Organic (non-salt) trace minerals have been reported to have positive effects on reproduction, immunity, overall productivity, and integrity of hair, skin and hooves. Organic trace minerals are widely used in the dairy, swine, and poultry industries by feeding consultants, and by large animal enterprises. Seemingly, these entities would only use these minerals if economic value was obtained. Unfortunately, there is not a lot of comparative scientific data to support these suggestions. In general, organic trace minerals (depending on source) are more bioavailable than inorganic minerals. This response is based primarily (but not always) on tissue mineral concentrations. However, the higher bioavailability of organic trace minerals would not seem to account for some of their proposed benefits. Examples of results among different species for the trace minerals Zn, Mn, Cu, Se, Cr, and I will be discussed. For example, organic Zn fed during lactation increased number of pigs born alive and improved immune status of the pigs, but data are limited. Organic Mn and Cu are usually more bioavailable, based on slope ratio technique for tissue concentrations, than inorganic Mn and Cu (usually the sulfate form), but there is very little comparative scientific data to assess the proposed benefits of organic Mn and Cu supplemented as the only organic mineral in the diet. Organic Se supplementation results in greater tissue concentrations of Se, but inorganic forms have equal or greater efficacy in affecting GPX activity. In broilers previously fed organic Se compared with those fed inorganic Se, tissue Se concentrations and GPX activity were greater when the broilers were subsequently fed a Se deficient diet. Organic Cr supplementation decreases plasma glucose levels, increases glucose clearance rate, and improves numbers of pigs born - these responses are relatively consistent. Excess iodine intake of dams at levels well below those considered toxic may have negative effects on the offspring. Where applicable, how these results may apply to companion animals and the selection of trace mineral form will be highlighted.

Key Words: Organic Trace Minerals

Dairy Foods: Chemistry and Microbiology

571 Protein interactions in heat-treated milk and effect on rennet coagulation. P. Kethireddipalli* and D. G. Dalgleish, *University of Guelph*, *Guelph, ON, Canada*.

The underlying molecular processes that cause impaired rennet clotting of heat-treated bovine milk were investigated. Firstly, the effect of whey protein(WP)/ κ-casein complexes bound to the casein micelle, on the elastic modulus (G') and gelation times (T) of renneted heat-treated milk was examined. Milks with different levels of micelle-bound WP (<5% to ~80%) were produced by heating skim milk at 90°C for 10 min at pH values ranging from 6.3 to 7.1. WP was quantified using SDS-PAGE. Lower pH produced higher micellar WP association and vice versa. Using oscillatory rheometry, we found that compared to unheated milk (G', 83.5 Pa; T, 10 min) all heat-treated milks, after renneting, showed a remarkable reduction in G' (0.1 to 2.1 Pa) and a large increase in T (55 to 130 min). It did not seem to matter if the WP/ κ-casein complexes were predominantly bound to the casein micelle (pH 6.3) or were largely present as soluble protein complexes in the lactoserum (pH 7.1). In the second part, the individual effects of casein micelles and lactoserum on the rennet gelation properties of heated milk were investigated. Two different milk systems were examined; one was prepared by re-suspending casein micelles from milk heated at pH values 6.3, 6.7, or 7.1 in native serum from unheated milk, and the other contained native micelles from unheated milk in the serum from the various pH- and heat-treated milks. Heat- and pH-modified casein micelles suspended in normal serum significantly lowered the G' values of the resulting rennet gels. With the exception of pH 6.3, the heated lactoserum also interfered with gelation of heat-treated milks. In the final part of the study, the serum from heat-treated milks was further examined after its ultrafiltration (removes WP/ κ-casein complexes) or its dialysis against unheated milk (restores ionic composition). Both these processes significantly improved serum performance. This clearly demonstrated that not only serum ionic factors, but also serum WP/ κ-casein complexes, and heat-modified casein micelles (with or without associated WP) significantly interfere with the rennet gelation of heat-treated milks.

Key Words: Heat-treated Milk, Rennet, WP/κ-casein Complexes

572 Flavor variability and stability of US-produced whole milk powder. M. A. Lloyd* and M. A. Drake, *North Carolina State University*, *Raleigh*.

