10 Manufacturing heat-stable whey protein isolate by clarification. G. Subbiah Prabhakaran*1, J. A. Lucey1,2, and M. Molitor2, 1University of Wisconsin, Madison, Madison, WI, 2Wisconsin Centre for Dairy Research, Madison, WI.

Residual fat (RF) in whey protein isolate (WPI) is detrimental for storage flavor and functional applications. Objective of this study was to remove RF without utilizing microfiltration. We believe that by demineralizing whey via ultrafiltration (UF), phospholipoprotein (PLP) fractions can be precipitated along with denatured proteins and removed by centrifugation. Ca is also removed which improves heat stability. By removing RF and hence reducing oxidation of PLPs, we expect the resultant WPI to have a longer shelf life. Demineralization was achieved by acidifying liquid WPC-34 to pH 4.5 using HCl and ultrafiltering it along with extensive diafiltration. Benchtop trials (n = 4, P < 0.05) studied effects of pH (4.1, 4.3, 4.5, 4.7, 4.9, 5.2), protein concentration (1, 2, 3, 5, 7, 9% wb) and conductivity (300, 500, 750, 1000, 1500 mS/cm) of demineralized UF retentate (UF-r) on precipitation of RF. The UF-r was adjusted to various combinations of pH, protein concentrations (PC) and conductivities and centrifuged on a bench scale at 7500 × g for 10 min. Sedimentation of RF was estimated by measuring turbidity of supernatants. RF precipitation was highest at PC ≤3% because of reduced viscosity. At pH 4.5–4.7, precipitation was maximum showing that denatured protein and PLPs coagulate at pH values close to isoelectric point of denatured proteins. Reducing conductivity, increased precipitation of RF indicating that more coagulation occurs with reduced charge screening. SDS-PAGE analysis indicated there was sedimentation of PLPs, caseins and denatured proteins while native whey proteins remained soluble. Thus, isoelectric precipitation was effective in removing RF from sweet whey. Results were used to scale up the process. 200 gal of sweet whey was demineralized and clarified using a pilot-scale desludging clarifier and spray dried to produce WPI powder (91% protein, 2.6% fat db). Test for heat stability showed that 98% protein remained soluble when a 5% protein solution was heated treated at 80°C for 30 min. Future work will compare functional, flavor and storage properties of this WPI with some commercially available WPIs.

Key Words: whey protein isolate, isoelectric precipitation, clarification.


Shelf-stable milk is consumed worldwide, and this market is expected to continue growing. One quality challenge for UHT milk shelf life is age gelation, which can be caused by bacterial heat-stable proteases (HSP) synthesized during the raw milk storage period before heat processing. Some Pseudomonas spp. are HSP producers and their ability to grow well at refrigeration temperature make them important spoilage organisms to control for UHT processors. Previous work from our group showed that lactose oxidase (LO), a commercial enzyme that produces hydrogen peroxide and lactobionic acid from lactose, can control bacterial growth in raw milk. In this research, we investigated the ability of LO to control HSP producer outgrowth and thus prevent, or delay, age gelation in UHT milk. Six strains of Pseudomonas spp. were selected based on their ability to synthesize HSP and used as a cocktail to inoculate both raw and sterile milk at a level of 1 × 105 cfu/mL. Samples were treated with and without LO, incubated for 3 or 4 d at 6°C, and monitored for cell count and pH. A heat treatment was applied after the incubation period, and particle size analysis and visual inspection were used to monitor gelation from HSP activity. Coagulation assessment -analyzed using Tukey’s HSD test- showed that in sterile milk, a LO treatment [0.12 g/L] was significantly different from the control (P < 0.05). In raw milk, however, a LO treatment of 0.24 g/L was needed to prevent gelation. The test was scaled up to 18.9 L pilot plant batches of raw milk, which were challenged with the Pseudomonas spp. cocktail, and treated with LO [0.24 g/L] for 3 d. Batches were then processed with a MicroThermics UHT unit, and bottles monitored for gelation at room temperature. Significant differences in particle size between the sample treated with LO and the control was observed as early as one month after processing (P < 0.05). These results demonstrate that LO can be used to prevent age gelation in UHT milk by HSP-producing Pseudomonas spp., representing an opportunity to improve quality and reduce post-production losses in the shelf-stable milk market sector.

