

756 Comparison of viscosity and stability of bovine fluid and evaporated milks with those of caprine milk counterparts stored under refrigeration. C. O. Maduko*¹, R. Shewfelt¹, R. Toledo¹, J. Frank¹, Y.-W. Huang¹, and Y. W. Park², ¹Department of Food Science and Technology, University of Georgia, Athens, ²Agricultural Research Station, Fort Valley State University, Fort Valley, GA.

Heat coagulation of milk results from a complex series of physico-chemical reactions that eventually lead to precipitation or gelation. Viscosity of milk tends to increase upon heating as it approaches the point of coagulation of the proteins, which is the basis for producing high viscosity in super-heated condensed milk. Although viscous property may have been studied extensively in cow fluid milk, relatively little information is available on viscosities of concentrated milks and comparison of those of different species milks. Commercial cow and goat fluid milks and their evaporated products were purchased from retail outlets to study characteristics of viscosity and physico-chemical stability of bovine milks compared to caprine counterparts stored at 4 and 13°C refrigeration for 4 weeks. The respective initial viscosity values for bovine fluid (BF), evaporated (BE), and caprine fluid (CF), evaporated (CE) milks were: 1.54, 1.55, and 1.72, 2.03 centipoises, indicating that goat milk products had higher viscosity values than cow counterparts. The viscosity of BF was gradually increased until day 12 at 4°C, and the increase continued to day 28 during which gelation occurred. The BE was stable till day 28 at 4°C storage and did not undergo gelation during this period. The viscosity of CF increased slightly under refrigeration until day 7, then decreased gradually to the lowest at day 11. A sharp increase in viscosity occurred from day 12, and continued to day 28 of storage, during which gelation of the CF milk occurred. However, CE did not show a noticeable decrease on day 9 and remained physically stable until day 28 of storage without any gelation. The refrigeration temperatures had minimal effect on the viscosities of the tested milks. The evaporated milks had greater physical stability under refrigerated storage relative to fluid milks. This may be attributable to the fact that heat treated milk products are actually electrodialysed before evaporation, whereby the partial removal of soluble salts by dialyzing milk against water increases the heat stability when the milk is subsequently concentrated.

Key Words: Viscosity, Bovine and Caprine Milk, Refrigerated Storage

757 Immunostimulatory activities of a novel AT oligonucleotide from “Immunobiotic” *Lactobacillus gasseri* on swine Peyer’s patch cells. H. Kitazawa*, T. Shimosato, S. Katoh, M. Tohno, Y. Kawai, and T. Saito, *Graduate School of Agricultural Science, Tohoku University, Sendai, Japan.*

Many works in the biological functions of dairy lactic acid bacteria (LAB) have contributed to the application of LAB as functional foods and supplements in the worldwide market. More recently, the new term “immunobiotics”, identifying probiotic bacteria that promote health through activation of the intestinal immunity compared to those with strictly local immunity, has been proposed and expected for an appropriate evolutionary development. We have studied specific effector molecules and their receptor targets. Recently, we have found that immunostimulatory AT oligonucleotide (ODN), but not CpG ODN, from *Lactobacillus gasseri* JCM1131^T triggered B cell mitogenic responses in mice. This finding suggests the possibility that the AT ODN could activate the immune responses in peyer’s patches, which belong to a gut associated lymphoid tissue. The present study was conducted to elucidate the immunostimulatory ability of AT ODN in swine peyer’s patches as a human model. The AT ODN significantly augmented the mitogenic activity of swine peyer’s patch cells, when they were stimulated at 10 μ M of ODN, as well as D25, which is known to be an optimal active CpG ODN for swine. The AT ODN strongly induced IL-6, 12p40 and IFN γ gene expressions in swine peyer’s patch cells. Preferential expression of Toll like receptor 9 (TLR9), which is a receptor for unmethylated CpG ODN, was detected in peyer’s patches by the real-time quantitative PCR and immunohistochemical analysis. These findings suggest a possibility that the AT ODN could regulate Th1/Th2 responses in the gut via TLR9 signaling as well as CpG ODN, which is expected play an important role in the prevention of infectious and allergic diseases. This study demonstrated that *Lactobacillus gasseri* JCM1131^T was a good

candidate for the production of new functional foods, “Immunobiotic Foods”.