Whole milk powder (WMP) produced in the United States (U.S.) is used both domestically and internationally. Much of the available literature on WMP was generated using internationally produced WMP. Flavor variability and stability of US-produced WMP has not been characterized. The objectives of this study were to characterize flavor and flavor variability of domestic WMP. Freshly produced WMP was collected from 4 production facilities at 5 timepoints over a year period. At each timepoint, two 23-kg bags from different production runs were collected. Each sample was analyzed initially and every 2 months for flavor profile, volatiles, color, water activity, and moisture over a year of storage. Samples were reconstituted to 10% solids using deodorized water for descriptive and volatile analysis. Volatile analysis was performed using solid phase microextraction (SPME) followed by gas chromatography/mass-spectrometry. Relative abundance was calculated for the following compounds, based on the internal standard recovery (2-methyl-3-heptanone): toluene, hexanal, 2-heptanone, heptanal, octanal, 2-nonanone, nonanal, 2-undecanone, delta-decalactone, and delta-dodecalactone. Descriptive analysis was conducted using a 10-member trained panel. All WMP were between 2-3% moisture and 0.11-0.25 water activity initially. WMP varied in flavor and volatile composition within and between production facilities ($p<0.05$). WMP had distinct flavor profiles initially, with varying levels of cooked, milkfat, and sweet aromatic notes ($p<0.05$). Several samples also had feed flavors. During storage, grassy and painty flavors developed while sweet aromatic flavor intensities decreased ($p<0.05$). Some WMP developed grassy or painty flavors as early as 4 months, and all samples developed painty flavor by 12 months. Painty and grassy flavors were confirmed by increased levels of lipid oxidation products such as hexanal, heptanal, and octanal ($p<0.05$). There is wide variation in flavor and flavor stability of U.S. WMP. Further research should be done to determine specific factors that can be controlled to optimize flavor and flavor stability.

Key Words: Whole Milk Powder, Flavor, Stability

573 The effect of pH and ionic calcium on the heat stability of sterilized and UHT milk. M. J. Lewis* and A. S. Grandison, *School of Chemistry, Food and Pharmacy, The University of Reading*, *Reading, Berkshire, UK*.

It has long been recognized that milk salts, in particular divalent cations and pH, are important factors in determining milk processing behavior, particularly in influencing the stability of the protein in aggregation, gelation and precipitation reactions, and in the fouling of heat exchanger surfaces with mineralized deposits. However, practical information supporting this is not so readily available and the much reported heat coagulation time is not easy to perform nor widely used in the dairy industry as a quality assurance indicator of heat stability. This paper investigates the role of pH and ionic calcium on heat stability issues during UHT processing and in-container sterilization of milk. It has been found that problems encountered at UHT conditions relate primarily to sediment formation and fouling, whereas those encountered during in-container sterilization relate to gelation and thickening, although a lesser amount of sediment is also formed. Thus it is hypothesized that different mechanisms are involved in the two processes. This is being further investigated by manipulating pH and ionic calcium in milk by methods such as calcium-removal, calcium fortification and addition of phosphate and citrate stabilizers. The changes in pH and ionic calcium brought about by these processes not only affect heat stability, but also influence Maillard reactions, especially during in-container sterilization, which might be detrimental to product quality. The aim is to provide some practical guidelines that can be used to predict the intrinsic stability of casein micelles to high temperature sterilization.

Key Words: Ionic Calcium, pH, Heat Stability

574 Isolation, composition and rennet-gelling functionality of milk fat globule membrane fractions from regular buttermilk, whey buttermilk, and washed cream buttermilk. B. Manion* and M. Corredig, *University of Guelph*, *Guelph, Ontario, Canada*.