Key Words: lactose oxidase, age gelation, UHT milk

12 Improving the extraction of dairy phospholipids by the combined effect of ultrasound pretreatment and switchable solvents. K. Rathnakumar*1, J. Ortega-Anaya*, R. Jimenez-Flores2, J. Reineke1, and S. I. Martinez-Monteaungdo1, 1South Dakota State University, Brookings, SD, 2The Ohio State University, Columbus, OH.

In the last decade, the consumption of phospholipids (PLs), a class of polar lipids, has been associated with important health benefits. Dairy by-products are an abundant source of PLs with potential for extraction and isolation. In this work, we report improvements in the extraction efficiency of PLs from Beta-serum, a dairy by-product, by the application of ultrasound (USD) before the extraction with switchable hydrophilicity solvent (SHS), these solvents can switch from hydrophobic to hydrophilic form by inserting the CO2. The ultrasound pretreatment consisted of the application of 3 levels of acoustic intensity (either 134.15 ± 1.20, 274.91 ± 2.47, or 385.67 ± 3.47 W/m2) for 4 min. After the pretreatment, the extraction of the lipid fraction was performed using a tertiary amine (N,N-dimethylcyclohexylamine, CyNMe2) as a switchable hydrophilicity solvent (SHS) at 3 different sample to SHS ratios (1/3, 1/6 and 1/12). The PLs were recovered by solid phase-microextraction, and the individual PLs were quantified by HPLC with charged aerosol detector. The effect of acoustic intensity (385.67 ± 3.47 W/m2) followed by SHS extraction using 1/12 ratio extracted 69.07 ± 0.11% of PLs, while only 7.57 ± 0.59% of the PLs were recovered without the ultrasound pretreatment. The recovered fraction of PLs mainly comprised of phosphatidylinositol (32%), phosphatidylethanolamine (30%), and sphingomyelin (37%) found higher compared with untreated samples. Scanning electron images, particle size, and gel electrophoresis revealed great disruption of the protein matrix that may release the PLs into the aqueous medium. The application of ultrasound prior the SHS extraction remarkably improved the recovery efficiency of PLs. The proposed method improves the extraction of dairy PLs and may help to expand the utilization of thereof.

Key Words: switchable solvent, ultrasound, phospholipids.


Milk protein concentrate (MPC) is a high-quality protein found in milk and a complete protein consisting of both casein and whey. MPC is known to have poor solubility and flowability due to physicochemical interactions. The cold plasma (CP) treatment is known to modify protein and thereby alter its functionality. The scope of this study is to improve functional characteristics of MPC after cold plasma treatment and understand mechanisms involved. In this study, MPC 85 powder was directly subjected to various treatment duration (15 min to 60 min) with cold plasma at 25°C, 120 W radio frequency (RF) power, and flow rates of 10 and 25 cm/s of argon and CO2. The samples (treated and untreated) were then subjected to various physicochemical tests including flowability, water activity, solubility, moisture content, dry mass, foaming capacity, stability, pH, wettability, zeta potential, water binding capacity and solubility (at 50, 22 and 5°C) in triplicates. The statistical analysis was done using ANOVA.
and the significant differences were calculated \( P \leq 0.05 \) using Tukey's HSD. Flow index significantly increased by 50% at 45 min. Foaming capacity and stability had a 63% \((157.33 \pm 257.33 \text{ mL/g})\) and 64% \((52.54 \text{ to } 86.11\%)\) increase till 30 min and then had a drop to 22% \((192.00 \text{ mL/g})\) at 60 min whereas the stability remained constant. The pH remained constant in the range of 6.8 - 7.0. There was an 80% decrease \((93 \text{ to } 18.67\%)\) in wettability and 38% \((45.16 \text{ to } 62\%)\) increase in water binding capacity after 45 min. Zeta potential did not follow a particular trend. The dry mass remained constant, but the moisture content decreased to 77% \((9 \text{ to } 2)\). The solubility increased significantly after CP treatment but the maximum increase was observed at different time points for each temperature. The solubility at 50 and 5°C had an increase of 16% and 97%, respectively. The flowability, wettability, foaming capacity and stability, water binding capacity improved following the CP treatment due to the change in protein structure. Results proved that the samples showed better functionality in comparison to untreated samples. RF cold plasma could provide an alternative non-thermal processing approach to modify functional properties of MPC 85 and can be tailored for specific applications.