Key Words: Immunobiotics, DNA, *L. gasseri*

758 AT oligonucleotide from “Immunobiotic” *Lactobacillus gasseri* augments immune responses via Toll-like receptor 9 signaling. T. Shimosato*, H. Kitazawa, M. Tohno, S. Katoh, Y. Kawai, and T. Saito, *Graduate School of Agricultural Science, Sendai, Japan.*

Toll-like receptor (TLR) 9 has been identified as a particular receptor for bacterial DNA that contains a specific sequence pattern, unmethylated CpG dinucleotide. From the discovery of TLR9, possible molecular mechanisms in immune responses through bacterial DNA have been rapidly revealed in mice. Recently, we have found the possibility that AT oligonucleotide (ODN), which was a non-CpG ODN from *Lactobacillus gasseri*, induced immunoactivation in peyers patches (Pps) via TLR9. In the present study, to elucidate the role of TLR9 with AT ODN in the intestinal immunity, we performed the following studies. First, we isolated a cDNA encoding a TLR9 from swine Pps, which are considered to be useful for human models. The total RNA was isolated from the Pps of adult swine intestine. The complete open reading frame of sTLR9 contains 3090bp coding and deduced 1029 amino acid residues with a calculated molecular mass of 115.7kDa. Next, we constructed a transfectant of swine TLR9 with mammalian cells for the development of an immunoassay against ODN. We demonstrated that the transfectant recognizing not only CpG but also non-CpG AT ODN from Lactic acid bacteria (LAB) resulted in the induction of NF- κ B activation by gene reporter assay. Furthermore, TLR9 was strongly detected in the follicle-associated epithelium including M cells as well as antigen presenting cells such as dendritic cells in Pps. These findings indicate that the TLR9 positive cells in Pps provide the host defense with the ability to respond to a variety of ODN from the Immunobiotic LAB. This study firstly shows that TLR9 is a receptor for not only CpG but also for non-CpG AT ODN and may help in understanding the intestinal immunoregulation mediated by Immunobiotic LAB DNA through TLR9 for a development of Immunobiotic Foods.

Key Words: TLR9, Swine, Oligonucleotide

759 Extending shelf life of skim milk with micro-filtration. M. W. Elwell* and D. M. Barbano, *Northeast Dairy Foods Research Center, Department of Food Science, Cornell University, Ithaca, NY.*

The objective of the research was to determine the efficacy of a micro-filtration and pasteurization process in extending the shelf life of skim milk. Raw skim milk (ca. 273 kg) from the Cornell University dairy plant was microfiltered at 50°C using a Tetra Alcross M7 Pilot Plant equipped with a ceramic Membralox membrane (pore diameter: 1.4 μ) and collected directly into a sterile container. Approximately 95 percent of the milk was collected as permeate. The collection container was connected directly to a shell and tube pasteurization system. The 50°C permeate was pasteurized at 72°C for 15 s, and collected directly into another sterile container. The experiment was replicated 3 times. Bacteria counts of raw skim were done by standard plate count. Bacteria counts of microfiltered and pasteurized microfiltered milk were determined using a most probable number (MPN) method. For the MPN method, five containers each with 1, 10, 100 and 500 mL of milk were incubated at 32°C for 6 days. Growth or no growth in each container was determined using a Foss BactoScanTM FC. The MPN bacteria count was calculated using a spreadsheet developed by FDA/CFSAN. Bacteria count of the raw milk was reduced from 2400, 3600, and 1475 cfu/mL to 0.2398, 0.9178, and 0.2398 cfu/mL, respectively, by microfiltration. Bacterial counts in the pasteurized microfiltered skim milk for the three trials were 0.0046, 0.0078, and 0.0045 cfu/mL respectively, demonstrating an average 5.6 log reduction from the raw count due to the combination of microfiltration plus pasteurization. The pasteurized microfiltered skim milk was pumped directly from the sterile collection container into 1L sterile sample containers for shelf life tests. Two sample containers were

incubated at each of four temperatures (0, 2, 4, and 6°C) and the total bacteria count was determined weekly until the count reached 10⁶ cfu/mL.

