W68 Effect of processing on the milk fat globule membrane constituents. X. Elías-Argote* and R. Jiménez-Flores, *California Polytechnic State University San Luis Obispo*.

The milk fat globule membrane (MFGM) proteins have been studied extensively using proteomic techniques to characterize them and to find out their structure within the membrane. The MFGM constituents have the potential to offer excellent health benefits; however, few studies have highlighted the effects of processing on the MFGM constituents and the repercussion it may have on their functionality. In this study, we have analyzed milk throughout traditional processing. These include collection, refrigeration, and 2 types of commercial pasteurization. In addition, this work also includes a potential pasteurization method using pulsed light treatment. Milk was collected before reaching the storage unit and kept at 37°C. Samples were processed at storage temperature (4°C), batch and high temperature short time (HTST) pasteurizations, and by pulsed light treatment. The cream was separated, and milk fat globule size distribution was determined using laser light scattering in the skim milk and cream fractions of the 4 treatments. The MFGM was then extracted and analyzed using 2D-PAGE and LC-MS. Microscopic evaluations were performed using confocal microscopy. As the heat treatment increased, more casein and whey proteins were absorbed onto the membrane and observed in the final product. The particle size reflects small diameter globules remaining in the skim milk and greater variability in the size distribution with heat treatments. We observed that MFGM proteins, especially adipophilin and butyrophilin, are affected differently by processing. Their concentration in the MFGM decreases as pasteurization temperature increases.

Key Words: MFGM, proteomics, protein interactions

W69 Evaluation of vacuum packaging on particle size, particle density and solubility of dry dairy powders. H. Eshpari* and P. S. Tong, *California Polytechnic State University*, *San Luis Obispo*.

Dry dairy ingredients can have a long shelf life if packaged and stored properly. Vacuum packaging can be an attractive method for keeping quality and provides added value; because of the inherent compactness of the products. Vacuum packaged dry dairy ingredients may also have added ease of handling for end users. However little is known about the impact of vacuum packaging on the properties of dry dairy ingredients. The objective of this study was to determine the effects of vacuum packaging on particle size, particle density and solubility of 4 types of dry dairy ingredients. Commercial samples of nonfat dry milk powder, whole milk powder, buttermilk powder, and milk protein Isolate were repackaged in duplicate using multi-wall foil side gusseted bags under varying degrees of vacuum (1, 0.7, 0.4 bar) and a control with no vacuum, and then stored for 3 mo at 37°C and 60% relative humidity. Each powder was sampled and analyzed in duplicate for particle size, particle density and solubility upon receiving and after 3 mo storage. After 3 mo storage there were no significant differences in solubility and particle density of the powders, regardless of the vacuum level, but some significant differences in particle size of the powders (Table 1). Moreover, the trend of change in particle size is different for different powders. The data suggest that the proposed vacuum packaging method may be beneficial to maintain the quality of the powders studied.

Table 1. Significant changes observed in the particle size of the samples after 3 months storage

Powder	Changes observed	P-value
Nonfat dry milk	no vacuum > before packaging	0.03
Whole milk	1 bar vacuum > before packaging	0.02
Whole milk	0.7 bar vacuum > before packaging	0.00
Whole milk	0.4 bar vacuum > before packaging	0.00
Whole milk	no vacuum < before packaging	0.00
Whole milk	no vacuum < 1 bar vacuum	0.00

Key Words: vacuum packaging, dry dairy powders, physical properties

W70 A new cold gelation method for producing calcium-fortified whey protein gels. Y. C. Tseng and C. L. Hicks*, *University of Kentucky*, *Lexington*.

Whey proteins, with versatile functionalities and excellent nutritional values, are widely utilized in food applications. In the presence of calcium ions or an acidulating agent, preheated whey proteins form gels at ambient temperature. However, to obtain a good texture, currently cold-set calcium-induced whey protein gels are prepared by introducing Ca^{2+} into a whey protein solution through dialysis, which is an inconvenient and time-consuming process. Thus, the objective of this research was to develop a new procedure for making homogeneous calcium-fortified cold-set whey protein gels. Whey protein isolate suspensions (7% w/v, pH 7) were prepared with distilled water and heated at 80°C for 30 min. After cooling to room temperature, calcium carbonate was added to the protein solutions at various concentrations (0, 5, 10, 15 m*M*). Gelation was induced by adding 30 m*M* glucono-delta-lactone (GDL) to the protein solutions at 24°C. The kinetic profile of the gelling system was studied using a Bohlin VOR rheometer. Finished gels were subjected to textural profile analysis and measurements of water holding capacity, pH and color. Within 2 h after the addition of GDL, all samples were shown to have significant increase in storage modulus (G'), indicating the formation of a viscoelastic gel network. Homogeneous cold-set gels were obtained from all treatments 5h after the initiation of gelation. Calcium level (0–15 m*M*) was shown to affect ($P < 0.05$) the pH, L value (lightness), and textural profile of the final gels. As Ca^{2+} increased, final pH values of the gels increased from 4.7 (calcium free samples) to 5.8 (samples w/ 15 m*M* $CaCO_3$) while the respective L values reduced from 72.4 to 70.8. Gel breaking strength was greatly reduced as calcium level increased, with values ranging from 595g to 349g, respectively. Results indicate that GDL combining with calcium carbonate can be utilized as an effective coagulating agent for making cold-set whey protein gels having fortified calcium levels, less cooked flavors and homogeneous texture, which may expand future food applications of whey proteins.

