



# Tech News Bulletin

126 N. Addison Ave., Elmhurst, IL 60126 ~ www.adpi.org ~ 630-530-8700

March 2014

Volume XXVIII No. 1

## **CHARACTERIZING ENDOGENOUS AND EXOGENOUS PEROXIDASE ACTIVITY FOR BLEACHING OF FLUID WHEY AND RETENTATE**

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*J. of Dairy Sci. 97(3): 1225. 2014.*

The lactoperoxidase (LP) system may be used to achieve the desired bleaching of fluid whey with the addition of low concentrations (<50 mg/kg) of hydrogen peroxide. The addition of an exogenous peroxidase (EP) to whey may also be used to aid in whey bleaching when the LP system is not fully active. The objectives were to monitor LP activity in previously refrigerated or frozen milk, fluid whey, and whey retentate (10% solids) and to evaluate peroxidase activity in fluid whey and whey retentate (10% solids), with and without additional EP (2, 1, or 0.5 dairy bleaching units), over a range of pH (5.5–6.5) and temperatures (4–60°C). Subsequent experiments were conducted to determine the relationship between enzyme activity and bleaching efficacy. Raw and pasteurized milk, fat-separated pasteurized whey, and whey retentate (10% solids) were evaluated for LP activity following storage at 4 or 20°C, using an established colorimetric method. A response surface model was applied to evaluate both endogenous and EP activity at various temperatures and pH in freshly manufactured whey and retentate. Refrigerated or frozen storage at the parameters evaluated did not affect LP activity in milk, whey, or retentate. In fluid whey, with and without added EP, as pH decreased (to 5.5) and temperature increased (to 60°C), peroxidase activity increased. Retentate with EP exhibited behavior similar to that of fluid whey: as pH decreased and temperature increased, activity increased. However, in retentate without EP, as pH increased and temperature increased, activity increased. Enzyme activity was negatively correlated to bleaching time (time for >80% norbixin destruction) in fluid whey but a linear relationship was not evident in retentate. When fluid whey is bleached enzymatically, if pH is decreased and temperature is increased, the rate of reaction increases (e.g., bleaching occurs in less time). When bleaching in retentate, a higher pH (pH 6.5 vs. pH 5.5) is desired for optimal bleaching by the LP system. Due to processing restraints, this may not be possible for all dairy producers to achieve and, thus, addition of EP could be beneficial to improve bleaching efficacy.

**POTENTIAL SOURCES OF MOUTH DRYING IN BEVERAGES FORTIFIED WITH DAIRY PROTEINS: A COMPARISON OF CASEIN- AND WHEY-RICH INGREDIENTS**

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J. of Dairy Sci. 97(3): 1233. 2014.*

Oral nutritional supplement drinks (ONS) are beverages high in dairy proteins that are prescribed to individuals at risk of malnutrition. Consumption of ONS is poor in elderly care facilities, with patients commenting that the sensory attributes of these drinks reduce their enjoyment and willingness to consume. Mouth drying is an attribute of ONS found to build with repeated consumption, which may further limit liking of these products. This study investigated the sources of drying sensations by sequential profiling, with a trained sensory panel rating a range of model milk systems and ONS over repeated sips and during after-effects. Sequential profiling found that fortification of milk with both caseinate and whey protein concentrate significantly increased the perception of mouth drying over repeated consumption, increasing by between 35 and 85% over consumption of 40 mL. Enrichment of ONS with either whey protein concentrate or milk protein concentrate to a total protein content of 8.7% (wt/wt) resulted in whey and casein levels of 4.3:4.4% and 1.7:7.0% respectively. The product higher in whey protein was substantially more mouth drying, implying that whey proteins may be the most important contributor to mouth drying in ONS. However, efforts to mask mouth drying of protein-fortified milk by increasing sweetness or fat level were unsuccessful at the levels tested. Increasing the viscosity of protein-fortified milk led to a small but significant reduction in mouth drying. However, this approach was not successful when tested within complete ONS. Further analysis is required into the mechanism of protein-derived mouth drying to mask negative sensations and improve the enjoyment and consumption of protein-rich ONS.

