T46 Rapid determination of Swiss cheese composition by infrared spectroscopy. N. Koca*[1,2], W. J. Harper[2], L. Rodriguez-Saona[2], and V. B. Alvarez[2], [1]*Ege University*, *Izmir, Turkey*, [2]*The Ohio State University*, *Columbus*.

Current methods for analyzing cheese composition are time consuming, expensive and use hazardous chemicals. Infrared spectroscopy is an attractive technology for the rapid, inexpensive, sensitive, and high-throughput analysis of food components without requiring special skills from the users. The objective of this research was to develop a simple and rapid screening tool for monitoring Swiss cheese composition by using FT-IR spectroscopy. Swiss cheese (16) samples from eight different manufacturers were evaluated. Direct measurements of Swiss cheese slices (about 0.5g) were made by using a MIRacle three reflection diamond attenuated total reflectance (ATR) accessory. Reference methods for moisture (vacuum oven), protein (Kjeldahl) and fat (Babcock) contents were used. Calibration models were developed based on a cross-validated (leave-one-out approach) Partial Least Squares (PLS) regression. The information-rich infrared spectral range for Swiss cheese samples was from 3000-2800 cm^{-1} and 1800-900 cm^{-1}. The range of 1800-900 cm^{-1} was used to develop calibration models for moisture and protein with performance statistics of 0.58% and 0.32% standard error of prediction (SEP), respectively and the correlation coefficients (R^2) were 0.96 and 0.92, respectively. Models for fat were generated by using all the information-rich spectral range and provided performance statistics of 0.35% SEP and R^2 of 0.97. FT-IR/ATR spectroscopy allowed for the rapid (about 3 min analysis time) and accurate analysis of composition of Swiss cheeses. This technique could contribute to the development of simple and rapid protocols for monitoring the complex chemical changes and predicting the final quality of the cheeses.

Key Words: Infrared Spectroscopy, Composition, Cheese

T47 Development of a combined sensor technology for monitoring coagulation and syneresis operations in cheese making. M. Castillo*, F. Payne, and A. Shea, *University of Kentucky*, *Lexington*.

The cheese making industry is a very important segment of the US agriculture and produces approximately 25% of world cheese production at an economic value of approximately $19 billion. Forming a gel, cutting that gel into cubes and stirring the mix of curd grains and whey to allow syneresis to occur are the first major unit operations in the cheese making process and exert a very significant impact on cheese quality. Indeed, it is well-known that milk composition and coagulation factors affect coagulation and gel properties, which ultimately have a decisive effect on curd firming and thus on syneresis properties of gels. There are several optical sensor technologies for monitoring milk coagulation based on either light backscatter or transmission, but unfortunately there are currently no technologies available for monitoring curd syneresis. The goal of this study is to develop an optical sensor technology for simultaneous monitoring of coagulation and syneresis operations to improve the control of curd moisture content during cheese making. We hypothesized that a light backscatter sensor having a large field of view relative to the curd size would accurately measure syneresis, and might hold the ability to monitor the coagulation too. A prototype sensor was fabricated and tested using a dual fiber optic spectrometer. The prototype signal increased by a 29% during coagulation and decreased by a 40% during syneresis. The proposed technology shows potential for monitoring changes in light backscatter during coagulation and syneresis. However, results suggest that the prototype must be redesigned to reduce the signal to noise ratio, especially during the first minutes of syneresis. Successful development of a sensor technology that is able to control curd moisture content will have a large impact on cheese manufacturing worldwide in terms of production efficiency and product quality and consistency.

Acknowledgements: This research was supported by the Kentucky Science and Engineering Foundation grant KSEF-407-RDE-004.

Key Words: Optical Sensor, Milk Coagulation, Syneresis

T48 Effect of the pH on the proteolysis of Prato cheese during ripening. V. S. Monteiro, R. T. A. N. Risse, and M. L. Gigante*, *State University of Campinas*, *Campinas, SP, Brazil*.

Although pH is an essential parameter characterizing the identity and quality of cheeses it is difficult to segregate its effect from the effect of pH-induced changes during cheese making. One way to overcome this difficulty is to change the pH after the cheese is manufactured. The objective of this study was to evaluate the effect of pH on the proteolysis of Prato cheese using a post-manufacture pH change method. Prato cheese was manufactured by the traditional method and one day after manufacture, the cheeses were shredded and mixed together so as to obtain a homogeneous sample, which was subsequently divided into 3 equal portions. The first portion was exposed to ammonium hydroxide for 5 minutes to raise the pH; the second portion was exposed to acetic acid lower the pH and the third portion was used as the control. Immediately after the pH alteration, portions of cheese were vacuum packed and stored at 12±1°C. To evaluate the effect of pH on proteolysis, randomly selected samples were analysed after 1, 8, 15, 22, 29, 44 and 58 days of storage for total nitrogen, soluble nitrogen at pH 4.6 and in 12% TCA and electrophoretic profile. The experimental design used was a split-plot arrangement of treatments in a randomised complete block of three replications. Pearson's correlation test was used to assess the correlation between pH and the other variables. The treatments had a significant impact on the pH of the cheese and generated three distinct pH groups: control (4.99±0.05), high pH (5.7±0.1) and low pH (4.79±0.08). The interaction between treatments and ripening time had a significant effect on proteolysis, increasing with time, but to a lesser extent in the low pH group of cheeses. Electrophoresis showed that the degradation of α_{s1} and β-caseins occurred at a slower rate and less extensively at the low pH. An analysis by Pearson's correlation showed that there was no significant association between pH and proteolysis. The effect of pH on proteolysis depends on storage time.