Key Words: cold gelation, whey proteins, calcium-fortified

W71 Use of caseinomacropeptide quantification by high performance liquid chromatography to estimate cheese whey addition in fermented milk beverages. E. H. P. Andrade, M. O. Leite, M. R. Souza, L. M. Fonseca*, M. M. O. P. Cerqueira, C. F. A. M. Penna, T. Roza, and N. M. A. Silva, *Federal University of Minas Gerais*, *Belo Horizonte, Brasil*.

The objective of this study was to evaluate quantification of caseinomacropeptide (CMP) as a method to estimate cheese whey addition in fermented milk beverages. Samples of fermented milk beverages were prepared in laboratory with 4 levels of whey (0, 10, 20 and 40%) and fermented with yogurt culture. After refrigerated storage (8–10°C) at different times (0, 7, 14, and 21 d) the samples were analyzed by high performance liquid chromatography (HPLC) according to official Brazilian method used to detect whey in milk and milk powder, based on caseinomacropeptide quantification. When the whey levels were analyzed along the storage time, there is no difference ($P > 0.05$) between fermented milk (0% of whey) and the fermented milk beverages added with 10 and 20% of whey for 0, 7, 14 and 21 d in refrigeration. However, for fermented milk beverage added with 40% of whey and stored by 21 d, the CMP concentration was higher than expected compared with the times before ($P < 0.05$). This increase in CMP quantity can be due to nonspecific proteolysis promoted for culture bacteria, especially *Lactobacillus*. Thus, it is possible to use CMP detection by HPLC according to official Brazilian method used to detect cheese whey in milk and milk powder as a method to quantify whey added to fermented milk beverages until 14 d of refrigerated storage.

Key Words: fermented milk beverages, whey, high performance liquid chromatography

W72 Comparison of solubility with methods for determining denaturation in whey protein. M. D. Allen* and P. S. Tong, *California Polytechnic State University*, *San Luis Obispo*.

Functional properties of whey proteins are very important for ingredient selection when incorporating in a food system. Solubility is one of the most important functional properties to consider when selecting a whey protein ingredient, especially for beverage systems. Processing parameters are known to have an effect on protein solubility and are often manipulated in efforts to improve solubility. Protein structures of whey proteins are considered to have an effect on solubility. Specifically, the degree of denaturation of whey proteins is thought to play a role in solubility. Previous studies concluded that bicinchoninic acid (BCA) assay and fluorescence spectroscopy are two relatively economical analytical methods that can be used to quantify denaturation of whey protein in liquid whey. The purpose of this current research is to compare these methods of quantifying denaturation to functional solubility of whey protein. A split plot experimental design was utilized with complete randomization. Raw bovine milk was skimmed and enzyme coagulated at natural pH to separate the whey. Liquid whey was then split into three plots and each received one of the following treatments: mild heat/ freeze dry, mild heat/spray dry and high heat/spray dry. Heat treatment was applied to liquid whey prior to a concentration step. Heat treated whey samples were then concentrated and dried accordingly. Powders were analyzed for denaturation using BCA and fluorescence spectroscopy and for solubility using an Insolubility Index. Statistical analysis of data indicates that there are differences among the three treatments for fluorescence spectroscopy, BCA and insolubility index, shown in Table 1. Peak intensity increases with denaturation in fluorescence spectroscopy, percent soluble at pH 4.6 decreases with denaturation in BCA analysis and mL soluble increases with denaturation by the insolubility index.

Table 1. Solubility and Measurements of Denaturation in Whey Powder

Treatment	Fluorescence Spectroscopy- Peak Intensity	BCA- %Soluble @ pH 4.6	Insolubility Index- mL Insoluble
Low Heat/Freeze Dry	173.85	93.24	0.587
Low Heat/Spray Dry	169.40	93.93	0.878
High Heat/Spray Dry	225.65	56.08	0.820

Key Words: whey protein, denaturation, solubility

W73 Whey protein fractionation with supercritical CO_2: Process optimization. L. M. Bonnaillie* and P. M. Tomasula, *USDA, Agricultural Research Services, Eastern Regional Research Center*, *Wyndmoor, PA*.