There has been increasing evidence of the health benefits associated with the consumption of some components of the milk fat globule membrane (MFGM) (i.e. phospholipids and immunoproteins). The objective of this work was to characterize the composition and processing functionality of MFGM-rich fractions. Three different fractions were prepared from industrially-upscalable processes, namely, microfiltration of buttermilk (MFGM), concentration of whey buttermilk (WR) and buttermilk derived from washed cream (WBM). MFGM was prepared by concentration and diafiltration through 1.4 µm ceramic membrane of fresh buttermilk with 2% sodium citrate. WR was obtained by concentration of fresh buttermilk from whey cream through 0.1 µm PVDF membranes. WBM was prepared from cream reconstituted back to the original volume with water and recentrifuged, and then churned to obtain the serum fraction. Proximate analysis, electrophoresis, and phospholipids analysis by HPLC were used to characterize the composition of each fraction. The amount of MFGM-derived material was MFGM>WR>WBM (from high to low), in both proteins and phospholipids, and the MFGM fraction contained the lowest level of caseins. Soy oil (10%) emulsions were prepared with 3% WR and WBM, 0.5% Tween 20 and 3% MFGM with various levels of Tween added. The renneting behavior of recombined milk (to 3.3% protein from skim milk powder) with 4% oil was tested. Milk containing MFGM-emulsions created the stiffest gels and showed the earliest onset of gelation compared to the other recombined milks. On the other hand, milk with oil droplets covered with WR-stabilized emulsions formed gels with low elastic modulus and showed longer gelation times than the other treatments. These results demonstrated that differences in the composition of the MFGM material at the interface affect the renneting behavior of recombined milk.

Key Words: Milk Fat Globule Membane MFGM, Buttermilk, Rennet Gelation

575 Fat globule interfacial composition affects the texture and microstructure of rennet-induced casein gels. Z. Gaygadzhiev*, M. Alexander, A. Hill, and M. Corredig, *University of Guelph*, *Guelph, ON, Canada*.

Model systems, containing both fat globules and casein micelles, were prepared to study the interactions occurring between filler particles and protein networks in rennet-induced casein gels. Anhydrous milk fat was emulsified in solutions of sodium caseinate (NaCas) or whey protein isolate (WPI). Pre-gelation stages of rennet coagulation were observed using Transmission Diffusing Wave Spectroscopy (DWS). Gelation was studied using small deformation rheological measurements and gel microstructure was characterized with laser confocal microscopy. Systems containing WPI-stabilized fat globules reached the gelling point, as determined by the cross-over of G'/G'', earlier than those containing no fat globules (control), although the values of final storage moduli were of a similar magnitude for both systems. The light scattering experiments also revealed that the changes in the DWS parameters (1/l*, apparent hydrodynamic radius, mean square displacement slope) occurred earlier for systems containing WPI emulsified fat globules In contrast, emulsification of fat globules with NaCas greatly retarded the gelation process and inhibited aggregation of casein micelles.

Key Words: Modified Milk Fat Globule Membrane, Diffusing Wave Spectroscopy, Rheology

576 Acoustical emissions generated by *E. coli* bacteria. C. L. Hicks*[1], J. M. Stencel[2], H. Song[2], and F. A. Payne[1], [1]*University of Kentucky*, *Lexington*, [2]*Tribo Flow Separations*, *Lexington, KY*.

Escherichia coli 15q and 15cc bacteria in TSB medium, at 32°C for greater than 5 h was monitored using contact acoustic sensors (20 to 50 kHz and 50 to 200 kHz) attached to the sides of the growth vessel. Acoustical emissions generated by the bacteria was picked up as a waveform and each waveform was referred to as a 'Hit'. Hits were analyzed for rate of accumulation and periodic cycles. Fast Fourier transform analysis was used to calculate average peak frequency emissions. Initial analysis showed that Hit detection from the 20 to 50kHz sensor began within 5 min after the medium was inoculated with *E. coli* 15cc. Hit detection from the 50 to 200 kHz sensor became more apparent as the organism entered the log phase and displayed a linear natural log increase in Hits during the log growth phase. Periodic cycles of 1.66 sec and 33.6 sec were observed during the early stages of growth suggesting that *E. coli* 15cc was involved in uniform sequenced activities, possibly quorum sensing. The average peak frequencies data showed shifting in frequencies as the bacteria

moved from the lag, log, and stationary phases. Differences between *E. coli* 15q and 15cc could be observed within the first 60 min of incubation (20 to 50 kHz sensor) with 15q producing 7average peak domains (frequencies) and 15cc producing only 5. After 5 h of incubation 15q produced only one broad peak domain while 15cc generated one defined domain and two smaller domains. Average peak frequencies for *E. coli* 15cc and 15q were sufficiently different in frequency and intensity during the lag, log and stationary phases that specific strain identification might be possible. Thus, acoustic emissions from bacteria may be unique enough to acoustically fingerprint bacteria and result in a rapid assay method.