**USE OF MICELLAR CASEIN CONCENTRATE FOR GREEK-STYLE YOGURT MANUFACTURING: EFFECTS ON PROCESSING AND PRODUCT PROPERTIES**

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J. of Dairy Sci. 97(3): 1259. 2014.*

The objective was to develop and optimize an alternative make process for Greek-style yogurt (GSY), in which the desired level of protein was reached by fortification with micellar casein concentrate (MCC) obtained from milk by microfiltration. Two MCC preparations with 58 and 88% total protein (MCC-58 and MCC-88) were used to fortify yogurt milk to 9.80% (wt/wt) protein. Strained GSY of similar protein content was used as the control. Yogurt milk bases were inoculated with 0.02% (wt/wt) or 0.04% (wt/wt)

direct vat set starter culture and fermented until pH 4.5. The acidification rate was faster for the MCC-fortified GSY than for the control, regardless of the inoculation level, which was attributed to the higher nonprotein nitrogen content in the MCC-fortified milk. Steady shear rate rheological analysis indicated a shear-thinning behavior for all GSY samples, which fitted well with the power law model. Dynamic rheological analysis at 5°C showed a weak frequency dependency of the elastic modulus ( $G_2'$ ) and viscous modulus ( $G_3''$ ) for all GSY samples, with  $G_2' > G_3''$ , indicating a weak gel structure. Differences in the magnitude of viscoelastic parameters between the 2 types of GSY were found, with  $G_2'$  of MCC-fortified GSY  $<$   $G_2'$  of control, indicating a different extent of protein interactions in the 2 types of yogurt. Differences were also noticed in water-holding capacity, which was lower for the MCC-fortified GSY compared with the control, attributed to lower serum protein content in the former. Despite some differences in the physicochemical characteristics of the final product compared with GSY manufactured by straining, the alternative process developed here is a feasible alternative to the traditional GSY make process, with environmental and possibly financial benefits to the dairy industry.

### **COLD ENZYMATIC BLEACHING OF FLUID WHEY**

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*J. of Dairy Sci. 96(12): 7404. 2013.*

Chemical bleaching of fluid whey and retentate with hydrogen peroxide (HP) alone requires high concentrations (100–500 mg of HP/kg) and recent studies have demonstrated that off-flavors are generated during chemical bleaching that carry through to spray-dried whey proteins. Bleaching of fluid whey and retentate with enzymes such as naturally present lactoperoxidase or an exogenous commercial peroxidase (EP) at cold temperatures (4°C) may be a viable alternative to traditional chemical bleaching of whey. The objective was to determine the optimum level of HP for enzymatic bleaching (both lactoperoxidase and EP) at 4°C and to compare bleaching efficacy and sensory characteristics to HP chemical bleaching at 4°C. Selected treatments were subsequently applied for whey protein concentrate with 80% protein (WPC80) manufacture. Fluid Cheddar whey and retentate (80% protein) were manufactured in triplicate from pasteurized whole milk. The optimum concentration of HP (0 to 250 mg/kg) to activate enzymatic bleaching at 4°C was determined by quantifying the loss of norbixin. In subsequent experiments, bleaching efficacy, descriptive sensory analysis, and volatile compounds were monitored at selected time points. A control with no bleaching was also evaluated. Enzymatic bleaching of fluid whey and retentate at 4°C resulted in faster bleaching and higher bleaching efficacy (color loss) than bleaching with HP

alone at 250 mg/kg. Due to concentrated levels of naturally present lactoperoxidase, retentate bleached to completion (>80% norbixin destruction in 30 min) faster than fluid whey at 4°C (>80% norbixin destruction in 12 h). In fluid whey, the addition of EP decreased bleaching time. Spray-dried WPC80 from bleached wheys, regardless of bleaching treatment, were characterized by a lack of sweet aromatic and buttery flavors, and the presence of cardboard flavor concurrent with higher relative abundance of 1-octen-3-ol and 1-octen-3-one. Among enzymatically bleached WPC80, lactoperoxidase-bleached WPC80 contained higher relative abundance of 2,3-octadienone, 2-pentyl furan, and hexanal than those bleached with added EP. Bleach times, bleaching efficacy, and flavor results suggest that enzymatic bleaching may be a viable and desirable alternative to HP bleaching of fluid whey or retentate.