Supercritical CO_2 (SCO_2) fractionation of commercial whey protein isolates (WPI), containing 20% α-lactalbumin (ALA) and 55% β-lactoglobulin (BLG) protein (w/w), into 2 fractions enriched with either ALA or BLG, generates new whey protein ingredients with enhanced functional and nutritional properties. For example, ALA-rich protein products have improved nutritional properties for use in infant and geriatric foods, while BLG-rich products have enhanced gelling properties. Prefatory studies with HCl showed that ALA formed aggregates under acidic conditions at 50–70°C, while BLG remained mostly soluble. The separation of aggregated ALA was optimized around pH 4.2 and 60°C where the difference between the rates of aggregation of ALA and BLG was maximized. SCO_2 dissolved in concentrated WPI solutions in a high-pressure 1-L reactor generates carbonic acid that causes the selective precipitation of ALA, without tainting the 2 protein fractions with residual acid (pH 6) after depressurization. A turbine impeller ensured fast thermodynamic equilibrium. Solution pH was lowered with increased pressure, P, and reduced WPI concentration, C, according to extensive calibration performed with 1–28% (w/w) WPI solutions up to 14 MPa and extrapolated to 2–10% WPI solutions up to 34 MPa (pH 4.2–5). The kinetics of aggregation of ALA and BLG were followed for up to 4 h as a function of time, T, C and pH. A systematic kinetic study and modeling of the SCO2-induced precipitation of both proteins in the multi-parameter process enabled the optimization of processing conditions to both maximize ALA aggregation while keeping BLG precipitation low, and the optimization of protein recovery and purity in both fractions. In the 60–65°C range, up to 60%-pure ALA and 80%-pure BLG were obtained, with total protein recoveries of up to 98% and 90%, respectively. ALA purity is limited due to a noted increase in BLG precipitation at high SCO_2 pressure compared with HCl-treatment, caused by possible pressure and/or anti-solvent effects. Kinetic models will be useful to design scaled-up batch or continuous versions of the WPI/SCO_2 fractionation process.

Key Words: whey protein, fractionation, carbon dioxide

W74 Effect of applying power ultrasounds during the thermal denaturation of whey proteins in the presence of buttermilk. M. Saffon*[1], M. Britten[2], and Y. Pouliot[1], [1]*STELA Dairy Research Center, Institute of Nutraceuticals and Functional Food (INAF), Université Laval*, *Québec, QC, Canada*, [2]*Food Research and Development Center (FRDC), Agriculture and Agri-Food Canada*, *St-Hyacinthe, Québec, Canada*.

The use of ultrasound treatments in food processing has been considered as an emergent potential alternative to heat treatments. It was hypothesized that power ultrasounds could affect heat-induced denaturation by

favoring aggregates remodeling or rearrangement during the aggregation process. Cheese whey and buttermilk were concentrated by ultrafiltration up to 9.5% (w/v) protein content. Mixtures with various whey to buttermilk protein ratios were adjusted to pH 4.6 and heated to 90°C for 25 min (including come-up time). Power ultrasounds (20 kHz probe) were applied for the last 15 min of the thermal denaturation treatment. After cooling, mixtures were homogenized 5 times at 9500 psi. Aggregated material was separated by centrifugation at 15000g for 20 min. Protein aggregation, water holding capacity (WHC), consistency index (k) and flow behavior index (n) were performed on the homogenized mixtures. All the experiments were repeated 3 times, statistical analysis of the data was performed using ANOVA and the results were considered significantly when $P < 0.05$. The use of power ultrasounds significantly increased the protein aggregation and this effect depended on the proportion of buttermilk protein in the mixture. The highest protein aggregation was obtained with 25:75 whey-buttermilk fractions (78.5% ± 1.8 and 87.0% ± 1.2 with ultrasound). Ultrasound treatment had no significant impact on the WHC of the aggregates and only a slight decrease (0.12 g water/g protein) was associated with increasing the buttermilk protein ratio. The use of ultrasound treatment significantly increased the k and decreased the n of the homogenized aggregate mixtures. The highest k was obtained with aggregates from 25:75 whey-buttermilk ratio (0.94 Pa.s ± 0.10 and 1.12 Pa.s ± 0.10 with ultrasound), which also displayed the lowest n (0.36 ± 0.04 and 0.30 ± 0.05 with ultrasound). Our results suggest that heat-denaturation in the presence of power ultrasounds may have affected the shape and size distribution of protein aggregates.

Key Words: whey, buttermilk, aggregation

W75 Partitioning of minerals and protein using dialysis at different temperatures. N. On-Nom*, A. Grandison, and M. Lewis, *University of Reading*, *Reading, Berkshire, UK*.