Key Words: Acoustic, Bacteria, Sensing

577 An assay system for probiotic lactic acid bacteria recognizing human blood type A-antigen that competitively excludes harmful intestinal bacteria. T. Saito*[1], N. Wakahara[1], H. Uchida[1], H. Kinoshita[1], Y. Kawai[1], H. Kitazawa[1], K. Miura[2], A. Horii[2], K. Kimura[3], and N. Taketomo[3], [1]*Graduate School of Agricultural Science, Tohoku University*, *Sendai, Miyagi, Japan*, [2]*Graduate School of Medicine, Tohoku University*, *Sendai, Miyagi, Japan*, [3]*Meiji Dairies Corporation*, *Odawara, Kanagawa, Japan*.

A new evaluation system for selecting probiotic lactic acid bacteria (LAB) with specific adhesion to human colonic mucin that recognizes different ABO-blood types was developed in our laboratory [1,2]. Sixteen strains including *L. gasseri* OLL2804 showed strong adhesion to human blood type-A antigen [GalNAc-α-1-3 (Fuc-α-1-2) Gal-] from the intestinal mucosa were selected from 283 probiotic strains using the biosensor, BIACORE, that employs surface plasmon resonance (SPR)[3]. Similarly, 16 and 11 strains of LAB were selected with strong affinity to human B-antigen [Gal-α-1-3 (Fuc-α-1-2) Gal-] and H-antigen [Fuc-α-1-2 Gal-], respectively [4]. At the same time, we surveyed for colonic harmful bacteria that recognize the blood type antigens (sugar moities on intestinal mucin) using the same BIOCORE recognition system as for OLL2804. Forty strains were isolated from the surface of human colon using TS, MRS, BL and XM-G agar medias incubating at 37°C for 24 hrs. Cells from isolated strains were analyzed using BIACORE against BSA-A (neoglycoprotein, BSA introduced A-antigen trisaccharide). After 16s RNA fragment sequencing, two strains of *Staphylococcus* sp. and one strain of *Escherichia coli* were identified as harmful bacteria recognizing the human blood type-A antigen. We are now determining competitive exclusion of them using OLL2804 in the human intestine. This new assay system will be useful in the selection of probiotic candidates for functional foods including yogurt.

Key Words: Probiotic Lactic Acid Bacteria, BIACORE, Human Blood Type

578 Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expressed on the cell surface of *Lactobacillus plantarum* LA 318 mediates adhesion to human colonic mucin. H. Kinoshita*[1], H. Uchida[1], T. Kawasaki[1], N. Wakahara[1], H. Matuo[1], Y. Kawai[1], H. Kitazawa[1], S. Ohmura[2], K. Miura[2], K. Shiiba[2], A. Horii[3], and T. Saito[1], [1]*Graduate School of Agricultural Science, Tohoku University*, *Sendai, Miyagi, Japan*, [2]*Department of Surgery, Tohoku University Graduate School of Medicine*, *Sendai, Miyagi, Japan*, [3]*Department of Molecular Pathology, Tohoku University School of Medicine*, *Sendai, Miyagi, Japan*.

