Milk whey proteins play a critical role in immune defense and are beneficial for human nutrition and health. The aim of this study was to characterize the milk whey proteins and their potential activities among different buffalo breeds. In this work, a tandem mass tags (TMT) proteomic approach was used to identify the differences in the proteomic profiles of milk whey proteins in Murrah, Nili-Ravi and Mediterranean water buffalos. Of the 589 identified proteins, there were 64 differentially expressed proteins identified by ANOVA analysis in buffalo milk whey proteomes. The most abundant proteins were polymeric immunoglobulin receptor, α1-antiprotease, heat shock cognate 71 kDa protein, Acl-CoA-binding protein, pigment epithelium-derived factor, antithrombin-III, and α2-HS-glycoprotein in Mediterranean water buffalo milk, fibroblast growth factor-binding protein 1 in Murrah buffalo milk, while clusterin, actin cytoplasmic 2, peroxiredoxin-2, and sortilin in Nili-Ravi buffalo milk. Gene ontology annotation revealed that molecular function of differentially expressed proteins were protein binding, enzyme regulator activity, and molecular function regulator. Furthermore, pathway analysis indicated that most differentially expressed proteins participated in complement and coagulation cascades pathway, which are strongly related to immune function. In addition to providing insight into the complexity of the buffalo milk whey proteome and their potential physiological functions, our study provided the molecular evidence of nutritive differences among different buffalo breeds. The presence of greater abundance of immune-protection whey protein in Mediterranean water buffalo milk indicated that this high nutritive milk is a high-quality resource for dairy-based functional food exploitation.

Key Words: whey protein, water buffalo, proteomics

280 Quantitative difference in proteomic profiles of milk whey protein in Murrah, Nili-Ravi, and Mediterranean water buffalo. S. Li1, L. Li1, J. Liu1, Y. Yang1, and D. Ren1, 1Institute of Dairy Science, College of Animal Science, Zhejiang University, Hangzhou, Zhejiang, China, 2Water Buffalo Institute, Chinese Academy of Agricultural Science, Nanning, Guangxi, China, 3Institute of Animal Science and Veterinary Medicine, Anhui Academy of Agricultural Sciences, Hefei, Anhui, China.

Testing functional boundaries of dairy ingredients in protein-fortified dairy gel systems. H. Zheng1, W. Wang2, J. Lin3, and T. Mendes Borges4, 1Dairy Innovation Institute, Animal Science Department, California Polytechnic State University, San Luis Obispo, CA, 2Department of Wine, Food and Molecular Biosciences, Lincoln University, Christchurch, New Zealand.

The global high protein based food market is strong and it has been increasing since the last decade. According to the latest projection from “technavio,” the size of the market for high protein foods (2017–2021) will reach to 91.07 billion USD by 2021. In the current research, Greek-style yogurt (GSY, 6.0% protein content) and Requeijão processed cheese (RPC, 10% protein content) were chosen as high protein food models. Milk protein concentrate (MPC80), micellar casein concentrate (MCC), Ca-caseinate (CaCN), and whey protein concentrate (WPC80) were used as protein fortifiers for increasing the total protein contents of the 2 food models. Using mixture design approach, we studied the functional features of different dairy ingredients in terms of modulating textural attributes and stability of the yogurt and processed cheese systems. The GSY gel was prepared using both fermentation and GDL (glucono delta lactone) induced acidification methods. Principal Component Analysis (PCA) showed that there is no discrimination between fermented GSY and GDL acidified GSY made from different ingredients in terms of textural attributes. Mixture design counter plots (MDCPs) (P < 0.05) showed that the GYS syneresis rate was significantly reduced when MPC, WPC, and CaCN mixed at optimum proportion as a protein-fortifier bundle rather than using individual ingredients along indicating the synergistic effect among dairy protein ingredients. The RPC was prepared using direct acidification method, the MDCPs (P < 0.05) showed that MPC and MCC had a similar capability in terms of
enhancing the adhesiveness, MCC has unique functionality in increasing firmness and viscosity of the processed cheese comparing with MPC and CaCN. The current research demonstrated the synergistic functionalities of different dairy protein ingredients in protein fortified dairy gel systems, such knowledge may be used for creating “clean-label” or “all dairy” formulations.

**Key Words:** dairy protein ingredient, Greek style yogurt, processed cheese

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**282** Micellar casein concentrate: Purity, serum protein removal, and sensory properties. D. M. Barbano*1, and M.A. Drake2, 1Cornell University, Ithaca, NY, 2North Carolina State University, Raleigh, NC.