**Key Words:** cold plasma, functionality, milk protein concentrate

14 **Shelf stability of milk protein gels created by pressure-assisted enzymatic gelation.** L. Wang* and C. I. Moraru, *Cornell University, Ithaca, NY.*

Recent studies showed that high-pressure processing \( (\text{HPP}) \) provides exciting opportunities for structure formation in high concentration protein foods, with minimal impact on their overall nutritional and sensory properties. In this work, a new concept of pressure-assisted enzymatic gelation of milk protein concentrates \( (\text{MPC}) \) was applied, with the goal of further enhancing the structure and stability of pressure-induced milk protein gels, utilizing their use for the manufacture of novel dairy products. MPC powder was reconstituted to form a 12.5% \((\text{wt/wt})\) protein solution. Calf chymosin \((45 \text{ IMCU/100g milk})\) was added to the samples. Immediately after chymosin addition, the samples were treated with HPP at 600 MPa at 5°C for 3 min, followed by a shelf life study of 28 d at 4 ± 0.2°C. Textural analysis and water holding capacity measurements were carried out on d 0, 7, 14, 21, and 28. The processing trials and measurements were conducted in triplicate. Statistical analyses were performed by ANOVA at a 95% confidence level. Pressurization of MPCs led to extensive protein aggregation and gel formation, in a much shorter time \( (3\text{min}) \) compared with conventional enzymatic coagulation \( (around 30 \text{ min}) \). The gel hardness of MPCs with added chymosin was 297 ± 61 g at the beginning of storage and remained at 410 ± 19 g after 7 d. The water holding capacity remained at 91 ± 1% during refrigerated storage. Without chymosin, HPP-created MPC gels had a lower gel hardness value, of 227 ± 54 g, which decreased by 5.9% to 213 ± 10 g during refrigerated storage. However, the water holding capacity remained at 100% during 28 d of refrigerated storage. These results clearly show that enzymatic coagulation under pressure can create gel structures that are stable during 28 d of refrigeration. These findings demonstrate that controlled, fast structural modification of high concentration protein systems can be obtained by pressure-assisted enzymatic treatment. Overall, this study provides insights into the possibility of using HPP for the development of milk-protein based products with novel structures and extended shelf life.

**Key Words:** high-pressure processing \( (\text{HPP}) \), milk protein gels, shelf stability

15 **A method to diagnose mid-infrared milk analyzer prediction equation performance.** M. Portnoy* and D. M. Barbano, *Department of Food Science, Northeast Dairy Food Research Center, Cornell University, Ithaca, NY.*

Mid-infrared \( (\text{MIR}) \) milk analyzers are used for milk payment and product testing. Our objective was to determine if a modified milk calibration sample set could be used to diagnose and identify weaknesses in both partial least squares \( (\text{PLS}) \) and traditional fixed-filter based predictions of milk component concentration. The modified milk calibration set \((14 \text{ samples with a wide range of fat, protein, lactose and urea})\) was formulated in an orthogonal design and all-lab mean reference chemistry that, allows the identification of specific weaknesses in MIR prediction equations that are due to incorrect compensation for variation in the background milk matrix effects of fat, protein, and lactose concentration. In the case of traditional fixed-filter prediction models, the calibration equations can be adjusted based on the results of analysis of the modified milk set to improve instrument accuracy, while in the case of PLS models, specific model weaknesses can be identified and pointed out to the PLS model developer. For traditional filter models that predict fat, protein, and lactose, the sensitivity of predicted values to a mismatch of the intercorrection factor settings with the instrument optic system characteristics caused the standard deviation of the difference \( (\text{SDD}) \) between instrument prediction and reference chemistry to be larger \((e.g., \text{SDD} \leq 0.004 \text{ vs } 0.021 \text{ for fat when intercorrection factor for protein on fat B differs by } 0.03)\) with systematic under or over estimation of the component being predicted at the ends of the concentration range of the interfering milk component. For PLS models, the inability of a PLS model to cancel out the background matrix variation effects of fat, protein, and lactose concentration on the parameter being predicted can be clearly identified and quantified. Based on this diagnostic data that can be produced by analysis of the modified milk samples, the population of milk sample spectra that need to be added to the PLS modeling population to improve the prediction accuracy of a PLS model measuring major milk components, or for prediction models for minor milk components \((e.g., \text{milk urea nitrogen or fatty acids})\) can be determined.