Key Words: Prato Cheese, pH, Proteolysis

T49 Effect of NaCl and pH on curd firmness, residual coagulant activity and chemical composition of soft white cheese. S. Awad*, *Alexandria University*, *Alexandria, Egypt*.

Domiati is the most popular soft white cheese produced in Egypt. This cheese is made from salted milk (8-15% NaCl). The high salinity level of whey obtained during Domiati cheese manufacture makes its disposal a problem. Characteristics of cheese made from unsalted milk has not been studied. The objectives of this work were a. to study the effect of sodium chloride concentration and pH of milk at renneting on the rennet clotting time (RCT) and curd firmness, and b. to study effect of milk salting on residual coagulant activity, expressible serum and chemical composition of cheese. The results showed that rennet clotting activity and curd firmness decreased with increasing NaCl concentration in milk. The RCT increased as the pH of the salted milk decreased. Milk containing 10 % NaCl did not coagulate at pH 5.0. The curd firmness increased with decreasing milk pH. However, the firmness decreased as the pH of milk at renneting dropped below 5.8. Cheese moisture and soluble proteins in expressible serum were lower in cheese made from unsalted milk than in that made from salted milk. Pre-acidification with citric acid increased the moisture content in cheese and reduced the amount of expressible serum. Cheese made from salted milk contained the lowest activity of residual coagulant, while cheese made from milk pre-acidified with citric acid contained the highest activity. In conclusion, Domiati cheese made from unsalted milk was much firmer than that produced from salted milk. Preacidification of unsalted milk reduced firmness and produced cheese with characteristics comparable to those of cheese made from salted milk.

Key Words: Soft White Cheese, Sodium Chloride, Residual Coagulant Activity

T50 The effect of calcium removal from milk on casein micelle stability and structure. H. Grimley*, A. Grandison, and M. Lewis, *The University of Reading*, *Reading, UK.*

Milk contains approximately 30 mM total calcium, but only about 5% of this is present as ionic calcium. Changing the amount of calcium in milk can influence its stability and structure. In this study calcium was removed from raw skimmed milk at levels of 10, 19, 29, 40 and 51% of total calcium using an ion exchange resin. During ion exchange, calcium must be removed from the micelle, before being converted to ionic calcium prior to its removal by the resin. After removal, ionic calcium, sodium and potassium were measured along with pH, ethanol stability, micelle size and zeta potential. Ionic calcium decreased with removal of calcium and pH increased. Calcium removal resulted in an increase in the ethanol stability from 88% to above 100%. Measurement of casein micelle size showed that the average micelle diameter increased as calcium was removed, from 198 nm to 213, 230, 254, 323 and 420 nm with 10, 19, 29, 40 and 51% total calcium removal, respectively. The zeta potential of the skimmed bulk milk was -24.4 mV, gradually becoming more negative with calcium removal to -30.6 mV for the sample with 51% calcium removal. The appearance of the milk became more translucent as calcium was removed.

To investigate the reversibility of this process, calcium was added back to each of the samples, as concentrated $CaCl_2$, to restore their original total calcium content. At 51% removal, restoration of the total calcium level resulted in formation of clots. At levels of 10 and 19% calcium removal the ethanol stability remained above 100%, but at higher levels of calcium removal the alcohol stability was reduced when the calcium was added back. Adding back calcium resulted in some restoration of the original casein micelle size. Where the micelle size was increased greatly, due to high removal of calcium, the addition of calcium reduced the micelle size but did not restore it to the original size. These results suggest that calcium plays a pivotal role in the stability of milk and reincorporating calcium into the micelle is not straightforward.

T51 A review of the models for the structure of the casein micelle. E. Ferrandini[1], M. Castillo*[2,1], M. B. López[1], and J. Laencina[1], [1]*University of Murcia*, *Murcia, Spain*, [2]*University of Kentucky*, *Lexington*.