Members of the genus *Lactobacillus* are often isolated from the alimentary canals and feces of man and animals and are used in fermented food as probiotics. We showed *L. plantarum* LA 318 isolated from human transverse colon is a potential probiotic bacterium that shows high adhesion to human colonic mucin (HCM) mediated by a surface cell wall protein (1). The adhesion test used the BIACORE assay. PBS-washed bacterial cells showed a significant decrease in adherence to HCM as did GHCl treated cells. The component in the PBS supernatant fraction adhered to the HCM and was shown to be a 40 kDa protein using SDS-PAGE. Using homology comparisons of N-terminal to sequence databases, this protein was identified as glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The purified GAPDH adhered to the HCM. The data strongly suggests the GAPDH of the LA318 strain is the adhesin. It has been reported that pathogens, such as *Candida albicans*, possess GAPDH on their cell surface that show binding activity to fibronectin etc. There is no report showing GAPDH exist on the cell surface of *Lactobacillus* probiotics that recognize HCM. This is the first report of GAPDH expressed on the cell surface of lactobacilli that also adhere to mucin; suggesting *L. plantarum* LA 318 adheres to HCM using GAPDH binding activity to colonize the intestine. Because LA 318 strain possesses the same adhesin as pathogens, this may prevent these pathogens from infecting the intestine. This suggests this probiotic can be ingested by mouth to work effectively by replacing pathogens in the intestine; and may be used in probiotic food products including functional yogurt.

Key Words: Probiotics, Adhesion, Human Colonic Mucin

579 Development and optimization of food-grade antimicrobial lactic acid bacteria isolated from raw milk. A. Ichinomiya*, K. R. Nauth, and V. V. Mistry, *South Dakota State University*, *Brookings*.

Producing safe products and extending the shelf life by reducing or eliminating foodborne pathogens are a challenge for the food industry. To meet the growing consumer demand for safe and natural foods, natural protection based on antimicrobial agents has been added to the product. Nisin is the one such natural food preservative. Nisin is a peptide produced by certain strains of *Lactcoccus lactis* subsp. *lactis* during fermentation. It has antimicrobial activity against broad range of Gram positive bacteria such as Clostridia and Listeria. Today nisin is permitted by law to be added to foods in more than 50 countries. The present study was performed to isolate nisin–producing *Lactcoccus lactis* subsp. *lactis* from raw milk, to evaluate the isolates for antimicrobial activity against food spoilage organisms and selected food pathogens, and to develop optimum conditions for maximal antimicrobial production. Milk samples from the raw milk tank or cows on the university dairy farm were collected and screened by the agar overlay method. Identification of the isolates was observed by Gram staining, 4% sodium chloride MRS media incubation, arginine catabolism and catalase test. The activity of nisin was calculated by agar diffusion method that nisin–producing lactic acid bacteria inhibit *Lactococcus lactis* subsp. *cremoris* ATCC 19257 as a nisin sensitive indicator strain. Several nisinproducing lactic acid bacteria strains were found in the raw milk. For optimal production of nisin, *Lactococcus lactis* needs complex nutrition such as nitrogen and phosphate in the medium. They affected the nisin production and biosynthesis. Their

addition to media increased the antimicrobial activity. The component of the antimicrobial activity will be characterized and optimized in substrate containing milk, whey, or, permeate.

Key Words: Antimicrobial Agents, Nisin, Natural Preservative

580 Challenge testing the lactoperoxidase system against against a range of bacteria using different activation agents. L. W. T. Fweja, A. S. Grandison*, and M. J. Lewis, *The University of Reading*, *Reading, Berkshire, UK*.

Lactoperoxidase (LP) exerts antimicrobial effects in combination with H_2O_2 and either SCN^- or a halide. Garlic extract (GE), in the presence of ethanol (E), has also been used to activate the LP system. This study aimed to determine the effects of three different LP activation systems (LP/SCN^-/H_2O_2; LP/I^-/H_2O_2; LP/GE/E) on the growth and activity of three test organisms (*Staphylococcus aureus; Pseudomonas aeruginosa; Bacillus cereus*). UHT milk was used as reaction medium and the growth pattern of the organisms and a range of keeping quality (KQ) indicators (pH, titratable acidity, ethanol stability, clot on boiling) were monitored during storage at the respective optimum growth temperature for each organism. LP/I^-/H_2O_2 reduced bacterial counts below the detection limit shortly after treatment for all three organisms, and no bacteria could be detected for the duration of the experiment (35-55 hours). The KQ data confirmed that the milk remained unspoilt at the end of the experiments. LP/GE/E, on the other hand, had no effect on the growth or KQ with *P. aeruginosa*, but gave a small retardation of growth of the other two organisms, accompanied by small increases (5-10 h) in KQ. The effects of the LP/SCN^-/H_2O_2 system were intermediate between the other two systems and differed between organisms. With *P. aeruginosa* the system exerted total inhibition within 10 h of incubation, but the bacteria regained viability after a further 5 h, following a logarithmic growth curve. This was reflected in the KQ indicators which implied an extension of 15 h. With the other two bacteria, LP/SCN^-/H_2O_2 exerted an obvious inhibitory effect giving a lag phase in the growth curve of 5-10 h and KQ extension of 10-15 h. When used in combination, I^- and SCN^- clearly competed for LP system intermediates and displayed negative synergy with respect to both bacterial growth and KQ.

