M1 Development and validation of a rapid method for measurement of casein in raw milk using front-face fluorescence spectroscopy and chemometrics. Y. B. Ma and J. K. Amamcharla, Food Science Institute, Animal Sciences and Industry, Kansas State University, Manhattan, KS.

The casein content in raw milk is important for the industry as it influences the cheese yield. The casein content is determined by the difference between true protein and non-casein protein in raw milk. The objective of this study was to develop a rapid quantification method for casein in raw milk using front-face fluorescence spectroscopy (FFFS). To prepare milk samples for calibration, raw skim milk was obtained from Kansas State University’s dairy farm and ultrafiltered to increase the protein concentration. The casein content of retentate and permeate were measured by a reference method. The retentate and permeate were combined at different ratios to make 10 calibration samples with casein content ranging from 0.37 to 3.7%. Sample preparation for the FFFS involved thoroughly mixing 7 mL calibration sample with 0.6 mL acetic acid (10% wt/wt) to precipitate the casein. Sample mixture was vortexed and transferred immediately to a quartz cuvette. Tryptophan emission spectrum of the mixture was immediately measured by a spectrofluorometer with a 1% attenuator (excitation wavelength at 280 nm; emission wavelength range from 300 to 440 nm) at 25°C. The process was repeated twice to obtain a sample size of 20 for the calibration model. Prediction models were developed using principal component regression and partial least square regression (PLSR) and validated with the leave-one-out cross-validation (LOOCV). The principal component regression and PLSR models showed LOOVC correlation coefficients of 0.970 and 0.988, root mean square error (RMSE) of 0.39% and 0.24%, and ratio of prediction to deviation of 4.5 and 4.7, respectively. The developed models were independently validated by 5 raw milk samples collected on different days. Principal component regression and PLSR predictions had a mean difference of 0.12% and 0.11% casein compared with the reference method and RMSE of 0.19% and 0.19%, respectively. The mean bias of 2 prediction models is not significantly different from 0 ($P > 0.05$). The FFFS method showed potential quantification of casein in raw milk, but validation on a large sample set is further required.

Key Words: partial least square regression (PLSR), principal component regression, front-face fluorescence spectroscopy (FFFS)

M2 Hunter versus CIE color measurement systems for analysis of milk-based beverages. N. Cheng, D. Barbano, and M. A. Drake, 1North Carolina State University, Raleigh, NC, 2Cornell University, Ithaca, NY.

Both Hunter (L, a, b) and International Commission on Illumination (CIE; L*, a*, b*) color measurement systems are used for instrumental measurement of food color but which system is best for fluid milk is not known. The objective of our work was to determine the differences in sensitivity of Hunter and CIE methods at 2 different viewer angles for measurement for whiteness, red/green, and blue/yellow color of milk based beverages. Sixty combinations of milk-based beverages were formulated (2 replicates) with a range of fat level from 0.2 to 2%, true protein level from 3 to 5%, and casein as a percent of true protein from 5 to 80% to provide a wide range of milk-based beverage color. In addition, commercial skim, 1% and 2% fat HTST pasteurized fluid milks were analyzed. All beverage formulations were HTST pasteurized and cooled to 4°C before analysis. Measurement viewer angle (2 versus 10°) had very little impact on objective color measures of milk-based beverages with a wide range of composition for either the Hunter or CIE color measurement system. Temperature (4, 20, and 50°C) of color measurement had a large impact ($P < 0.05$) on the results of color measurement in both the Hunter and CIE measurement systems. The effect of milk beverage temperature on color measurement results was the largest for skim milk and the least for 2% fat milk ($P < 0.05$). This highlights the need for proper control of beverage serving temperature for sensory panel analysis of milk-based beverages with very low fat content and for control of milk temperature when doing objective color analysis for quality control in manufacture of milk-based beverages. The Hunter system of color measurement was more sensitive to differences in whiteness among milk based beverages than the CIE system ($P < 0.05$), while the CIE system was much more sensitive to differences in yellowness among milk based beverages ($P < 0.05$). There was little difference between the Hunter and CIE system in sensitivity to green/red color of milk based beverages. In defining milk based beverage product specifications for objective color measures for dairy product manufacturers, the viewer angle, color measurement system (CIE versus Hunter) and sample measurement temperature should be specified along with type of illuminant.

Key Words: color, milk, whiteness

M3 Optimizing the emulsification properties of heated whey protein isolate (WPI)-pectin complexes for emulsions containing 20% oil at pH 5.0. A. Kotchabhakdi and B. Vardhanabhuti, University of Missouri, Columbia, MO.

