

## THE EFFECT OF BLEACHING AGENTS ON THE DEGRADATION OF VITAMINS AND CAROTENOIDS IN SPRAY-DRIED WHEY PROTEIN CONCENTRATE

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Previous research has shown that bleaching affects flavor and functionality of whey proteins. The role of different bleaching agents on vitamin and carotenoid degradation is unknown. The objective of this study was to determine the effects of bleaching whey with traditional annatto (norbixin) by hydrogen peroxide (HP), benzoyl peroxide (BP), or native lactoperoxidase (LP) on vitamin and carotenoid degradation in spray-dried whey protein concentrate 80% protein (WPC80). An alternative colorant was also evaluated. Cheddar whey colored with annatto (15 mL/454 L of milk) was manufactured, pasteurized, and fat separated and then assigned to bleaching treatments of 250 mg/kg HP, 50 mg/kg BP, or 20 mg/kg HP (LP system) at 50°C for 1 h. In addition to a control (whey with norbixin, whey from cheese milk with an alternative colorant (AltC) was evaluated. The control and AltC wheys were also heated to 50°C for 1 h. Wheys were concentrated to 80% protein by ultrafiltration and spray dried. The experiment was replicated in triplicate. Samples were taken after initial milk pasteurization, initial whey formation, after fat separation, after whey pasteurization, after bleaching, and after spray drying for vitamin and carotenoid analyses. Concentrations of retinol,  $\alpha$ -tocopherol, water-soluble vitamins, norbixin, and other carotenoids were determined by HPLC, and volatile compounds were measured by gas chromatography-mass spectrometry. Sensory attributes of the rehydrated WPC80 were documented by a trained panel. After chemical or enzymatic bleaching, WPC80 displayed 7.0 to 33.3% reductions in retinol,  $\beta$ -carotene, ascorbic acid, thiamin,  $\alpha$ -carotene, and  $\alpha$ -tocopherol. The WPC80 bleached with BP contained significantly less of these compounds than the HP- or LP-bleached WPC80. Riboflavin, pantothenic acid, pyridoxine, nicotinic acid, and cobalamin concentrations in fluid whey were not affected by bleaching. Fat-soluble vitamins were reduced in all wheys by more than 90% following curd formation and fat separation. With the exception of cobalamin and ascorbic acid, water-soluble vitamins were reduced by less than 20% throughout processing. Norbixin destruction, volatile compound, and sensory results were consistent with previous studies on bleached WPC80. The WPC80 colored with AltC had a similar sensory profile, volatile compound profile, and vitamin concentration as the control WPC80.

**STRUCTURAL CHANGES INDUCED BY HIGH-PRESSURE PROCESSING IN MICELLAR CASEIN AND MILK PROTEIN CONCENTRATES**

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Reconstituted micellar casein concentrates and milk protein concentrates of 2.5 and 10% (wt/vol) protein concentration were subjected to high-pressure processing at pressures from 150 to 450 MPa, for 15 min, at ambient temperature. The structural changes induced in milk proteins by high-pressure processing were investigated using a range of physical, physicochemical, and chemical methods, including dynamic light scattering, rheology, mid-infrared spectroscopy, scanning electron microscopy, proteomics, and soluble mineral analyses. The experimental data clearly indicate pressure-induced changes of casein micelles, as well as denaturation of serum proteins. Calcium-binding  $\alpha$ S1- and  $\alpha$ S2-casein levels increased in the soluble phase after all pressure treatments. Pressurization up to 350 MPa also increased levels of soluble calcium and phosphorus, in all samples and concentrations, whereas treatment at 450 MPa reduced the levels of soluble Ca and P. Experimental data suggest dissociation of calcium phosphate and subsequent casein micelle destabilization as a result of pressure treatment. Treatment of 10% micellar casein concentrate and 10% milk protein concentrate samples at 450 MPa resulted in weak, physical gels, which featured aggregates of uniformly distributed, casein substructures of 15 to 20 nm in diameter. Serum proteins were significantly denatured by pressures above 250 MPa. These results provide information on pressure-induced changes in high-concentration protein systems, and may inform the development on new milk protein-based foods with novel textures and potentially high nutritional quality, of particular interest being the soft gel structures formed at high pressure levels.