Key Words: Microfiltration, Shelf Life, Pasteurization

760 Descriptive analysis of processed cheese manufactured by extrusion technology. A. C. Cole*¹, K. A. Adhikari², I. U. Gruen¹, and H. Heymann³, ¹University of Missouri, Columbia, ²California State Polytechnic University, Pomona, ³University of California, Davis.

The use of extrusion technology to manufacture processed cheese has rarely been utilized in the US dairy industry. Extrusion technology ought to be explored for the continuous manufacture of processed cheeses, which might result in greater uniformity of the product as well as use in unique applications that are not feasible with current technology. The objective of this study was to determine and compare the physical and sensory characteristics of extruded processed cheese to those of a conventional processed cheese. Processed cheese was manufactured by blending one month, three month, and six month old Cheddar cheeses in the ratio 1:2:1, respectively. A twin screw co-rotating extruder was used to manufacture the experimental cheeses at two temperatures (80 and 90°C), two moisture levels (44 and 48%) and two melting salt levels (1 and 1.5%). Velveeta was used as the control. In total, nine processed cheese samples were tested. Descriptive sensory analysis was performed to identify the sensory characteristics of the processed cheeses by a panel of nine trained judges. Texture profile analysis (TPA) was performed to determine the physical characteristics of the cheese samples. Canonical variate analysis (CVA) was done to find differences among the processed cheeses using SAS, and partial least square (PLS2) regression was done to correlate the sensory data to the physical data. The CVA biplot showed that the high moisture and the low moisture cheeses were separated into two distinct clusters. The control was an outlier. The cheeses with high moisture levels were judged to have a cooked flavor, a sour aftertaste, and to be bitter, moist, and chewy. The cheeses with low moisture levels were found to be buttery, hard, and springy. The control was judged to be sweeter than the experimental cheeses. PLS2 indicated that the physical characteristics of hardness, springiness, and chewiness were highly correlated with the same sensory characteristics, while the physical and sensory characteristics of adhesiveness, gumminess, and cohesiveness were not correlated with physical texture measures.

Key Words: Descriptive Analysis, Processed Cheese, Extrusion Technology

761 Developments in the small scale manufacture of process cheese using RVA. R. Kapoor*, A. Pollard, P. Upreti, and L. E. Metzger, MN-SD Dairy Foods Research Center, University of Minnesota, Saint Paul.

Recently, methodology utilizing a Rapid Visco Analyzer (RVA) was developed that could be used to manufacture and analyze process cheese on a small scale (25 g). Although this methodology was successfully used to manufacture process cheese, a significant difference in the functionality of the process cheese was observed when compared to process cheese produced on a pilot scale. In the present study, adjustments in the RVA methodology involving the RVA processing conditions, preblend preparation and Texture Profile Analysis (TPA) techniques for the final process cheese were investigated. Three replicates of process cheese food (PCF) were manufactured from eight different pre-blends. Each pre-blend was prepared using eight different natural cheeses and was balanced for moisture (43.5%), fat (25%) and salt (2%). These pre-blends were split into three portions and each portion was subjected to three different manufacturing treatments. Treatment 1 (T1) was manufactured in the Blentech twin screw (BTS) cooker, whereas treatment 2 (T2) and treatment 3 (T3) were manufactured in the RVA using different processing profiles. T2 and T3 were produced in triplicate. The resulting process cheeses were analyzed for their chemical and functional properties. PCF produced in the BTS was analyzed for moisture, fat and salt whereas the PCF produced in the RVA was analyzed for moisture. There were no significant differences ($p \#8805 .05$) in the moisture content of PCF among the 3 treatments with each of the eight batches (produced from the eight different pre-blends). TPA and RVA melt analyses were performed on all the PCF. The batch of natural cheese

used had a significant effect ($p < .05$) on both the TPA-hardness and hot apparent viscosity of the PCF manufactured using the three treatments. Additionally, all three treatments indicated a similar ranking for the eight batches. The adjustments in the RVA methodology produced process cheese with functionality similar to the process cheese produced in the BTS and the RVA methodology was able to identify differences in functional properties of PCF caused by the use of different natural cheeses.