There has been increasing interest in developing food ingredients for clean-label applications. We have previously shown that heated whey protein and pectin complexes (HCPX) formed at pH above pI have improved emulsification properties and stability when emulsions contained 5% oil. However, it is not fully understood whether these HCPX could stabilize emulsions containing higher oil content as in sauces and salad dressings. The objective of this study was to optimize the emulsification properties of HCPX in emulsions containing 20% oil at pH 5.0. The HCPX were formed by heating mixed 3 wt% whey protein isolate (WPI) and pectin (0, 0.3, 0.45 wt%) at pH 5.5, 5.8, and 6.2 at 85°C for 15 min. Emulsions were made, followed by pH adjustment to 5.0. Final emulsions contained 20 wt% protein and 0 to 0.3 wt% pectin. Emulsification properties were assessed by measuring droplet size, ζ-potential, rheological properties and creaming stability. Emulsions stabilized by heated WPI without pectin had the average droplets sizes >36 μm and ζ-potentials ranging from −27.2 to −19.8 mV. They were not stable and separated into 2 layers within a few hours. The HCPX-stabilized emulsions showed significant improvement in emulsification properties and stability. Mean droplet sizes significantly decreased ($P < 0.05$) and ranged from 1.6 to 21 μm while droplets became more negatively charged with ζ-potential ranging from −37 to −40.9 mV. Both heating pH and pectin concentration during HCPX formation played important roles on the emulsification properties of the HCPX. The most stable emulsions (>30 d) were those stabilized by HCPX formed with 0.45% pectin at heating pH of 5.5 and 5.8. Formation pH also influenced the rheological behavior of the emulsions with those stabilized by HCPX formed at pH 6.2 being more viscous. These results indicate that emulsification properties of heated WPI and pectin complexes formed at pH > pI can be optimized to stabilize
emulsions containing higher oil content. They can be utilized as clean-label ingredients in applications such as sauces and dressings.

**M4 Evaluation of the drying kinetics of micellar casein concentrate and reduced-mineral micellar casein concentrate at different solids concentrations.** H. N. Vora* and L. E. Metzger, Dairy and Food Science Department, South Dakota State University, Brookings, SD.

Micellar casein concentrate (MCC) was prepared by microfiltration and diafiltration of skim milk to produce a retentate with approximately 22% total solids (95% casein as a percentage of true protein). Modified (reduced mineral) micellar casein concentrate (MMCC) was prepared by diluting the MCC retentate to 7% solids and injecting CO2 to pH 5.9 and ultrafiltered to produce a retentate with approx. 22% total solids. Three sets of trials were performed on separate skim milk lots for both MCC and MMCC. The drying kinetics of the MCC and MMCC from each trial were then studied using single droplet drying (SDD). The SDD approach involves a single droplet suspended on the tip of a glass filament, where changes in droplet diameter, mass, and temperature can be measured during drying. The aim of this study was to develop and compare a predictive model generated using SDD for MCC and MMCC which can be used as a tool to optimize the drying conditions and reduce costly plant trials when developing new ingredients with unique functional properties. In this study, 2 ± 0.05 μL droplets of MCC and MMCC were dried using SDD at 2 different levels of total solids: 10% and 20% at 90°C with hot air at a velocity of 0.8 m/s. Droplet diameter and mass change data were collected and processed using Adobe After Effects 7.0 to enable the extraction of images. Although the pattern of change in average diameter data obtained from SDD was same for both MCC and MMCC, there was a significant difference observed during the average diameter change between MCC and MMCC (P < 0.05) at both the solids level MCC showed a rapid change in average diameter compared with MMCC. The curves of average mass change obtained from SDD were plotted against time. It was observed that as the total solids level increases the drying time increases, which is mainly due to the formation of crust on the particle and subsequent slower moisture migration to the surface of the particle with higher total solids level in both MCC and MMCC.

**Key Words:** single droplet drying, micellar casein concentrate, reduced-mineral micellar casein concentrate

**M5 Whey proteins enhance color and stability of anthocyanin pigments.** G. Miyagusuku-Cruzado*, R. Jimenez-Flores, and M. M. Giusti, The Ohio State University, Columbus, OH.