**Key Words:** mid-infrared, intercorrection factor, partial least squares models

16 **Impact of milk protein type and concentration on the composition, physical, and sensory properties of low-fat, high-protein ice cream.** L. R. Sipple*1, D. M. Barbano2, and M. A. Drake1, 1North Carolina State University, Raleigh, NC, 2Cornell University, Ithaca, NY.

The market for frozen desserts with added protein has grown in the last decade. Milk proteins are important in the development of ice cream structure. The objective of this study was to determine the effect of liquid dairy proteins on the composition, physical, and sensory properties of low-fat \((about 4\%)\), high-protein ice cream. Ice creams were formulated in duplicate with liquid micellar casein concentrate \( (\text{MCC}) \), milk protein concentrate \( (\text{MPC}) \), or whey protein isolate \( (\text{WPI}) \) to contain 3, 6, or 9% crude protein \( (\text{CP}) \), total nitrogen \( (X 6.38)\) for each protein type. The composition, color, particle size, and viscosity of ice cream mixes were determined. Mix was frozen on a continuous freezer, and the overrun and particle size of frozen ice creams were determined. The color, meltdown rate, and trained panel flavor and texture attributes were determined for ice creams following 0, 1, 2, and 3 mo storage. Ice cream made with WPI had higher mix viscosity \((P < 0.05)\), and lower overrun than MCC or MPC ice cream \((P < 0.05)\). WPI ice cream also had a larger mean particle size and wider particle size distribution before and after freezing than MCC and MPC ice creams \((P < 0.05)\). Vanilla flavor was highest in MCC ice creams \((P < 0.05)\) followed by MPC and then WPI ice cream mixes. Ice creams with lower protein had higher vanilla flavor \((3 > 6 > 9\% \text{CP})\) \((P < 0.05)\). Vanilla flavor also declined over time for all protein types and concentrations \((P < 0.05)\). Astringency increased with increasing CP, and WPI ice creams were higher in astringency than MCC or MPC ice creams \((P < 0.05)\). Cardboard flavor developed in ice creams over time, and this flavor intensity was higher in higher CP ice creams \((P < 0.05)\). Firmness, denseness, mouth coating, and melted viscosity increased with increasing CP \((P < 0.05)\), whereas crumbliness had an inverse relationship with CP. MCC ice cream was lower in mouth coating and melted viscosity than MPC and WPI ice creams \((P < 0.05)\). Over time, a decrease in crumbliness and mouth coating was observed in ice creams while denseness and smoothness increased.
Food flavor and aroma are significantly impacted by the presence of 4-vinylphenols (4VPs), volatile compounds with very low-perception thresholds produced by decarboxylation of hydroxycinnamic acids (HCAs) ubiquitous in nature. 4VPs can be found in dairy products such as cheese and yogurt, and when in low concentrations, they contribute positively to the flavor by adding complexity and uniqueness. In HCA-rich foods subjected to bacterial fermentation, it is key to select strains that will produce the desired sensory properties, highlighting the need for screening lactic acid bacteria (LAB) for decarboxylating ability. The decarboxylating activity of LAB strains from the Ohio State University–Parker Endowed Chair collection (137 strains) with potential to produce phenolic acid decarboxylase (22 strains) was evaluated after incubation with HCAs for 36 h at 32°C. Decarboxylation was monitored using a high-throughput spectrophotometric method based on hypsochromic shifts when HCAs are transformed into 4VPs. Spectrophotometric results were confirmed by HPLC-DAD-MS analyses, looking for longer retention times and shorter λ_{230-500} than their precursor HCA, and characteristic m/z. Enterococcus mundtii, Lactobacillus plantarum and Pediococcus pentosaceus were capable of decarboxylating p-coumaric, caffeic and ferulic acids producing their 4VP derivatives. Seven other strains were only capable of biotransforming p-coumaric and caffeic acid, 1 was able to decarboxylate only caffeic acid and 1 was able to decarboxylate only p-coumaric acid, while 10 strains were not able to biotransform any HCA. No strain in this study was capable of decarboxylating sinapic acid. p-Coumaric acid had the highest biotransformation efficiency, followed by caffeic acid and lastly ferulic acid. This is the first study showing decarboxylating activity by the E. mundtii strain. This work can help improve LAB strain selection for food applications, improving the sensory characteristics of fermented dairy products such as cheese and yoghurt, especially the ones formulated with HCA-rich fruit and vegetable extracts.

Key Words: microbial biotransformation, fermented food, flavor compound