Key Words: Process Cheese, Rapid Visco Analyzer

762 Evaluation of process cheese spread meltability using a Rapid Visco Analyzer. L. A. Prow* and L. E. Metzger, University of Minnesota, St. Paul.

The melt characteristics of process cheese spread are an important functional attribute. Currently, there remains a need for a fast, accurate and low cost test to evaluate cheese meltability. The objective of this study was to develop a melt test for process cheese spread using a Rapid Visco Analyzer (RVA). The melt properties of 32 commercial process cheese spread and process cheese product samples from four different manufacturers were analyzed with the RVA, tube melt test and dynamic stress rheometry (DSR). Three replicates using the RVA, five replicates using the tube melt test and two replicates using DSR were performed on each sample. In the RVA melt test a 15 g cylinder of cheese was packed into the RVA canister and then subjected to a heating, holding, and cooling profile during continuous mixing. During the test an apparent viscosity vs. time graph was obtained. The melt time, hot viscosity, and time at 5000 cP were determined from each apparent viscosity vs. time curve. The tube melt test determines cheese flow in mm, whereas the DSR data was used to calculate cheese melt temperature (temperature at $\tan \delta = 1$). There was a high correlation (.91) between the DSR cheese melt temperature and the tube melt test. There was also a high correlation between the RVA melt time and the other melt tests (.84 and .74 for the DSR and tube melt test respectively). The RVA melt test also measures the apparent viscosity of melted cheese at a constant temperature (hot viscosity). The data obtained for hot viscosity had a low correlation (< .44) with the data from other melt test, which indicates that an additional cheese melting property may be measured. The results of this study indicate that RVA melt analysis of process cheese spread or process cheese product is correlated with the results from other melt test, but it may also provide additional information on cheese meltability which is not quantified in other melt tests.

Key Words: Process Cheese, Melt Analysis

763 A microfiltration (MF) process to maximize the removal of serum proteins from skim milk prior to cheese making. B. K. Nelson* and D. M. Barbano, Northeast Dairy Foods Research Center, Cornell University, Ithaca, NY.

MF of milk before cheese making using a 0.1- μm pore size (ceramic) partially removes serum proteins (SP). The SP (mostly β -lactoglobulin and α -lactalbumin) do not contain residual starter, color, coagulant, and lactic acid from cheese making. Diafiltration (DF) has been used to remove lactose from whey protein retentates during ultrafiltration (UF) using water as the diafiltrant. Water as a diafiltrant has been used to remove SP from skim milk during MF, but this produces a retentate with lower lactose and unbound mineral content than milk and may have a negative impact on conventional cheese making. We hypothesize that high recovery of the SP from skim milk prior to cheese manufacture could be achieved by using permeate from the UF of MF permeate as the diafiltrant instead of water to maintain the lactose and unbound mineral balance of the retentate for cheese making. Pasteurized skim milk was single pass processed to a 3X concentration factor using MF. Permeate from MF was collected and ultrafiltered to produce UF permeate that was used as a diafiltrant. The polysulfone UF membranes had a 10-kDa molecular weight cut-off. The retentate collected during the MF step was diluted back to the original weight of the skim milk with UF permeate. The diluted skim retentate was then processed using MF and this constituted the first DF. After the first DF to 3X, the retentate from MF was again diluted with UF permeate and processed (3X) using MF. The UF permeate used for DF contained a low concentration (ca. 0.07%) of SP. Thus, the total SP in the process was that in the skim plus the SP added with the UF permeate. MF plus the two subsequent DF steps removed about 94% of the total SP. The observed SP removal

was close to the expected theoretical value of 96% serum protein for the process.