Current trends show that the food industry is moving away from synthetic colorants and looking for natural alternatives. Anthocyanins (ACN) are plant flavonoids with vivid colors that range from red to blue, but their application as food colorants is restricted by their limited stability, particularly at pH close to neutral. Nevertheless, some studies have shown that ACN have higher stability in dairy systems than in buffers at the same pH. We hypothesized that milk components such as proteins can interact with ACN, and that certain ACN may have a higher affinity for proteins due to specific structural conformations. Our objective was to evaluate whether whey proteins can interact with ACN leading to enhanced color and stability. Model solutions were prepared by diluting ACN from different sources with pH 3 citric acid – Na2HPO4 buffer until a λvis-max absorbance of 0.7 was reached, followed by addition of whey protein isolate (WPI) at different concentrations (0, 0.01, 0.05, 0.1, 0.5 and 1.0 mg/mL model solution). Absorption spectra was measured after 15 min and color parameters (CIELab) were calculated using ColorBySpectra software. Model solutions were heated to 90°C for up to 500 min to test heat stability, with samples taken every 50 min. ACN content was measured using the pH differential method. Addition of WPI resulted in a significant absorption increase (P < 0.05) at the λvis-max up to 17%. Color of the model solutions was enhanced (ΔE >5), becoming noticeably darker with WPI concentrations as low as 0.05 mg/mL for Berberis holiviana, 0.5 mg/mL for purple corn and grape skin, and 1.00 mg/mL for black carrot and red cabbage. The absorption increase at the λvis-max was dependent on WPI concentration, some fitting a linear model while others an exponential one, suggesting that some ACN may have higher affinity for WPI than others. Also, WPI addition significantly increased thermal resistance of ACN (P < 0.05). Further studies will focus on different anthocyanin chemical structures and the possible utilization of acid whey to stabilize ACN pigments. This could facilitate the transition from synthetic colorants to natural and healthier alternatives.

**Key Words:** whey protein isolate (WPI), acid whey, natural colorant

**M6 Production and storage stability of liquid micellar casein concentrate.** A. R. A. Hammam* and L. E. Metzger, South Dakota State University, Brookings, SD.

Micellar casein is a high protein ingredient that can be used as a valuable source of intact casein in process cheese formulations. The objective of this study was to produce a highly concentrated micellar casein (HC-MC) and evaluate its storage stability. Skim milk was pasteurized at 72°C for 16 s and kept at ≤ 4°C until the following day when it was heated in a plate heat exchanger to 50°C and microfiltered with a ceramic GP MF system (0.1μm) in a feed and bleed mode to produce a 3 × MF retentate (1 kg of retentate:2 kg of permeate). Subsequently, the retentate was diluted 2× with soft-water (2 kg of water:1 kg of retentate) and again microfiltered at 50°C to a 3× concentration as described previously. The retentate was then cooled to 4°C, and stored overnight. The following day, the retentate was heated to 65°C and microfiltered in a recirculation mode until the total solid reached approximately 22%. Subsequently, the temperature was increased to 74°C and microfiltration was continued until the permeate flow rate reached less than 5 L/h. The HC-MC retentate was transferred at 74°C to sterilized vials and stored at 4°C. This trial was repeated 3 times using 3 separate batches of raw milk. During microfiltration, the mean cumulative SP removal in the first, second, and third stages was 46, 77, and 83%, respectively. The mean HC-MC at time zero contained 25.42% total solids (TS), 20.20% true protein (TP), 0.09% NPN, 0.55% NCN, 19.80% CN, 2.0% ash, 97.70% CN%TP, and 0.45% SP. The NCN content increased significantly (P < 0.05) from 0.55 to 0.76% during 2 mo of storage. The NPN also increased over time from 0.095% at time zero to 0.12% after 2 mo of storage. The mean aerobic bacterial count in HC-MC at time zero was 2.6 ± 0.16 log cfu/mL and increased to 3.5 ± 0.89 and 4.3 ± 0.97 log cfu/mL after 1 and 2 mo of storage, respectively. Coliform, yeasts, and mold were not detected at any time point. This study determined that HC-MC could be manufactured using ceramic MF membranes with over 25% TS and greater than 95% CN%TP. The impact of the small increase in NCN and NPN during 2 mo of storage on process cheese characteristics will be evaluated in subsequent studies.