Partitioning of minerals and protein in pasteurized milk has been measured at different temperatures, using dialysis with PVDF membrane (MWCO of 250 kdal). Dialysis conditions of 4, 20 and 40°C from 24 to 96 h and at 60 and 80°C from 1 to 5 h were used to prepare a suitable soluble phase for estimating pH, ionic calcium and protein partitioning. Dialysis bags were placed into baby cans which contained milk sample, which were then heated for the required temperature and time. Immediately after heating, dialysates were removed as quickly as possible and then cooled for 24 h before pH and ionic calcium were measured. The results showed that pH and ionic calcium decreased as temperature increased. It was also observed there are slight increases in pH and ionic calcium when the time of dialysis was increased at the same temperature. To measure protein partitioning, reducing SDS-PAGE was used. Results showed that at 4°C, β-casein and traces of α-casein and κ-casein, as well as whey proteins (α-lactalbumin and β-lactoglobulin) were detected while only whey protein was found at 20, 40 and 60°C. No soluble protein was observed at all conditions of 80°C. However, it was found that the intensity of the protein bands increased with increasing dialysis time. In contrast, no proteins were detected in dialysates produced using Visking tubing, which had a lower MWCO of 12 kdal.

Key Words: dialysis, minerals, proteins

W76 Measurement of pH and ionic calcium at high temperatures and their effect on the heat stability of milk supplemented with calcium chloride. N. On-Nom*, M. Lewis, and A. Grandison, *University of Reading*, *Reading, Berkshire, UK*.

Calcium chloride was added to milk in the range (0 - 20 m*M* Ca) with no pH adjustment. These milk samples where then subjected to in-container heating in the range 60 to 120°C for 1 h. Dialysis was performed on these samples during heating to estimate pH and ionic calcium at each heating temperature. Dialysis bags were removed as soon as possible after the heat treatment. The heat treated samples were inspected to assess their heat stability and those which had coagulated were centrifuged to produce a coagulum and supernatant. The results showed that calcium addition decreased pH and increased ionic calcium and that further reductions in both pH and ionic calcium occurred as temperature increased. Coagulation was observed to take place at lower calcium additions as the temperature increased. Furthermore, no coagulation took place if the pH was maintained above 6.30 and ionic calcium was below 0.46 m*M*, respectively, both measured at the heating temperature. However, when the milk samples cooled, pH and ionic calcium recovered, but not quite to their original value. Dialysis allows measurement of pH and ionic calcium at the heating temperature, which should improve understanding of their role in heat stability. Analysis of supernatants from coagulated milk samples heated at 115°C showed that over 90% of the milk protein coagulated, with only small amounts of whey protein and soluble casein remaining in the supernatant.

Key Words: heat stability, calcium, dialysis

W77 Production of single cell oil during growth of *Aspergillus* species on whey. A. Akpinar-Bayizit*, L. Yilmaz-Ersan, and T. Ozcan, *Uludag University, Department of Food Engineering*, *Bursa, Turkey*.

Oils and fats, lipids, form a class of natural compounds that serve as sources of energy and are considered an important component of our food. The demand for oils and fats, in general, is largely met from plant and animal sources. In view of this, utilization of industrial waste material into high value biological material is a cause of raw material limitation and increasing world population. Thus, conversion of food processing wastes into high-value and beneficiary products via microbial and enzymatic processes is gaining importance as they are ready-to-use substrates. Microbial lipids (SCO) with similar properties to vegetable or animal oils can be produced by microorganisms utilizing various carbon sources. Dairy industry wastes are good substrates for waste valuation as they contain appropriate ingredients that support microbial growth. Whey is the serum separated during the curding of milk for cheese production and contains 5% lactose. This research was planned in order to use cheese whey in production of high-value microbial lipids by five *Aspergillus* species, namely *A. niger*, *A. oryzae*, *A. ruber*, *A. versicolor*, *A. parasiticus*. The lipid accumulation in biomasses of examined fungi varied from 1.10 to 7.72 g/100 g. The amount of saturated fatty acids was found highest in *A. parasiticus* (65.70%), whilst the highest amount of unsaturated fatty acids was in *A. niger* (62.72%). The fatty acid profile of SCO obtained from *A. parasiticus* and *A. versicolor* revealed a high percentage of unsaturated 20-carbon fatty acids. It was observed that longer chain polyunsaturated fatty acids, which are not found in whey, have been detected in SCOs of *Aspergillus* species. This sheds light on the possibilities of exploring these fungi, having the ability to synthesize longer chain fatty acids from oleic (octadecenoic) or linoleic (octadecadienoic) acids through desaturase and elongase activity, to be used as supplement to edible fats and oils, and for other non-edible industrial purposes.

Key Words: whey, single cell oil, *Aspergillus*