Casein micelles have been defined by Dalgleish as the association colloid in fresh milk. They consist of four types of aggregated casein combined with significant amount of colloidal calcium phosphate (CCP). It is widely accepted that casein micelles are sterically stabilized by an external hairy layer of κ-casein. The fact that casein micelles constitute a very stable colloidal system, as compared to almost any other synthetic colloidal system, has significant implications especially with regard to casein gel formation and stability of dairy products during heating, concentration and storage. For that reason, the structure of the casein micelle has been intensively investigated during the last five decades. However, specific questions about micelle structure and stability are still arising as new instrumental methodologies become available. This work reviews the current state of knowledge of the casein micelle structure. Traditionally, casein micelle models have been classified in three different groups: coat-core models, internal structure models, and subunits models. The subunit models have become widely accepted, probably because the existence of submicelles has been supported by different techniques: casein dissociation, formation of submicelles from sodium caseinate and subsequent polymerization in presence of calcium, microscopic observation of submicelles, small-angle neutron scattering measurements and ultrasonic spectroscopy. However, several studies by Holt have made the existence of submicelles doubtful. This controversy is stimulating the apparition of alternative models such as the model based in CCP nanoclusters proposed by Holt, the dual-binding model proposed by Horne, and a modified submicellar model proposed by Walstra. New studies based on field emission scanning electron microscopy have shown no evidence of the presence of either micellar coating or spherical subunits, but have shown evidence for the organization of caseins into tubular structures within the micelles. The new models might improve our knowledge about the technological and functional properties of casein micelles, enhancing the development of novel structures and textures in foodstuffs.

Key Words: Casein, Micelle, Structure

T52 Porcine milk proteins throughout lactation and isolation of lactoferrin and immunoglobulin. J. Gunness*[1], M. Monaco[1], B. Lonnerdal[2], and S. Donovan[1], [1]*University of Illinois*, *Urbana*, [2]*University of California*, *Davis*.

The piglet is commonly used to assess the impact of milk-borne components on neonatal development. Whey proteins exert functional roles within the neonate, but have not been extensively studied in the pig. Our goals were to develop methods to isolate porcine whey proteins and to characterize the protein content and composition of porcine milk throughout lactation. Milk samples were collected from sows (n=3) at farrowing (0 h), 12 h and d 1-4, 7, 14, 18, 21 and 24 postpartum. To separate porcine whey and casein proteins, whole milk was pH-adjusted to 4.0, 4.3 or 4.6 or left unadjusted (6.5) and centrifuged at 40,000xg or 190,000xg. Whole milk, whey and casein pellets were compared by SDS-PAGE. The pH 4.3 and 40,000xg treatment produced the best separation of porcine whey and casein proteins. Proteins in whole porcine milk and whey were visualized throughout lactation by SDS-PAGE and compared to purified milk proteins. Bands of similar molecular weight as IgG, lactoferrin, α-lactalbumin, β-lactoglobulin and α, β and κ-casein were visualized in porcine milk. The density of IgG bands decreased with lactation, while casein increased from colostrum (0-24h) to mature milk (>24h). Whey protein and IgA and IgG were measured by Lowry protein and ELISA, respectively. Colostral protein (15.9 ± 0.8 g/dL) was 3-fold higher ($p<0.001$) than mature milk (5.1 ± 0.2 g/dL). IgG decreased 100-fold ($p<0.001$) from colostrum to mature milk and accounted for 80% and 2.3% of total whey protein, respectively. IgA was highest at farrowing (0.5 ± 0.02 g/dL), decreased 50% ($p<0.05$) within 12h, but rose again at d21 and d24 of lactation. Lastly, lactoferrin (Lf) was isolated from porcine whey by fast protein liquid chromatography using Heparin-Sepharose and IgG was isolated by affinity chromatography using Protein A. SDS-PAGE analysis of purified proteins and Lf- and IgG-depleted milk samples demonstrated quantitative isolation of high purity milk proteins. Thus, methods established for human and bovine milks were optimized for porcine milk, which will enable future studies directed towards assessing bioactivity of porcine milk proteins.

Key Words: Milk, Protein, Immunoglobulin

T53 Interactions of whey proteins during heat treatment of oil-in-water emulsions formed with whey protein isolate and hydroxylated lecithin. A. Ye[2], H. Singh[2], and R. Jimenez-Flores*[1], [1]*California Polytechnic State University, DPTC.*, *San Luis Obispo*, [2]*Riddet Crntre, Massey University.*, *Palmerston North, New Zealand*.