**EFFECT OF SKIM MILK TREATED WITH HIGH HYDROSTATIC PRESSURE ON PERMEATE FLUX AND FOULING DURING ULTRAFILTRATION**

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Ultrafiltration (UF) is largely used in the dairy industry to generate milk and whey protein concentrate for standardization of milk or production of dairy ingredients. Recently, it was demonstrated that high hydrostatic pressure (HHP) extended the shelf life of milk and improved rennet coagulation and cheese yield. Pressurization also modified casein micelle size distribution and promoted aggregation of whey proteins. These changes are likely to affect UF performance. Consequently, this study determined the effect of skim milk pressurization (300 and 600 MPa, 5 min) on UF performance in terms of permeate flux decline and fouling. The effect of HHP on milk proteins was first studied and UF was performed in total recycle mode at different transmembrane pressures to determine optimal UF operational parameters and to evaluate the effect of pressurization on critical and limiting fluxes. Ultrafiltration was also performed in concentration mode at a transmembrane pressure of 345 kPa for 130 or 140 min to evaluate the decline of permeate flux and to determine fouling resistances. It was observed that average casein micelle size decreased by 32 and 38%, whereas  $\beta$ -lactoglobulin denaturation reached 30 and 70% at 300 and 600 MPa, respectively. These results were

directly related to UF performance because initial permeate fluxes in total recycle mode decreased by 25% at 300 and 600 MPa compared with nonpressurized milk, critical flux, and limiting flux, which were lower during UF of milk treated with HHP. During UF in concentration mode, initial permeate fluxes were 30% lower at 300 and 600 MPa compared with the control, but the total flux decline was higher for nonpressurized milk (62%) compared with pressure-treated milk (30%). Fouling resistances were similar, whatever the treatment, except at 600 MPa where irreversible fouling was higher. Characterization of the fouling layer showed that caseins and  $\beta$ -lactoglobulin were mainly involved in membrane fouling after UF of pressure-treated milk. Our results demonstrate that HHP treatment of skim milk drastically decreased UF performance.

### **EFFECT OF PASTURE VERSUS INDOOR FEEDING SYSTEMS ON QUALITY CHARACTERISTICS, NUTRITIONAL COMPOSITION, AND SENSORY AND VOLATILE PROPERTIES OF FULL-FAT CHEDDAR CHEESE**

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The purpose of this study was to investigate the effects of pasture-based versus indoor total mixed ration (TMR) feeding systems on the chemical composition, quality characteristics, and sensory properties of full-fat Cheddar cheeses. Fifty-four multiparous and primiparous Friesian cows were divided into 3 groups (n =18) for an entire lactation. Group 1 was housed indoors and fed a TMR diet of grass silage, maize silage, and concentrates; group 2 was maintained outdoors on perennial ryegrass only pasture (GRS); and group 3 was maintained outdoors on perennial ryegrass/white clover pasture (CLV). Full-fat Cheddar cheeses were manufactured in triplicate at pilot scale from each feeding system in September 2015 and were examined over a 270-d ripening period at 8°C. Pasture-derived feeding systems were shown to produce Cheddar cheeses yellower in color than that of TMR, which was positively correlated with increased cheese  $\beta$ -carotene content. Feeding system had a significant effect on the fatty acid composition of the cheeses. The nutritional composition of Cheddar cheese was improved through pasture-based feeding systems, with significantly lower thrombogenicity index scores and a greater than 2-fold increase in the concentration of vaccenic acid and the bioactive conjugated linoleic acid C18:2 cis-9,trans-11, whereas TMR-derived cheeses had significantly higher palmitic acid content. Fatty acid profiling of cheeses coupled with multivariate analysis showed clear separation of Cheddar cheeses derived from pasture-based diets (GRS or CLV) from that of a TMR system. Such alterations in the fatty acid profile resulted in pasture-derived cheeses having reduced hardness scores at room temperature. Feeding system and ripening time had a significant effect on the volatile profile of the Cheddar cheeses. Pasture-derived Cheddar cheeses had significantly higher concentrations of the hydrocarbon toluene, whereas TMR-derived cheese had significantly higher concentration of 2,3-butanediol. Ripening period resulted in significant alterations to cheese volatile profiles, with increases in acid-, alcohol-aldehyde-, ester-, and terpene-based volatile compounds. This study has demonstrated the benefits of pasture-derived feeding systems for production of Cheddar cheeses with enhanced nutritional and rheological quality compared with a TMR feeding system.

