation at 37°C for 72 h was found suitable for all the three strains of *L. acidophilus* used in the study.

Key Words: *L. acidphilus*, Enumeration, Goat's milk yogurt beverage

T68 Evaluation of adherence of *Bifidobacterium* and *Lactobacillus* strains to cell membranes by blot analysis and optical tweezers. C. Iñiguez*[2,1], J. Sharpe[1], E. Acedo-Félix[2], and R. Jiménez-Flores[1], [1]*California Polytechnic State University*, *San Luis Obispo*, [2]*Centro de investigación en Alimentación y Desarrollo*, *Hermosillo, Sonora, Mexico*.

Bacterial adhesion to intestinal mucosa is one of the most important criteria for selection of any potentially probiotic strain. This adhesion is mediated by specific molecules present on the bacterial cell surface. Little information is available on the effect that milk fat globule membrane (MFGM) components have on the adhesion of probiotic bacteria. We have combined two techniques, dot blot analysis and laser tweezers to characterize the binding properties of several strains of *Bifidobacterium* and *Lactobacillus*. Dot blot analysis has been proven to be helpful in the determination of the affinity of bacteria to specific components in milk or the surface of bacteria. Optical tweezers have many applications in measuring biological forces due to their ability to exert piconewton scale force and to manipulate biological material with minimal damage. The optical trap consisted of a laser near infrared region Ti:saaph at 1064 nm, that was strongly focused through a lens with a very short focal length (it was used a numerical aperture microscope objective =1.25). To perform an adhesion evaluation, a bacterium was optically trapped and brought in contact with a 10 µm diameter polystyrene microsphere coated with intestinal mucosa or MFGM by passive adsorption technique. Various calibration procedures were implemented in order to provide absolute force measurements. These measuremnts were compared to the relative intensities of binding obtained by dot blot assays. In this method, the intensity of binding is proportional to the color intensity developed in a dot blot assay from biotinilated bacteria adhering to a specific concentration of intestinal mucosa or MFGM immobilized in a membrane. This data will demonstrate the efficacy of this technique of adhesion force measuring and this research will contribute to knowledge of optical tweezers and their applications to investigations of living biological systems.

Key Words: Probiotic bacteria, Laser tweezers, Adhesion

T69 Probiotic weight loss yogurt. M. Guillory and K. J. Aryana*, *Louisiana State University Agricultural Center*, *Baton Rouge*.

Probiotics microorganism exert health beneficial effects on the gasterointestinal tract by improving the balance of intestinal microflora. A novel ingredient namely "Super Citrimax" is being marketed as a weight loss ingredient (WLI). The objective was to study the effect of various amounts of this weight loss ingredient on the physico-chemical,

microbiological and sensory characteristics of fat free probiotic yougurt over its shelf life. Super Citrimax incorporated into the yogurt mixes at the rate of 0, 1.5, 3.0 and 4.5 g per 228 g of yogurt. *Lactobacillus acidophilus* was incorporated at the rate of 0.054% v/v into all the homogenized, pasteurized mixes cooled to 104°F containing yogurt culture. Fat free plain yogurt manufacture was replicated three times. The WLI*storage-time interaction effect was significant for viscosity, body and texture, L* and b*. The a* values were significantly the highest for yogurts with 4.5g followed by yogurts with 3.0 g of the WLI. The a* values were the least for the yogurts with 1.5 g of WLI and the control which were not significantly different from each other. There were no differences in flavor scores between the control and the yogurts containing 1.5 g which were significantly higher than the yogurts containing 3.0 and 4.5 g WLI. Control yogurts had significantly the most syneresis followed by the yogurts with 1.5g of the WLI. The yogurts with the 3.0 and 4.5 g of WLI had significantly the least amount of syneresis. The WLI did not affect lactobacilli counts, appearance and color scores and yogurt pH, these three attributes steadily declined over yogurt shelf life. The optimum amount of WLI to be used in probiotic yogurt manufacture would be 1.5 g per 228 g yogurt.

Key Words: Probiotic, Weight loss, Fermented

T70 Health beneficial bacterial influence on the characteristics of fat free plain set yogurt. M. R. Faciane and K. J. Aryana*, *Louisiana State University Agricultural Center*, *Baton Rouge*.