The interactions of proteins during the heat treatment of whey-protein-isolate (WPI)-based oil-in-water emulsions with and without added hydroxylated lecithin were studied by examining the changes in droplet size distribution and the quantity and type of adsorbed and unadsorbed proteins. Heat treatment at 90°C of WPI emulsions resulted in an increase in total adsorbed protein; unadsorbed β-lactoglobulin (β-LG) was the main protein interacting with the adsorbed proteins during the first 10 min of heating, but, after this time, unadsorbed α-lactalbumin (α-LA) also associated with the adsorbed protein. In emulsions containing hydroxylated lecithin, the increase in total adsorbed protein during heat treatment was much lower and the unadsorbed β-LG did not appear to interact with the adsorbed proteins during heating. However, the behavior of α-LA during heat treatment of these emulsions was similar to that observed in the emulsions containing no hydroxylated lecithin. In the presence of NaCl, the particle size of the emulsion droplets and the quantities of adsorbed protein increased markedly during heating. Emulsions containing hydroxylated lecithin were less sensitive to addition of NaCl. These results suggest that the binding of hydroxylated lecithin to unfolded monomers or intermediate products of β-LG reduces the extent of heat-induced aggregation of β-LG and consequently decreases the interactions between unadsorbed β-LG and adsorbed protein. This was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of heated whey protein and hydroxylated lecithin solutions.

Key Words: Emulsions, Whey Proteins, Lecithin

T54 A novel two-dimentional gel electrophoresis for studing the cress-linking between β-Lactoglobulin and milk proteins. W. L. Chen*, M. T. Huang, and S. J. T. Mao, *National Chiao Tung University*, *Hsinchu, Taiwan*.

β-Lactoglobulin (LG) is one of the major protein moiety in milk. It forms aggregates from the heating in milk processing. The aggregation, however, may attenuate some physiological function such as binding to fatty acids and retinols. In addition to self aggregation, LG also conjugates with other milk proteins mediated by disulfide bond linkage. There have been no specific methods in separating and identifying the proteins cross-linked to LG using gel electrophoresis. In this study, we developed a novel 2D-SDS-polyacrylamide gel electrophoresis (PAGE). In the first dimension, sample was run without reducing reagent, after which time the gel with separated proteins was immersed in reducing reagent. The gel was then placed horizontally and run the second dimension. Under this condition, the aggregated proteins were reduced and dissociated from the LG and could be identified by a Western blot. This 2D-SDS-PAGE assay allows us to analyze cross-linkings between LG and other milk proteins. The results show that LG interacted with casein, lactalbumin, and BSA by disulfide bond linkage during the heating process. We also mixed LG and casein, lactalbumin, with BSA, and heated respectively, followed by analyzing 2D-gel. It confirms that LG associated with casein, lactalbumin, and BSA by thiol group upon the heating. Finally, the LG conjugates in heated milk were isolated from LG antibody affinity-column, the conjugated proteins contained casein, lactalbumin, and BSA. Thus, we conclude that the 2D-gel assay can be used for analyzing protein-protein interaction.

Acknowledgements: This work was supported by a grant 90-2313-B-009-001, 91-2313-B-009-001, 92-2313-B-009-002 and 93-2313-B-009-002 from the National Science Council (NSC) Taiwan, ROC.

Key Words: β-Lactoglobulin, Two-Dimentional Gel Eelectrophoresis, Cross-Linkings

T55 Concentration of polar MFGM lipids from buttermilk using supercritical carbon dioxide. A. Spence*[1,2], J. Yee[1], M. Qian[2], and R. Jimenez-Flores[1], [1]*California Polytechnic State Universtiy*, *San Luis Obispo*, [2]*Oregon State University*, *Corvalis*.

Buttermilk is a unique source of milk fat globule membrane (MFGM), a material that contains many complex lipids that function as nutritionally valuable components. Milk derived phospholipids not only function as integral components of the membrane, but have been shown to be involved with other biological processes, including cell regulation and development, cell to cell interactions, immune recognition, transmembrane signaling, and as cell receptors. Previous work has shown that polar MFGM lipids can be concentrated using a two-step method by microfiltration and supercritical fluid extraction (SFE) using CO2. We have optimized the SFE concentration process for efficient non-polar lipid extraction from buttermilk powder without compromising the remaining components. First, we evaluated pressure and temperature parameters using a general full factorial design. Three pressure levels (150, 250 and 350 bar) were evaluated with three temperature levels (40, 50 and 60°C). SFE treatments at 60°C show an increased whey protein Mr (relative molecular mass) indicating likelihood of lactosylation at higher temperatures. Optimal extraction conditions were 350 bar and 50°C at a flow rate of 20 g/min and three consecutive runs of 75 min. Second, we assessed an alternative to adsorbent addition; removable Teflon® beads are a suitable substitute to the addition of biosilicate materials. Third, we assessed modified CO2. Using a co-solvent can further concentrate or fractionate polar lipids. Isopropanol, ethanol and methanol at three concentrations (10%, 20%, and 50% w/w) displayed different extraction affinities for buttermilk lipids. Methanol showed the highest level of total lipid extraction at higher concentrations whereas at 20% concentration, ethanol and methanol had the highest level of polar lipid extraction. Little to no polar lipids was extracted using isopropanol or a 10% co-solvent concentration.