**Key Words:** microfiltration, micellar casein, shelf life

**M7 Use of micro- and nano-bubbles for improving the functional properties of Greek-style yogurt.** K. S. Babu*, D. Z. Liu, and J. K. Amamcharla, Kansas State University, Manhattan, KS.
Increased awareness of health benefits has driven the popularity of Greek-style yogurt (GSY) in recent years. However, increased protein content in the GSY leads to increased graininess, higher viscosity, and chalky mouthfeel. The objective of this study was to investigate the efficiency of micro- and nano-bubbles for improving the physical, rheological, and functional properties of GSY. In this study, a custom-built system was used to incorporate micro- and nano-bubbles (MNB) in GSY. The base for GSY was formulated to a protein content of 10% (wt/wt) using nonfat dry milk, micellar casein concentrates, and water. Control GSY (C-GSY; GSY pumped through the positive displacement pump without attaching the MNB generator) and MNB-treated GSY (MNB-GSY) were compared and evaluated for physical, rheological characteristics such as apparent viscosity, % loss-of-structure (measure of the rate of thixotropic breakdown), syneresis, water-holding capacity (WHC) before and after storage at 5°C for 1, 2, 3, and 4 weeks. Two replicates of C-GSY and MNB-GSY were manufactured and the data were analyzed as repeated measures (SAS Institute Inc.). The density of freshly prepared MNB-GSY and C-GSY was 0.97 and 1.04 g/cm³, respectively. When compared with C-GSY, the syneresis, WHC, and grain counts were significantly different (P < 0.05) after the MNB treatment and subsequent storage time. After the wk 2, 3, and 4, the MNB-GSY samples showed ~58.4%, ~43.1%, and ~50.1% lesser apparent viscosity compared with the corresponding weeks C-GSY samples. The syneresis of the MNB-GSY was significantly lower (P < 0.05), ~19% than C-GSY after storage for 4 wk. After storage for 4 wk, the % loss-of-structure for the C-GSY and MNB-GSY was 32% and 20.1%, respectively. Before storage, the grain counts of C-GSY and MNB-GSY were ~143 and ~37 grain counts/g of yogurt, respectively. After storage for 4 wk, the grain counts of C-GSY and MNB-GSY were ~178 and ~4 grain counts/g of yogurt, respectively. Overall, the incorporation of MNB into GSY showed significant improvements in the rheological and functional properties of GSY.

Key Words: micellar casein concentrate, rheology, micro- and nano-bubbles

M8 Ratiometric fluorescence spectroscopy—a novel technique for rapid detection of bacterial endospores. N. Awasthi* and S. Anand, Midwest Dairy Food Research Center, Dairy and Food Science Department, South Dakota State University, Brookings, SD.

The current spore detection methods rely on cultural techniques, having limitations of time, efficiency, and sensitivity. Spore coat contains calcium dipicolinic acid (CaDPA) as a major constituent, which can serve as a biomarker for bacterial endospores. We report a rapid and sensitive technique for detection of bacterial endospores by using ratiometric fluorescence-based sensors. This method is based on the detection of CaDPA that enhances luminescence of lanthanide ion, when complexed with a semiconducting polymer. A CaDPA standard curve was generated at excitation-emission wavelength of λ284-λ528 by using Synergy 2 fluorescence spectrophotometer. Intensity was recorded after chelating semiconducting fluorescent polyfluorene (PFO) dots with terbium ions, sensitized by different volumes of CaDPA (0.1 μM). All trials were conducted in the replicates of 3 and mean ± SE were calculated. The standard curve so generated showed a linear relationship (R² = 0.98) in experimental concentration range of 2.5 to 25 nM of CaDPA, with corresponding intensity (a.u.) of 545 to 2130. Endospores of an aerobic spore former, Bacillus licheniformis ATCC 14580, were produced at 37°C for 15 d, on Brain Heart Infusion agar. The efficiency of sporulation was evaluated by spore staining and plating techniques. Total CaDPA content in spores was estimated after suspending reducing concentrations of spores (logs 9.0 through 1.0 cfu/mL., at 1-log intervals) in HPLC-grade water. For higher spore spiking levels such as 9.2 ± 0.03, 8.4 ± 0.05, 7.1 ± 0.13 and 6.3 ± 0.02 logs, the mean CaDPA content values, observed from the standard curve, were 9.4, 7.2, 6.2 and 5.3 nM, whereas, for lower levels of 4.2 ± 0.05, 3.1 ± 0.04, 2.0 ± 0.11, and 1.36 ± 0.09 logs, we observed 3.8, 3.3, 2.2 and 1.3 nM mean CaDPA content. Our results indicated a linear relationship of the CaDPA content of endospores with that of the endospore counts, and the standard curve of CaDPA concentration. This study provides a proof of concept for a potential application of this technique to rapidly detect bacterial endospores in dairy and food industry. Further studies are in progress in our laboratory to standardize this technique for dairy product matrices such as cheese, whey proteins, and powders.

Key Words: spore, fluorescence, Bacillus