Micellar casein concentrate (MCC) and serum protein (SP) isolate (SPI) can be made from skim milk (SM) using ceramic (C) or spiral wound (SW) polymeric microfiltration (MF) membranes. MCC can be made at different purities (i.e., different SP removal). The purpose of this work was to demonstrate the relationship between values for SP removal and MCC purity. High quality raw SM contains about 3.2% true protein (TP), 2.6% casein, and 0.576% SP and contains about 82% casein as a percent of TP (CN%TP). SP removal (%) with SW and C MF membranes differs, with C membranes having higher SP removal in a 3 stage, 3X MF process at 50°C (72 versus 95% respectively). SP removal is expressed as total Kg SP in permeate removed divided by Kg SP in the original skim multiplied by 100 (measured by Kjeldahl). MCC purity is often expressed as CN%TP. A MF process at 72% versus 95% SP removal in a factory processing 908,000 kg of SM in a day would have a higher yield of 3768 versus 4967 kg of SP per day and a MCC purity of 94.2 and 98.9% CN%TP, respectively. Differences in MCC concentrate purity will have different effects depending on the application (e.g., cheese making versus shelf stable beverages). In retorted or ultrapasteurized beverages, flavor is important. Lower purity of MCC in high-heat-treated beverages may cause more heat induced off flavors due to thermal degradation of milk SP. The lower purity MCC (94.2 versus 98.9%) has a 5 to 6 fold higher SP concentration. The impact of MCC purity is magnified in higher protein beverages as the absolute concentration of the SP increases. Heat induced SP degradation products (e.g., H2S, methional) have very low sensory thresholds and higher concentrations of residual SP in MCC will be correlated with higher intensities of heat induced off flavors. In addition, the amount of SP in the final MCC will be influenced by thermal denaturation of SP in the pasteurization skin milk before MF, regardless of the type of membrane. Minimum HTST pasteurization will have an apparent increase in CN%TP from 82 to about 83% by Kjeldahl due to SP denaturation. Heat induced covalent binding of SP to casein micelles causes over estimation of MCC purity when measured by classical Kjeldahl methods, so HPLC or SDS-PAGE would provide more correct determination of MCC purity.

**Key Words:** microfiltration, micellar casein concentrate (MCC), serum protein concentrate

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**283** Effects of microfluidization on the enzyme coagulation properties of milk. A. J. Bucci1, D. L. Van Hekken*1, M. H. Tunick1,2, and P. M. Tomasula1, 1USDA, ARS, Wyndmoor, PA, 2Drexel University, Philadelphia, PA.

The chymosin-induced coagulation of microfluidized milk was evaluated to determine its potential in making high-moisture cheese. Raw, thermized (65°C, 15s) and HTST pasteurized (75°C, 15s) 3.0% (wt/wt) fat milk samples were microfluidized at 4 treatment conditions: 42C/75 MPa, 42C/125 MPa, 54C/125 MPa, and 54C/170 MPa; controls consisted of homogenized and homogenized (2-stages at 105 MPa) milk. Microfluidized and non-microfluidized milk samples were evaluated for alkaline phosphatase activity and median particle size. After chymosin addition, coagulation time, curd firmness and microstructure were examined. Microfluidization had varied and significant effects (P < 0.05) on the size reduction of fat droplets, coagulation time, and curd firmness, while either heat treatment alone did not. Alkaline phosphatase was inactivated in all samples except for the raw and thermized milk non-microfluidized controls and the 75 MPa treatment. Particle size decreased by half during 2-stage homogenization, and 15- to 20-fold after microfluidization yielding median area-weighted values, $d(3,2)$, of 7.9, 4.2, and 0.39 to 0.50 μm, respectively. Milk samples microfluidized at 42C and 75 or 125 MPa were similar to the controls in coagulation times and curd firmness. Compared with the controls, milk microfluidized at 54C and 125 or 170 MPa took 3 to 8 times longer to coagulate and had lower curd firmness, which indicated that protein matrix formation, a critical step in the production of cheese, had been altered. Scanning electron microscopy images of the chymosin curds illustrated that, compared with the controls, the use of nonthermal microfluidization at different pressures resulted in modified casein-lipid structures that reflected the altered interactions of the smaller sized lipid droplets and intact or fractured casein micelles. Use of this technique will help meet consumer demand for novel dairy products such as cheeses with yogurt-like textures, cheese snacks, and desserts.

**Key Words:** milk, microfluidization, chymosin coagulation

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**284** Effect of milk protein solution viscosities on electrospun fiber formation. S. Akkurt1,2, L. M. Bonnallie2, and P. M. Tomasula2, 1Food Science Department, Rutgers, The State University of New Jersey, New Brunswick, NJ, 2Dairy & Functional Foods Research Unit, United States Department of Agriculture, Agricultural Research Unit Service, Eastern Regional Research Center, Wyndmoor, PA.