Key Words: Buttermilk, Supercritical Fuid, MFGM

T56 Quantitative determination of thermally derived volatile compounds in milk using solid-phase microextraction and gas chromatography. P. Vazquez-Landaverde*[1], G. Velazquez[1,2], J. Torres[1], and M. Qian[1], [1]*Oregon State University*, *Corvallis.*, [2]*Universidad Autonoma de Tamaulipas*, *Reynosa, Tamaulipas, Mexico.*

Many volatile compounds are generated during the heat processing of milk and their association to the development of cooked, stale and sulfurous notes have been reported. A headspace solid phase microextraction/gas chromatographic (HS-SPME/GC) technique for their quantitative analysis was developed in this study. The extraction temperature, time and sample weight were optimized using a randomized 2^3 central composite rotatable design with two central replicates and two replicates in each factorial point along with response surface methodology. High correlation coefficient calibration curves were obtained for twenty volatile compounds in milk using the standard addition technique and then used to quantify their concentration in raw, pasteurized and UHT milk samples with various fat contents. Concentrations of dimethyl disulfide, 2-hexanone, 2-heptanone, 2-nonanone, and 2-undecanone, 2-methylpropanal, 3-methylbutanal, heptanal, and decanal were present at much higher concentrations in UHT milk as compared to raw and pasteurized samples.

The concentration of volatiles in raw and pasteurized milk samples was not significantly different, except for dimethyl disulfide in raw and one of the pasteurized milk brands analyzed. Fat content had an effect on the concentration of volatiles in heat-processed milk, generally increasing with fat content.

Key Words: Milk, SPME, Heat

T57 Quantification of volatile sulfur compounds in milk by solid-phase microextraction and gas chromatography coupled to pulsed-flame photometric detection. P. Vazquez-Landaverde*[1], G. Velazquez[1,2], J. Torres[1], and M. Qian[1], [1]*Oregon State University*, *Corvallis*, [2]*Universidad Autonoma de Tamaulipas*, *Reynosa, Tamaulipas. Mexico.*

The sulfurous off-flavor generated during thermal processing of fluid milk affects the consumer sensory perception of milk. A wide variety of sulfur compounds have been identified as the responsible of this off-flavor; however, no quantification of these sulfur containing compounds has been reported due to their high reactivity and volatility. A headspace solid phase microextraction/gas chromatographic (HS-SPME/GC) technique coupled to a pulsed-flame photometric detector for their quantitative analysis in milk was developed in this study. Calibration curves with highly significant correlation

coefficients were obtained for seven sulfur-containing milk volatiles using the standard addition technique and then used to quantify their concentration in raw, pasteurized and UHT milk samples with various fat contents. All calibrated compounds were stable in the milk matrix and no artifact formation was observed. UHT milk contained significantly higher concentrations of hydrogen sulfide, carbon disulfide, dimethyl trisulfide, methanethiol and dimethyl sulfoxide when compared to raw and pasteurized milk with the two latter ones being the most abundant. The concentration of dimethyl sulfone was lower for the UHT 3% sample when compared to the raw and one of the pasteurized brands analyzed. Pasteurized samples had the same concentration of sulfur volatile compounds when compared to raw milk, except for carbon disulfide found at a higher concentration in one pasteurized brand. In UHT milk, the concentrations of hydrogen sulfide, methanethiol, dimethyl trisulfide, and dimethyl sulfoxide increased with fat content level.

Key Words: Milk, Sulfur, SPME

T58 Novel reporter molecule for the development of rapid assay probes. I. Surjawan*, H. Karacelik, S. Neelakantan, P. A. Crooks, and C. L. Hicks, *University of Kentucky*, *Lexington*.

A novel rapid assay reporter molecule (4-{[6-aminohexyl]-ethyl-amino}-2,3-dihydro-phthalazine-1, 4 dione) was prepared using a 5 step syntheses process.

The novel synthesis procedure placed the 6 amino hexyl group in the 4 position rather than the 3 position of the traditional 9 step syntheses procedure. Overall yield was 8% for the novel procedure compared 2% for the traditional method. The limit of detection (LOD) for the traditional probe and novel probe were determined by comparing fluorescent readings of the serially diluted probes (0.1, 1, 10, & 100 ppm) against those obtained from blank samples and establishing the minimum detectable level of each reporter molecule. The stock of each probe (100 ppm) was initially dissolved in DMSO (2 drops) and then adjusted to volume (2.0 ml) with 0.01 N phosphate buffer (pH 6.6). Each dilution of probe (2.5 ml) was placed in cuvette for fluorescent readings with excitation and emission wavelengths set at 360 and 430 nm, respectively. The means (n=5) of the highest fluorescent intensity for each diluted probes were recorded. The noise level of each diluted probe was determined by dividing the mean of the highest intensity by the average peak- to-peak distance for 60 sec. or RMS (n=54 fluorescent readings). Results showed that the LOD for the traditional and novel probes fell between 1.006 to 1.09 ppm. Thus the LOD for either probe would be approximately 1.1 ppm. Since no difference in sensitivity existed between the reporter molecules, all new rapid assay probes could be prepared utilizing the novel synthesis procedure.