### **EFFECT OF MICROFILTRATION CONCENTRATION FACTOR ON SERUM PROTEIN REMOVAL FROM SKIM MILK USING SPIRAL-WOUND POLYMERIC MEMBRANES**

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*J. of Dairy Sci. 96(10): 6199. 2013.*

The objective was to determine the effect of concentration factor (CF) on the removal of serum protein (SP) from skim milk during microfiltration (MF) at 50°C using a 0.3- $\mu$ m-pore-size spiral-wound (SW) polymeric polyvinylidene fluoride (PVDF) membrane. Pasteurized (72°C for 16 s) skim milk was MF (50°C) at 3 CF (1.50, 2.25, and 3.00 $\times$ ), each on a separate day of processing starting with skim milk. Two phases of MF were used at each CF, with an initial startup-stabilization phase (40 min in full recycle mode) to achieve the desired CF, followed by a steady-state phase (90-min feed-and-bleed with recycle) where data was collected. The experiment was replicated 3 times, and SP removal from skim milk was quantified at each CF. System pressures, flow rates, CF, and fluxes were monitored during the 90-min run. Permeate flux increased (12.8, 15.3, and 19.0 kg/m<sup>2</sup> per hour) with decreasing CF from 3.00 to 1.50 $\times$ , whereas fouled water flux did not differ among CF, indicating that the effect of membrane fouling on hydraulic resistance of the membrane was similar at all CF. However, the CF used when microfiltering skim milk (50°C) with a 0.3- $\mu$ m polymeric SW PVDF membrane did affect the percentage of SP removed. As CF increased from 1.50 to 3.00 $\times$ , the percentage of SP removed from skim milk increased from 10.56 to 35.57%, in a single stage bleed-and-feed MF system. Percentage SP removal from skim milk was lower than the theoretical value. Rejection of SP during MF of skim milk with SW PVDF membranes was caused by fouling of the membrane, not by the membrane itself and differences in the foulant characteristic among CF

influenced SP rejection more than it influenced hydraulic resistance. They hypothesize that differences in the conditions near the surface of the membrane and within the pores during the first few minutes of processing, when casein micelles pass through the membrane, influenced the rejection of SP because more pore size narrowing and plugging occurred at low CF than at high CF due to a slower rate of gel layer formation at low CF. It is possible that percentage removal of SP from skim milk at 50°C could be improved by optimization of the membrane pore size, feed solution composition and concentration, and controlling the rate of formation of the concentration polarization-derived gel layer at the surface of the membrane during the first few minutes of processing.

### **COMPARISON OF FUNCTIONAL PROPERTIES OF 34% AND 80% WHEY PROTEIN AND MILK SERUM PROTEIN CONCENTRATES**

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J. of Dairy Sci. 96(9): 5522. 2013.*

This study compared the functional properties of serum protein concentrate (SPC) with whey protein concentrate (WPC) made from the same milk and with commercial WPC. The experimental SPC and WPC were produced at 34% or 80% protein from the same lot of milk. Protein contents of WPC and SPC were comparable; however, fat content was much lower in SPC compared with WPC and commercial WPC. The effect of drying methods (freeze vs. spray drying) was studied for 34% WPC and SPC. Few differences due to drying method were found in turbidity and gelation; however, drying method made a large difference in foam formation for WPC but not SPC. Between pH 3 and 7, SPC was found to have lower turbidity than WPC; however, protein solubility was similar between SPC and WPC. Foaming and gelation properties of SPC were better than those of WPC. Differences in functional properties may be explained by differences in composition and extent of denaturation or aggregation.

### **CRYSTALLIZATION IN LACTOSE REFINING—A REVIEW**

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J. of Food Sci. 79(3): R257. 2014.*

In the dairy industry, crystallization is an important separation process used in the refining of lactose from whey solutions. In the refining operation, lactose crystals are separated from the whey solution through nucleation, growth, and/or aggregation. The rate of crystallization is determined by the combined effect of crystallizer design, processing parameters, and impurities on the kinetics of the process. This review summarizes studies on lactose crystallization, including the mechanism, theory of crystallization,

and the impact of various factors affecting the crystallization kinetics. In addition, an overview of the industrial crystallization operation highlights the problems faced by the lactose manufacturer. The approaches that are beneficial to the lactose manufacturer for process optimization or improvement are summarized in this review. Over the years, much knowledge has been acquired through extensive research. However, the industrial crystallization process is still far from optimized. Therefore, future effort should focus on transferring the new knowledge and technology to the dairy industry.