**EFFECT OF DENATURED WHEY PROTEIN CONCENTRATE AND ITS FRACTIONS ON CHEESE COMPOSITION AND RHEOLOGICAL PROPERTIES**

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The objectives were (1) to assess the effect of a denatured whey protein concentrate (DWPC) and its fractions on cheese yield, composition, and rheological properties, and (2) to separate the direct effect of the DWPC or its fractions on cheese rheological properties from the effect of a concomitant increase in cheese moisture. Semihard cheeses were produced at a laboratory scale, and mechanical properties were characterized by dynamic rheometry. Centrifugation was used to induce a moisture gradient in cheese to separate the direct contribution of the DWPC from the contribution of moisture to cheese mechanical properties. Cheese yield increased and complex modulus ( $G^*$ ) decreased when the DWPC was substituted for milk proteins in milk. For cheeses with the same moisture content, the substitution of denatured whey proteins for milk proteins had no direct effect on rheological parameters. The DWPC was fractionated to evaluate the contribution of its different components (sedimentable aggregates, soluble component, and diffusible component) to cheese yield, composition, and rheological properties. The sedimentable aggregates were primarily responsible for the increase in cheese yield when DWPC was added. Overall, moisture content explained to a large extent the variation in cheese rheological properties depending on the DWPC fraction. However, when the effect of moisture was removed, the addition of the DWPC sedimentable fraction to milk increased cheese complex modulus. Whey protein aggregates were hypothesized to act as active fillers that physically interact with the casein matrix and confer rigidity after pressing.

**THE EFFECT OF HOMOGENIZATION PRESSURE ON THE FLAVOR AND FLAVOR STABILITY OF WHOLE MILK POWDER**

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Flavor is one of the key factors that can limit the application and shelf life of dried dairy ingredients. Many off-flavors are caused during ingredient manufacture that carry through into ingredient applications and decrease consumer acceptance. The objective was to investigate the effect of homogenization pressure on the flavor and flavor stability of whole milk powder (WMP). Whole milk powder was produced from standardized pasteurized whole milk that was evaporated to 50% solids (wt/wt), homogenized in 2 stages with varying pressures (0/0, 5.5/1.4, 11.0/2.8, or 16.5/4.3 MPa), and spray dried. Whole milk powder was evaluated at 0, 3, and 6 mo of storage at 21°C. Sensory properties were evaluated by descriptive analysis. Volatile compounds were analyzed by sorptive stir bar extraction with gas chromatography-mass spectrometry. Fat globule size in condensed whole milk and particle size of powders were measured by laser diffraction. Surface free fat, inner free fat, and encapsulated fat of WMP were measured by solvent extractions. Phospholipid content was measured by ultra-high-performance liquid chromatography–evaporative light scattering. Furosine in WMP was analyzed by ultra-high-performance liquid chromatography–mass spectrometry. Increased homogenization pressure

decreased cardboard and painty flavors, volatile lipid oxidation compound concentrations, fat globule size in condensed milk, surface free fat, and inner free fat in WMP. Encapsulated fat increased and phospholipid-to-encapsulated fat ratio decreased with higher homogenization pressure. Surface free fat in powders increased cardboard flavor and lipid oxidation. These results indicate that off-flavors were decreased with increased homogenization pressures in WMP due to the decrease in free fat. To decrease off-flavor intensities in WMP, manufacturers should carefully evaluate these parameters during ingredient manufacture.

### **EFFECTS OF ULTRASOUND TREATMENT ON PHYSIOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITIES OF WHEY PROTEIN–TOTAROL NANOPARTICLES**

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Totarol is a natural antimicrobial compound extracted from the heartwood of *Podocarpus totara*, a conifer native to New Zealand. The effects of whey protein–totarol nanoparticles treated with ultrasound on the physiochemical properties and the growth of *Staphylococcus aureus* were investigated. The particle size of whey protein–totarol nanoparticles was reduced by ultrasound treatment from  $31.24 \pm 5.31$  to  $24.20 \pm 4.02$  nm, and the size distribution was also narrowed by the treatment. Viscosity and modulus data indicated that the flow behaviors of whey protein–totarol nanoparticles seemed to be Newtonian and exerted a typical viscoelastic fluid at protein content of 15% (w/v). Rheological properties were more insensitive to ultrasonic time. Time-killing assays, agar diffusion tests, the cell membrane damage analysis, and microstructure were totarol nanoparticles after ultrasound treatment decreased from 4 to 2  $\mu\text{g/mL}$  compared with that without ultrasound treatment. Whey protein–totarol nanoparticles treated with ultrasound resulted in a significant ( $P < 0.05$ ) decrease in time killing after 24 h. The agar diffusion results showed that the inhibition zones of whey protein–totarol nanoparticles were 12 and 36 mm for untreated and treated with ultrasound, respectively. The cell membrane damages and the microstructure changes also proved that whey protein–totarol nanoparticles treated with ultrasound had strong antibacterial activities against *S. aureus* and that the antibacterial effectiveness enhanced with the increasing of ultrasonic time. These findings suggested that whey protein–totarol nanoparticles treated with ultrasound were more effective against *S. aureus* than untreated nanoparticles.