Key Words: Rapid Assay Probe, Fluorescent, Limit of Detection

T59 Spectrophotometry and DSC correlate with fatty acid differences in milk fat crystallization behavior. L. Lassonde*[1], E. DePeters[2], and R. Jimenez-Flores[1], [1]*California Polytechnic State University, DPTC*, *San Luis Obispo*, [2]*University of California*, *Davis*.

The purpose of this study is to test if common methods used in food thermodynamic analysis can detect differences between samples when a single fatty acid component is altered. Butter and buttermilk samples taken from individual cows on high and low palmitic acid (C16:0) diets were received from UC Davis. Genotypic information from herd records was provided for each cow for the following genes, k-CN, β-LG, α-LA, DST, and DGAT to correlate with individual variation between samples. Samples were shipped to Cal Poly, DPTC for analysis. Milk fat crystallization studies were performed on the anhydrous milk fat, which was separated from the serum fraction by melting the butter, then centrifuging. Isothermal turbidimetric absorbance was measured on melted (60°C) anhydrous milk fat (AMF) at 610nm. Measurements were taken in 30s intervals until nucleation occurred. Of the analysis conducted, between individual cows low-palmitic AMF resulted in slower rates of crystallization and longer induction times until catastrophic nucleation. Conversely, high palmitic AMF resulted in faster crystallization rates and shorter induction times until nucleation. This data was positively correlated with DSC (differential scanning calorimetry) melting profiles of heat flow from -40°C to 65°C, at 5.00°C/min. DSC melting profiles of anhydrous milk fat showed a lower melting temperature and lower crystallization temperature in low palmitic AMF, when compared to high palmitic AMF. Texture analysis on low palmitic AMF of hardness (force g) at refrigeration and ambient temperatures yielded a softer AMF. The high palmitic acid content resulted in a much harder AMF at both refrigeration and ambient temperatures. Subjectively, differences in the milk fats could be observed visually, as melted, equilibriated high palmitic AMF solidified at room temperature much quicker than low palmitic AMF. These net effects were seen on pooled data regardless of the genotype of the individual cows. However, between individuals, genotype either increased or decreased the net effect of the palmitic acid on AMF behavior.

Key Words: Palmitic Acid, DSC of Lipids, Milk Fat Crystallization

T60 Optimization of cholesterol removal in milk by crosslinked β-cyclodextrin. E. M Han, S. H. Kim, J. Ahn, and H. S. Kwak*, *Sejong University*, *Seoul, Korea*.

This study was examined to find optimum conditions of crosslinked β-cyclodextrin (β-CD) concentration, mixing temperature, mixing time, and mixing speed for cholesterol removal in milk and to examine the recycling efficiency of the crosslinked β-CD. Crosslinked β-CD was made by adipic acid. When milk was treated with 1% crosslinked β-CD with 400 rpm mixing speed at 10°C for 5 min, 93.13% of cholesterol was removed. At the lower than 10°C mixing, the rate of cholesterol removal was significantly lower than that above 10°C mixing temperature ($p<0.05$). Mixing speed did not affect the rate of cholesterol removal . In recycling study, when crosslinked β-CD was repeatedly used five times, the rate of cholesterol removal was about 92.32% in the range of 92.13 to 92.51%. Therefore, the present study indicated that optimum conditions for cholesterol removal in milk using crosslinked β-CD were 1% β-CD, 10°C mixing temperature, 5 min mixing time, and 400 rpm mixing speed, which resulted in 92.39% of cholesterol removal. In conclusion, the crosslinked β-CD could be efficiently recyclable for cholesterol removal in milk.

Key Words: Crosslinked β-CD, Cholesterol Removal, Recycling

T61 Effect of crosslinked β-cyclodextrin on cholesterol removal in cream. E. M. Han, S. H. Kim, J. Ahn, and H. S. Kwak*, *Sejong University*, *Seoul, Korea*.