Electrospinning has been used to produce edible fibrous mats from micro- or nanosized fibers of sodium caseinate (NaCAS) and calcium caseinate (CaCAS) with pullulan (PUL), a polysaccharide added to facilitate formation of the caseinate fibers. The effects of rheological properties on electrospun fiber morphology are known for many synthetic and water-soluble polymers; however, this information is not available for proteins in aqueous solution such as the milk proteins or in blends with polysaccharides, such as PUL. The objective of this study was to determine the dependence of specific viscosity on aqueous protein concentration, C, for neat NFDM, CaCAS and NaCAS and in blends with PUL, to identify the entanglement concentrations, Ce, the point at which the respective protein chains begin to interact, and to identify the C regions where fibers are formed. In this study, aqueous solutions of NFDM, CaCAS, NaCAS, and PUL were prepared at 20°C at C ranging from 1 to 20 wt% and then 1:1 blends of each protein and PUL solution were prepared with total C also ranging from 1 to 20 wt%. A syringe containing 3 mL of each solution was used to feed an electrospinning unit at flow rate of 3mL/h, and at voltage of 20 kV, with fibers forming a fibrous mat. From plots of specific viscosity as a function of C for the neat and blended protein solutions, 3 regions were identified: the semidilute unentangled (C < C*) semidilute entangled (C > C > C*) and the concentrated entangled (C > C > C*). In general, electrospaying (drops) was observed for the blended solutions at C < C*; followed by semidilute entangled region of beaded fibers, and fully formed fibers just after the transition to C. Fully formed fibers were not identified at C > C* for the neat protein solutions but formed powders.
since electrospraying behavior predominated over the C range due to the lower solution viscosities. This is the first detailed examination of the dependence of milk protein-based nanofiber morphology on the viscosity and concentration regimens.

**Key Words:** nanofibers, rheological properties, entanglements

285 **Comparison of yogurt gels made from various types of milk proteins.** N. Trusler*1, J. Lucey1,2, and M. Molitor1,2,1University of Wisconsin-Madison, Madison, WI, 2Center for Dairy Research, Madison, WI.

We wanted to explore if the concentration of minerals in milk protein products affected the functional properties such as in acid milk gels. Microfiltration (MF) was used to deplete whey proteins and minerals from acidified milk to create soluble casein isolate (SCI), which was compositionally similar to sodium caseinate. Yogurt gels were made from SCI, sodium caseinate, nonfat dry milk, and 4 commercial milk protein concentrates (MPC) that had some level of mineral reduction. All powders were rehydrated and standardized to 5% protein. Yogurt gels were made by inoculating milk with yogurt culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, and fermented at 40°C until pH 4.6 for the MPC, and to pH 4.3 for sodium caseinate and SCI (both of which were supplemented with peptone to aid fermentation). Rheological properties such as gel stiffness and loss tangent were measured during fermentation, and yield stress was measured upon reaching the target pH. Buffering capacity was measured using acid-base titrations to indicate levels of insoluble calcium phosphate. Soluble calcium levels in rehydrated samples were determined in ultrafiltered permeate by performing inductively coupled plasma spectrometry (ICP) on the permeate. Calcium depleted MPC had a lower buffering capacity than nonfat dry milk, sodium caseinate and SCI had similar (very low) buffering capacities. Calcium depleted MPC also had lower soluble calcium levels than nonfat dry milk, whereas sodium caseinate and SCI had similar but very low levels of soluble calcium. Gels made from MPC had similar gel stiffness to nonfat dry milk, but had higher yield stress, indicating a more cross-linked yogurt gel. Sodium caseinate and SCI had similar (weak) gel stiffness during fermentation, but sodium caseinate gels exhibited higher yield stress values than SCI. Overall, this MF process produced SCI with similar acid gelation characteristics to sodium caseinate.

**Key Words:** microfiltration, calcium depletion, functionality

286 **Performance of dairy and plant proteins in a model high-acid beverage system.** H. Jiang* and K. Burrington, Wisconsin Center for Dairy Research, Madison, WI.

The protein drink market reached more than $600 million wholesale in 2016 and is expected to continue to grow (Beverage Industry, May 2017). Increases in consumer interest in protein and an insurgence of new protein ingredients has led to the development of new protein based foods and beverages. Each protein ingredient has its unique set of functional and sensory properties, which can contribute to formulation challenges. In this study, the functional properties (heat stability, emulsion, viscosity, and foaming) of 30 commercial dairy- and plant- based protein powders were investigated. Based on heat stability testing at pH 3, 7 protein ingredients (whey protein concentrate and isolate, milk-derived whey, potato protein, soy protein, pea protein, and rice protein) were selected for a high acid beverage application. A mango flavored beverage formula with 87% water, 7% sugar and 5% protein was chosen. All the beverages were adjusted to pH 3 with phosphoric acid before heat treatment at 82.2°C for 2min through an HTST process in a pilot plant. The beverages were cold-filled into pre-sanitized bottles and stored at 4°C. One set of beverages was stored in a higher temperature (45°C) incubator for 2 weeks to simulate an accelerated shelf life study. Rice protein beverages showed phase separation immediately after processing. Pea and soy protein beverages phase separated when stored at 45°C. Whey protein and potato protein beverages stayed stable during storage at 45°C. Changes in color (measured by a colorimeter) with storage was observed for all beverages except the rice protein beverage. Viscosity of beverages was measured by a rheometer at one day after processing and after the 45°C storage. No significant difference in viscosity was observed before and after the shelf life test of non-separated beverages. Sensory evaluation showed that the plant protein beverages had more bitter and beany flavors compared with dairy protein. Whey proteins provide better functionalities, and cleaner taste in high acid beverages. Plant proteins may need more modifications to produce improved functional characteristics and sensory properties in high acid beverages.

**Key Words:** dairy protein, plant protein, beverage

286 **See Dairy Foods Processing Symposium (page 376)**