### **UTILIZING WHEY PROTEIN ISOLATE AND POLYSACCHARIDE COMPLEXES TO STABILIZE AERATED DAIRY GELS**

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Heated soluble complexes of whey protein isolate (WPI) with polysaccharides may be used to modify the properties of aerated dairy gels, which could be formulated into novel-textured high-protein desserts. The objective was to determine the effect of polysaccharide charge density and concentration within a WPI-polysaccharide complex on the physical properties of

aerated gels. Three polysaccharides having different degrees of charge density were chosen: low-methoxyl pectin, high-methoxyl type D pectin, and guar gum. Heated complexes were prepared by heating the mixed dispersions (8% protein, 0 to 1% polysaccharide) at pH 7. To form aerated gels, 2% glucono- $\delta$ -lactone was added to the dispersions of skim milk powder and heated complex and foam was generated by whipping with a handheld frother. The foam set into a gel as the glucono- $\delta$ -lactone acidified to a final pH of 4.5. The aerated gels were evaluated for overrun, drainage, gel strength, and viscoelastic properties. Without heated complexes, stable aerated gels could not be formed. Overrun of aerated gel decreased (up to 73%) as polysaccharide concentration increased from 0.105 to 0.315% due to increased viscosity, which limited air incorporation. A negative relationship was found between percent drainage and dispersion viscosity. However, plotting of drainage against dispersion viscosity separated by polysaccharide type revealed that drainage decreased most in samples with high-charge-density, low-methoxyl pectin followed by those with low-charge-density, high-methoxyl type D pectin. Aerated gels with guar gum (no charge) did not show improvement to stability. Rheological results showed no significant difference in gelation time among samples; therefore, stronger interactions between WPI and high-charge-density polysaccharide were likely responsible for increased stability. Stable dairy aerated gels can be created from WPI-polysaccharide complexes. High-charge-density polysaccharides, at concentrations that provide adequate viscosity, are needed to achieve stability while also maintaining dispersion overrun capabilities.

**Short communication: SENSITIVE DETECTION OF NORBIXIN IN DRIED DAIRY INGREDIENTS AT CONCENTRATIONS OF LESS THAN 1 PART PER BILLION**

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Norbixin is the water-soluble carotenoid in annatto extracts used in the cheese industry to color Cheddar cheese. The purpose of norbixin is to provide cheese color, but norbixin is also present in the whey stream and contaminates dried dairy ingredients. Regulatory restrictions dictate that norbixin cannot be present in dairy ingredients destined for infant formula or ingredients entering different international markets. Thus, there is a need for the detection and quantification of norbixin at very low levels in dried dairy ingredients to confirm its absence. A rapid method for norbixin evaluation exists, but it does not have the sensitivity required to confirm norbixin absence at very low levels in compliance with existing regulations. The current method has a limit of detection of 2.7  $\mu\text{g}/\text{kg}$  and a limit of quantification of 3.5  $\mu\text{g}/\text{kg}$ . The purpose of this study was to develop a method to extract and concentrate norbixin for quantification in dried dairy ingredients below 1  $\mu\text{g}/\text{kg}$  (1 ppb). A reverse-phase solid-phase extraction column step was applied in the new method to concentrate and quantify norbixin from liquid and dried WPC80 (whey protein concentrate with 80% protein), WPC34 (WPC, 34% protein), permeate, and lactose. Samples were evaluated by both methods for comparison. The established method was able to quantify norbixin in whey proteins and permeates (9.39  $\mu\text{g}/\text{kg}$  to 2.35  $\text{mg}/\text{kg}$ ) but was unable to detect norbixin in suspect powdered lactose samples. The newly developed method had similar performance to the established method for whey

proteins and permeates but was also able to detect norbixin in powdered lactose samples. The proposed method had a >90% recovery in lactose samples and a limit of detection of 28 ppt (ng/kg) and a limit of quantification of 94 ppt (ng/kg). The developed method provides detection and quantification of norbixin for dairy ingredients that have a concentration of <1 ppb.