This study was carried out to determine optimum conditions of four different factors (β-cyclodextrin (β-CD) concentration, mixing temperature, mixing time, and mixing speed) for cholesterol reduction in cream by crosslinked β-CD and to examine the recycling efficiency of the crosslinked β-CD. Crosslinked β-CD was manufactured with adipic acid. When cream was treated with 10% crosslinked β-CD at 40°C for 30 min with 1,400 rpm mixing speed, 90.72% of cholesterol was removed ($p < 0.05$). The rate of cholesterol removal was not significantly different among 10, 15 and 20% crosslinked β-CD addition, but those in 1 and 5% crosslinked β-CD addition resulted in a significantly lower rate ($p < 0.05$). There was no difference in the rate of cholesterol removal by mixing temperature (40 to 55°C), mixing time (30 to 50min), and mixing speed (1,200 to 1,600rpm). In subsequent study, the used crosslinked β-CD was applied into cream five times for recycling. The rates of cholesterol removal were in the range of 90.42 to 91.45% with no significant difference. Therefore, the present study indicated that optimum conditions for cholesterol removal of cream using the crosslinked β-CD were 10% β-CD concentration, 40°C mixing temperature, 30 min mixing time and 1,400 rpm mixing speed, and the average cholesterol removal reached 91.42%. In addition, the crosslinked β-CD could be efficiently recyclable for cholesterol removal in cream.

Key Words: Crosslinked β-CD, Cholesterol Removal, Cream

T62 The comparison of freeze drying and stirring processes for recycling of crosslinked β-cyclodextrin used for cholesterol removal in milk and cream. S. H. Kim, E. M. Han, J. Ahn, and H. S. Kwak*, *Sejong University*, *Seoul, Korea*.

This study was performed to compare two different processes on the recycling of crosslinked β-cyclodextrin (β-CD). The mixture of acetic acid and isopropanol (3:1) was used as a solvent. Freeze Drying is known as an efficient method in recycling β-CD because it can preserve ingredients for a long time, stabilize and make ingredients to rehydrate fast and completely. Stirring is the reverse way of removing cholesterol from milk and cream with using β-CD. When organic agitation is applied, it gets back cholesterol which is attached to the β-CD. For freeze drying process; cholesterol - crosslinked β-CD complex was freeze-dried for 6 hr, and solvent was added, ultrasonicated for 30min, stirred for 2hr with 100rpm for 50°C, and centrifuged. For stirring process, the complex was mixed with solvent and mixture, stirred at 400 rpm in room temperature for 20 min, and centrifuged. Freeze drying process needed more reaction time, while stirring process was rapid and easy. However, the rate of average cholesterol removal was 97.3% in milk and 97.82% in cream when the crosslinked β-CD recycled by freeze drying process was applied ten times repeatedly. In comparison, when stirring process was applied, it was significantly lower as 90.18% and 91.63% in milk and cream, respectively. In conclusion, the present study indicated that crosslinked β-CD could be efficiently recyclable in milk and cream over 90%.

Key Words: Crosslinked β-CD, Cholesterol Removal, Recycle

T63 Microencapsulated isoflavone to apply into milk and hypocholesterolemic effect. B. J Jeon, N. C. Kim, E. M. Han, and H. S. Kwak*, *Sejong University*, *Seoul, Korea*.

This study was designed to develop the microencapsulation of water-soluble isoflavone to apply into milk and to examine the hypocholesterolemic effect in rats. Coating material was medium-chain triglyceride (MCT) and core material was water-soluble isoflavone. The microencapsulation efficiency was 70.2% when the ratio of coating material to core material was 15:1. The isoflavone released from microcapsules was 8% at 4°C for 3 day storage. In vitro study, water-soluble isoflavone from microcapsules was released 4.0-9.3% at the range of 2 to 5 pHs for 60 min incubation. In simulated intestinal fluid, 87.6% of isoflavone was released at pH 8 for 40 min incubation. In sensory analysis, the scores of bitterness, astringency, and off-taste in encapsulated isoflavone-added milk were slightly but not significantly different from those in uncapsulated isoflavone-added milk. In blood analysis, total cholesterol was significantly decreased in isoflavone-added group compared with in control for 6 week feeding, however, no difference was found in blood HDL-cholesterol. The present study suggested that as a coating material, MCT was suitable for the microencapsulation of water-soluble isoflavone, and isoflavone showed blood cholesterol lowering effect.

Key Words: Microencapsulation, Water-Soluble Isoflavone, Hypocholesterolemic Effect

T64 Hydrolysis of isoflavone glycoside by β-galactosidase and stability in the form of microcapsule. N. C. Kim, B. J. Jeon, J. Ahn, and H. S. Kwak*, *Sejong University*, *Seoul, Korea*.