Health benefits of *Lactobacillus acidophilus* include providing immune support for infections or cancer, providing a healthy replacement of good bacteria in the intestinal tract following antibiotic therapy, reducing occurrence of diarrhea in humans, aiding in lowering cholesterol and improving the symptoms of lactose intolerance. The objective was to study the effect of a slightly gradual increment in incorporation of *L. acidophilus* in yogurt manufacture on the physico-chemical, microbiological and sensory characteristics of yogurt over its shelf life. Yogurts were manufactured using standard procedure *L. acidophilus* was incorporated after yogurt culture addition at the rate of 0, 0.024, 0.048 and 0.144 % w/w yogurt mix. Product manufacture was replicated three times. The treatment * time interaction effect and the treatment effect were not significant for all characteristics. The incorporation of slightly increasing amounts of *L. acidophilus* did not influence viscosity, flavor, appearance, body and texture, L*,a*,b* syneresis and pH. The time effect was significant for some characteristics. Flavor scores were significantly the highest for week 0, lower for weeks 1 and 3 and significantly the lowest for week 5. Appearance scores were significantly the highest for week 0. Yogurts had best body and texture at weeks 3 and 5. Product pH at weeks 0 and 1 were not different yet significantly higher compared to weeks 3 and 5. *L. acidophilus* can be incorporated at a rate as high as 0.144% w/w without altering yogurt characteristics.

Key Words: Fermented, Health, *Lactobacillus acidophilus*

T71 A novel yogurt manufactured with probiotic bacteria at various levels. S. Ganesh* and K. J. Aryana, *Louisiana State University Agricultural Center*, *Baton Rouge*.

There are various types of yogurts namely, set curd, stir curd, drinking yogurt. *Lactobacillus acidophilus* has several health benefits. The objective was to study the dose effect of *L. acidophilus* on the physical and microbiological characteristics of a novel type of yogurt. A novel yogurt was manufactured and details of its manufacture will be discussed. *L. acidophilus* was incorporated in the yogurts at the rate of 0, 1, 10 and 100 g of frozen concentrate per gallon of final product. *L. acidophilus* counts, lactobacilli counts with MRS agar, coliform counts and yeast and mold counts were enumerated. Texture of the novel yogurt was determined by Texture Profile Analysis using an Instron. Product manufacture was replicated three times. The *L. acidophilus* counts in the novel yogurt were significantly lower for 1g compared to 10 and 100g incorporation. There were no differences in *L. acidophilus* counts of the product containing 10 and 100 g *L. acidophilus*. Hardness of the product with 100 g *L. acidophilus* was significantly lower than the product with 10 g. Coliform counts were less than 10 in all treatments. Different doses of *L. acidophilus* did not affect lactobacilli counts, yeast and mold counts, springiness, cohesiveness, gumminess, chewiness and adhesiveness of the product. Data indicates that the optimum level of *L. acidophilus* incorporation in the product is 10 g / gallon.

Key Words: Yogurt, Probiotic, Health

T72 Effect of *Lactobacillus acidophilus* inoculation level on yogurt properties during storage. D. W. Olson* and K. J. Aryana, *Louisiana State University Agricultural Center*, *Baton Rouge*.

The effect of *Lactobacillus acidophilus* inoculation level on the Lactobacilli counts, sensory (flavor, body and texture, and appearance) scores, amount of syneresis, color (L*, a*, and b* values), and pH of the resulting yogurt was determined. Plain yogurt was manufactured with skim milk, nonfat dry milk, yogurt cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus*), and with or without *L. acidophilus* (0 (control), 0.0239, 0.238, or 2.33% (w/w)). After homogenization at 10.3 MPa first stage and 3.45 MPa second stage and batch pasteurization at 85°C for 30 min, yogurt mixes were incubated at 40°C in 355 mL cups to pH 4.5 and then cooled to 4°C. Yogurt samples containing 2.33% *L. acidophilus* typically took about an additional 3 h to reach pH 4.5 compared to the control yogurt. Lactobacilli counts with MRS agar, amount of syneresis, and pH were determined at 0, 1, 2, 4, 6, and 8 wk of storage, and color measurements and sensory evaluations were performed at 0, 1, 2, 4, and 6 wk. Yogurts containing 2.33% *L. acidophilus* generally had significantly lower sensory scores, more syneresis, lower Lactobacilli counts, lower L* values, and higher a* and b* values than the remaining yogurts. The flavor scores and the a* values at 0 wk were significantly higher than the corresponding values for the remaining storage times, and the pH decreased from 0 wk to either 4 or 6 wk for each type of yogurt. The body and texture and appearance scores, amount of syneresis, Lactobacilli counts, and L* and b* values were not significantly affected by aging. High inoculated levels of *L. acidophilus* (2.33%) prolonged the yogurt incubation time and resulted in an inferior quality yogurt during storage.

Key Words: *Lactobacillus acidophilus*, Sensory, Yogurt