Key Words: Lactoperoxidase, Keeping Quality, Antibacterial

581 Characterization of immuno active peptides present in cell free preparations obtained from milk fermented by L. Helveticus. A. M. Tellez*[2,1], M. Corredig[3,1], L. Brovko[2,1], and M. Griffiths[2,1], [1]*University of Guelph*, *Guelph, Ontario, Canada*, [2]*Canadian Research Institue for Food Safety*, *Guelph, Ontario, Canada*, [3]*Food Science Department*, *Guelph, Ontario, Canada*.

Interest in the ability of bioactive peptides to impact on immune system has grown considerably in the past decade. Fermented milk has been proposed as a source of those bioactive compounds. The objectives of this research were to confirm the effect of bioactive compounds from milk fermented by Lactobacillus helveticus (LH-2) on the nonspecific host defense system, and purify and characterize the active peptides. For this reason, the cell free supernatant obtained from centrifugation of the fermented milk was tested and an in vitro study using macrophages (RAW 264.7 cell line) was performed. Cytokines production (IL-6, TNF-α±, and IL -1β), Nitric Oxide (NO) production and Phagocytosis effect were used as biomarkers. Cytokine production in culture supernatants was assessed by ELISA. Trypsin-hydrolyzed fermented milk was used as negative control, and bacterial lipopolysaccharide (LPS) was the positive control. Macrophages stimulated with supernatant showed higher production of cytokines and NO compare with LPS. Phagocytosis effect was positive for macrophages stimulated with supernatant (50.75 % ±1.2). The supernatant from fermented milk was analyzed using size exclusion chromatography (SEC) and nine fractions were collected. All fractions were tested for activity. Two fractions (excluded volume and a fraction eluting at 15 minutes) produced higher response when used to stimulate macrophages compared with the other fractions (0.37 and o.25 ng/µg of protein). These results confirmed fermenting milk with Lactobacillus helveticus (LH-2) improves bioactivity, and suggested that specific peptides released during fermentation enhance immune response by modulating macrophage activity.

Key Words: Fermented Milk, Bioactive Peptides, Biomarkers

Dairy Foods: On the Road from Analysis and Discovery of Functional Milk Bioactives to New Products and Health Outcomes

582 An approach to capturing and translating the biological activities and health outcomes of milk components. S. L. Freeman*, *University of California*, *Davis*.

Chronic disease, complex metabolic disorders and obesity dominate the current health landscape. Food has contributed to the problem and therefore food-based interventions offer great potential for not only preventing disease, but also promoting health. To translate the knowledge from analysis and discovery of functional milk bioactives to new products & health outcomes requires different methods, approaches and techniques for studying and validating benefits of these different milk components in a scientifically substantiated manner. Successfully modulating metabolism and immune protection through rational food ingredients and products offers novel solutions to lifestyle and food choices. Milk is an appropriate model for delivering health benefits because it has evolved to nourish, protect and promote infants to not only survive but also thrive. Fundamentally, the components of milk guide health at a time of extreme vulnerability following birth. Understanding milk from this perspective of how it interacts with different biological processes will enable the development of new food products to guide health in different target population. Integrating food science, molecular biology, physiology, nutritional and clinical aspects to capture and apply the knowledge generated is a key to the development and documentation of effective dairy products. The emphasis has to be on documenting the biological activity of different milk components and how they can be applied to measurable health benefits.

Key Words: Health Outcomes, Bioactive Ingredients, Translational Process