The objectives of this study were to find conditions for conversion to aglycone form which showed a high biological activity using β-galactosidase and to examine the stability of microencapsulated β-galactosidase and isoflavone in the simulated gastric and intestinal conditions in vitro. Three different β-galactosidases were tested and the conversion of isoflavone glycoside by selected β-galactosidase was determined by different factors, such as pH, temperature and time in incubation, and enzyme activity. The rate of conversion was about 35% in optimum conditions. For stability study, isoflavone and β-galactosidase were microencapsulated to prevent beany flavor and sweetness in milk. When microencapsulated isoflavone was incubated in simulated gastric fluid with the pH range of 2 to 4, isoflavone was released 6.5 to 5.9mg(9.3 to 8.4%) and the release of β-galactosidase was 0.41 to 0.40 unit/ml (13.7 to 13.3%), respectively. However, 84.4% of isoflavone was released and 80.7% of β-galactosidase were in simulated intestinal fluid after 3 hr incubation(pH7). The contents of aglycone converted from isoflavone glycoside were 2.6mg in control and 18.8mg in β-galactosidase containing sample. Finally, this study showed that the converted high amount of aglycone was found by microencapsulated β-galactosidase when incubated in simulated intestinal fluid. In addition, the result may improve milk digestion.

Key Words: Isoflavone, β-Galactosidase, Microencapsulation

Forages and Pastures: Additives, Nutrient Content, and Quality

T65 Addition of enzyme or/and wheat bran on fermentation characteristics and in vitro gas production of rice straw silage. J.-M. Lv*, W.-L. Hu, and J.-X. Liu, *Zhejiang University*, *Hangzhou, China*.

A two- way factorial trial was designed to study the technique aspects of ensiling rice straw (RS) mixed with Strawzyme (an experimental preparation of cell-wall degrading enzymes containing cellulase and xylanase) and wheat bran (WB). The WB was added at levels of 0, 3, 6 or 9 % (fresh basis), respectively, and the RS was untreated (C-0, C-3, C-6 and C-9), or treated with Strawzyme at level of 1300g/t DM (T-0, T-3, T-6, and T-9). The fermentation characteristics of ensiled rice straw were evaluated for pH value, percentage of ammonia N in total N and organic acid (lactic acid, butyric acid, acetic acid and propionate acid) content. The in vitro gas production technique (Menke et al, 1988) was utilized to assess the nutritive value of silages.

Addition of WB improved the fermentation quality and nutritive value of RS silage. The pH value, percentage of ammonia N in total N and butyric acid content were decreased ($p<0.05$) and the lactic acid content and in vitro gas production (GP48) were increased with the increasing levels of WB ($p<0.01$). Compared to the silages added with WB alone, the RS silages added with WB along with Strawzyme treatment had a higher 48h GP and a faster rate of GP ($p<0.01$). The content of NDF was 3.3 and 5.3 percent unit lower in treatments T-6 and T-9 than in C-6 and C-9, respectively ($p<0.01$). Proportion of ammonia N of total N was decreased by 37.9 or 15.5% ($p<0.05$), and the lactic acid was increased by 67.8 or 5.7% respectively ($p<0.01$), when Strawzyme plus 6 or 9% WB was added. It can be concluded that combination of Strawzyme with WB was more effective in the improvement of RS silage quality than addition of WB alone.

Key Words: Rice Straw Silage, Enzyme, Wheat Bran

T66 Effect of adding enzyme on fermentation quality and nutritive value of corn stover silage. J.-M. Lv*, W.-L. Hu, and J.-X. Liu, *Zhejiang University*, *Hangzhou, China*.

This experiment was carried out to assess the effect of adding enzyme on fermentation quality and nutritive value of corn stover silage. About 120 kg corn stover was used as materials. The DM content of the corn stover was 22.1% The CP, NDF and WSC in corn stover (on DM basis) were 8.1, 74.2 and 3.5%, respectively. The enzyme containing cellulase (3500 IU/g) and xylanase (450 IU/g) was added to corn stover to ensile at four levels: 0(control, C), 800(1), 1300(2), 1800(3) g/kgDM.

Materials for each treatment were ensiled in triplicate in experimental silos with capacity of 50 L. After ensiled for 40 days, silages were taken for analysis in terms of chemical composition, pH value, ammonia nitrogen and organic acid. The in vitro gas production technique (Menke et al, 1988) was utilized to assess the nutritive value of silages.

The result showed that while all corn stover silages were of good quality with low pH, low ammonia nitrogen (NH3-N) and high lactic acid content, enzyme addition reduced the NDF content, pH value and the ratio of NH3-N to the total N, meanwhile increased the concentration of lactic acid as well as the total organic acid. Compared to control, the NDF content was 6.5% and 3.2% ($p<0.05$) lower, and lactic acid content was 32.6% and 29.1% ($p<0.01$) higher for the silages in group 2 and 3, respectively. PH value and the ratio of NH3-N to total N in group 3 were lower than those in group C ($p<0.01$). Enzyme addition improved the silages's nutritive value be increasing in vitro gas production (GP) parameters. The organic matter digestibility (OMD) estimated from the GP was higher ($P<0.01$) for the enzyme added silages than for group C. In conclusion, enzyme addition can improve both fermentation quality and nutritive value of corn stover silage, in this experiment, the best addition level of enzyme was 1800g/tDM.

Key Words: Corn Stover Silage, Enzyme, Nutritive Value