### **THE EFFECT OF FEED SOLIDS CONCENTRATION AND INLET TEMPERATURE ON THE FLAVOR OF SPRAY DRIED WHEY PROTEIN CONCENTRATE**

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*J. of Food Sci. 79(1): C19. 2014.*

Previous research has demonstrated that unit operations in whey protein manufacture promote off-flavor production in whey protein. The objective of this study was to determine the effects of feed solids concentration in liquid retentate and spray drier inlet temperature on the flavor of dried whey protein concentrate (WPC). Cheddar cheese whey was manufactured, fat-separated, pasteurized, bleached (250 ppm hydrogen peroxide), and ultrafiltered (UF) to obtain WPC80 retentate (25% solids, wt/wt). The liquid retentate was then diluted with deionized water to the following solids concentrations: 25%, 18%, and 10%. Each of the treatments was then spray dried at the following temperatures: 180 °C, 200 °C, and 220 °C. The experiment was replicated 3 times. Flavor of the WPC80 was evaluated by sensory and instrumental analyses. Particle size and surface free fat were also analyzed. Both main effects (solids concentration and inlet temperature) and interactions were investigated. WPC80 spray dried at 10% feed solids concentration had increased surface free fat, increased intensities of overall aroma, cabbage and cardboard flavors and increased concentrations of pentanal, hexanal, heptanal, decanal, (E)2-decenal, DMTS, DMDS, and 2,4-decadienal ( $P < 0.05$ ) compared to WPC80 spray dried at 25% feed solids. Product spray dried at lower inlet temperature also had increased surface free fat and increased intensity of cardboard flavor and increased concentrations of pentanal, (Z)4-heptenal, nonanal, decanal, 2,4-nonadienal, 2,4-decadienal, and 2- and 3-methyl butanal ( $P < 0.05$ ) compared to product spray dried at higher inlet temperature. Particle size was higher for powders from increased feed solids concentration and increased inlet temperature ( $P < 0.05$ ). An increase in feed solids concentration in the liquid retentate and inlet temperature within the parameters evaluated decreased off-flavor intensity in the resulting WPC80.

**THE INFLUENCE OF BLEACHING AGENT AND TEMPERATURE ON BLEACHING EFFICACY AND VOLATILE COMPONENTS OF FLUID WHEY AND WHEY RETENTATE**

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*J. of Food Sci. 78(10): C1535. 2013.*

Fluid whey or retentate are often bleached to remove residual annatto Cheddar cheese colorant, and this process causes off-flavors in dried whey proteins. This study determined the impact of temperature and bleaching agent on bleaching efficacy and volatile components in fluid whey and fluid whey retentate. Freshly manufactured liquid whey (6.7% solids) or concentrated whey protein (retentate) (12% solids, 80% protein) were bleached using benzoyl peroxide (BP) at 100 mg/kg (w/w) or hydrogen peroxide (HP) at 250 mg/kg (w/w) at 5 °C for 16 h or 50 °C for 1 h. Unbleached controls were subjected to a similar temperature profile. The experiment was replicated three times. Annatto destruction (bleaching efficacy) among treatments was compared, and volatile compounds were extracted and separated using solid phase microextraction gas chromatography mass spectrometry (SPME GC-MS). Bleaching efficacy of BP was higher than HP ( $P < 0.05$ ) for fluid whey at both 5 and 50 °C. HP bleaching efficacy was increased in retentate compared to liquid whey ( $P < 0.05$ ). In whey retentate, there was no difference between bleaching with HP or BP at 50 or 5 °C ( $P > 0.05$ ). Retentate bleached with HP at either temperature had higher relative abundances of pentanal, hexanal, heptanal, and octanal than BP bleached retentate ( $P < 0.05$ ). Liquid wheys generally had lower concentrations of selected volatiles compared to retentates. These results suggest that the highest bleaching efficacy (within the parameters evaluated) in liquid whey is achieved using BP at 5 or 50 °C and at 50 °C with HP or BP in whey protein retentate.

**USE OF WHEY PROTEIN SOLUBLE AGGREGATES FOR THERMAL STABILITY—  
A HYPOTHESIS PAPER**

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*J. of Food Sci. 78(8): R1105. 2013.*

Forming whey proteins into soluble aggregates is a modification shown to improve or expand the applications in foaming, emulsification, gelation, film-formation, and encapsulation. Whey protein soluble aggregates are defined as aggregates that are intermediates between monomer proteins and an insoluble gel network or precipitate. The conditions under which whey proteins denature and aggregate have been extensively studied and can be used as guiding principles of producing soluble aggregates. These conditions are reviewed for pH, ion type and concentration, cosolutes, and

protein concentration, along with heating temperature and duration. Combinations of these conditions can be used to design soluble aggregates with desired physicochemical properties including surface charge, surface hydrophobicity, size, and shape. These properties in turn can be used to obtain target macroscopic properties, such as viscosity, clarity, and stability, of the final product. A proposed approach to designing soluble aggregates with improved thermal stability for beverage applications is presented.