Key Words: Microfiltration, Diafiltration, Milk

764 Isolation and identification of *Micrococcus* spp from Egyptian soft cheese. A. S. Zahran*, *Minia University.*

Micrococci are the predominant bacteria of milk drawn aseptically from the udder. They were also isolated from different cheese varieties. These bacteria constitute a major portion of the secondary cheese microflora and contribute to its flavour through their proteolytic, lipolytic and esterolytic enzymes activities. Fifty strains of *Micrococcus* spp were isolated from Egyptian soft cheese (Domiat). They were identified into four different species as follows *M. varians*, *M. roseus*, *M. sedentarius* and *M. luteus*. The species most often isolated was *M. varians* as it represents 55% of the total isolates, strain *M. varians* DC6 was very active producer of extracellular proteinase and lipase. Immobilized cell culture is a widely used technique for achieving high volumetric efficiency and sustained productivity from microorganisms. The entrapment of bacterial cells in 2% agar substantially improved their enzyme activity. Immobilized cells produced from 40-50% more enzymes than free cells. The agar entrapment method appeared to have no adverse effects on the activity of the cells. The agar beads were structurally stable over five usage cycles. Synthesis of the extracellular enzymes by *M. varians* DC6 appeared to be inducible as no enzymes were detected in the absence of organic nitrogen.

Key Words: *Micrococcus*, Egyptian Soft Cheese, Immobilized Cells

ASAS - Growth and Development II

766 Influence of dietary protein and lactose levels on protein synthesis and translation initiation factor activation in neonatal pigs. J. W. Frank*¹, J. Escobar¹, A. Suryawan¹, H. V. Nguyen¹, C. W. Liu¹, S. R. Kimball², L. S. Jefferson², and T. A. Davis¹, ¹USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, ²College of Medicine, The Pennsylvania State University, Hershey.

Parenteral infusion of insulin (INS) and amino acids increases protein synthesis (PS) and eukaryotic translation initiation factor (eIF) activation in skeletal muscle and liver. Pigs (N = 25; BW = 1.6 kg) were enterally fed isocaloric milk diets with three levels of protein (5, 10, and 15 g/kg/d) and two levels of lactose (11 and 23 g/kg/d) from 1 to 7 d of age. On d 7, pigs were gavage fed after a 4 h fast and blood samples were collected every 30 min for 1.5 h. Pigs were then euthanized and tissues harvested. Daily gain and PS in the longissimus dorsi and gastrocnemius muscles and liver were not influenced by lactose level, but increased with dietary protein and plateaued at the 10 g/kg/d level (P < 0.01). Plasma INS was greater in the high lactose fed pigs (P < 0.01) and lower in pigs fed the lowest protein diet (P < 0.001). Plasma branched-chain amino acids were influenced by dietary protein level (P < 0.001). Liver and muscle protein kinase B phosphorylation was greater in the high lactose fed pigs (P < 0.05). Liver and muscle ribosomal protein S6 kinase and liver 4E-BP1 phosphorylation increased with dietary protein and plateaued at the 10 g/kg/d level (P < 0.01), while muscle 4E-BP1 phosphorylation continued to increase to the highest protein level (P < 0.001). The association of eIF4G to eIF4E increased with dietary protein level (P < 0.05) and was not influenced by lactose level. The results suggest that growth and PS in neonatal pigs are influenced by dietary protein intake. These changes involve modulation of the availability of eIF4E for eIF4F complex assembly and may be mediated by plasma insulin and amino acid levels. (NIAMS AR 44474, USDA 58-6250-6-001)

Key Words: Pigs, Protein Synthesis, Translation Initiation

765 Use of exopolysaccharides producing lactic acid bacteria for the production of buffalo milk dahi (yogurt). N. Pandya*¹, S. Kanawjia¹, and R. Dave², ¹Dairy Technology Department, National Dairy Research Institute, Karnal, India, ²Dairy Science Department, South Dakota State University, Brookings.

Exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) have generated increasing attention among researchers and considered novel and safe food additives. In this experiment, two EPS⁺ cultures (*Lactococcus lactis* subsp. *lactis* NCDC 191 & mixed thermophilic strains NCDC 260) in combination with standard dahi culture (mixed mesophilic strains NCDC 167, EPS⁻) were studied for their effects on incubation pattern, rheology and sensory properties of the dahi made from buffalo milk standardized at 4.5% fat and 9.5% SNF. Both cultures were inoculated at 2% (v/v) rate in three different ratios (1:1, 2:1 and 3:1 of EPS⁻ and EPS⁺ cultures respectively) and incubated at 27 and 32°C for NCDC 191 and NCDC 260, respectively. It was observed that the rate of drop in pH and rate of increase in titratable acidity declined with increasing proportion of EPS⁺ cultures. Rheology and overall sensory properties were improved with increasing ratio of EPS⁺ culture. However, the improvement was significant (P<0.05) only with NCDC 260, but not with NCDC 191. With the increasing proportion of NCDC 260, the viscosity increased from 0.384 to 0.596 Pa.s., the curd tension increased from 35.52 to 46.60 g, and the syneresis reduced from 1.48 to 0.33 ml per 10 g of dahi. The flavor scores decreased significantly (P<0.05) with increasing ratio of NCDC 191 culture whereas, effect of NCDC 260 culture on flavor scores was not significant (P<0.05). The body and texture scores improved up to 2:1 ratio (NCDC 167: NCDC 260) but at a higher ratio of EPS⁺ culture the dahi developedropy consistency and resulted into decline in sensory scores. The appearance scores were improved for both EPS⁺ cultures. A combination of NCDC 167 and NCDC 260 with (2:1) ratio was suggested for commercial production of dahi.

Key Words: Exopolysaccharides, Buffalo Milk, Dahi

767 Infusion of a physiological dose of leucine stimulates muscle protein synthesis in neonatal pigs by enhancing the activity of translation initiation factors. J. Escobar*¹, J. W. Frank¹, S. R. Kimball², A. Suryawan¹, H. V. Nguyen¹, C. W. Liu¹, L. S. Jefferson², and T. A. Davis¹, ¹USDA/ARS, Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, ²Cellular and Molecular Physiology, College of Medicine, Pennsylvania State University, Hershey.

In adult rats, skeletal muscle protein synthesis increases in response to pharmacological doses of leucine (Leu) administered orally. The effect of a physiological rise in plasma Leu on skeletal muscle protein synthesis has not been investigated in neonatal pigs, which are highly sensitive to amino acid and insulin stimulation. Thus, 24 crossbred pigs were food-deprived for 12 h and intra-arterially infused with Leu (0 or 400 mmolkg⁻¹h⁻¹). Protein synthesis was measured after 60 or 120 min in liver, and longissimus dorsi and gastrocnemius muscles. Infusion of Leu increased (P < 0.01) plasma Leu 2.5- to 3.4-fold while plasma insulin and glucose were unchanged. Infusing Leu for 120 min, but not for 60 min, reduced (P < 0.05) plasma essential amino acids levels. Infusing Leu for 60 and 120 min increased (P < 0.05) phosphorylation of eukaryotic initiation factor (eIF) 4E binding protein-1 (4E-BP1), ribosomal protein (rp) S6 kinase (S6K1), and rpS6, and decreased the amount of eIF4E associated with its repressor, 4E-BP1, in longissimus dorsi muscle. In liver, phosphorylation of 4E-BP1, S6K1 and rpS6, as well as eIF4E associated with 4E-BP1 were not affected by Leu infusion. Leucine infusion for 60 min increased protein synthesis in longissimus dorsi (38%, P = 0.04) and gastrocnemius (67%, P = 0.005) muscles, but not in liver (P = 0.11). Leucine infusion for 120 min did not increase protein synthesis in skeletal muscle and reduced protein synthesis in liver (25%, P < 0.01). Thus, a physiological increase in plasma Leu stimulates protein synthesis in skeletal muscle of neonatal pigs by increasing eIF4E availability for eIF4F assembly. Moreover, this response appears to be insulin-independent, substrate-dependent, and tissue-specific (NIAMS AR 44474, USDA58-6250-6-001).

Key Words: Leucine, Translation Initiation Factors, Protein Synthesis