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DETERMINATION OF MELAMINE IN MILK AND DAIRY PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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A simple, precise, accurate, and validated reverse-phase HPLC method was developed for the determination of melamine in milk (pasteurized and UHT milk) and dairy products (powdered infant formula, fruit yogurt, soft cheese, and milk powder). Following extraction with acetonitrile:water (50:50, vol/vol), samples were purified by filter (0.45 μ m), separated on a Nucleosil C8 column (4.6mm \times 250mm, 3 μ m) with acetonitrile:10mmol/L sodium α -octane sulfonate (pH 3.1; 15:85, vol/vol) as mobile phase at a flow rate of 1mL/min, and determined by a photodiode array detector. A linear calibration curve was obtained in the concentration range from 0.05 to 5mg/kg. Milk and dairy products were fortified with melamine at 4 levels producing average recovery yields of 95 to 109%. The limits of detection and quantification of melamine were 35 to 110 and 105 to 340 μ g/kg, respectively. The method was then used to analyze 300 samples of milk and dairy products purchased from major retailers in Turkey. Melamine was not found in infant formulas and pasteurized UHT milk, whereas 2% of cheese, 8% of milk powder, and 44% of yogurt samples contained melamine at the 121, 694 \pm 146, and 294 \pm 98 μ g/kg levels, respectively. These findings were below the limits set by the Codex Alimentarius Commission and European Union legislation. This is the first study to confirm the existence of melamine in milk and dairy products in Turkey. Consumption of foods containing these low levels of melamine does not constitute a health risk for consumers.

SHORT COMMUNICATION: ANNATTO IN CHEDDAR CHEESE-DERIVED WHEY PROTEIN CONCENTRATE IS PRIMARILY ASSOCIATED WITH MILK FAT GLOBULE MEMBRANE*D. Zhu & S. Damodaran**J. of Dairy Sci. 95(2): 614. 2012.*

The yellow color of Cheddar cheese whey arises from a residual amount of annatto that partitions into the whey during Cheddar cheese manufacture. Bleaching of the color using hydrogen peroxide or benzoyl peroxide is often a prerequisite to produce an acceptable neutral-colored whey protein concentrate and isolate. However, the use of these strong oxidizing agents often generates off-flavors as a result of lipid oxidation and results in loss of nutritive value due to protein oxidation. The objective was to determine the extent of partitioning of annatto between protein, milk fat globule membrane (MFGM), and aqueous (serum) phases of cheese whey so that a simple method can be developed to remove annatto from cheese whey. The MFGM was separated from Cheddar cheese whey using a recently developed novel method. Quantitative analysis of the distribution of annatto in the fat-free whey protein isolate (WPI), the MFGM fractions, and the serum phase revealed that annatto was not bound to the protein fraction but was mostly distributed between the serum phase and the MFGM fraction. The results showed that a colorless WPI or whey protein concentrate could be produced from Cheddar cheese whey by separation of MFGM from the whey, followed by diafiltration. This approach will negate the need for using bleaching agents.

EFFECT OF TEMPERATURE AND BLEACHING AGENT ON BLEACHING OF LIQUID CHEDDAR WHEY*M.A.D. Listiyani, R.E. Campbell, R.E. Miracle, D.M. Barbano, P.D. Gerard & M.A. Drake**J. of Dairy Sci. 95(1): 36. 2012.*

The use of whey protein as an ingredient in foods and beverages is increasing, and thus demand for colorless and mild-tasting whey protein is rising. Bleaching is commonly applied to fluid colored cheese whey to decrease color, and different temperatures and bleach concentrations are used. The objectives were to compare the effects of hot and cold bleaching, the point of bleaching (before or after fat separation), and bleaching agent on bleaching efficacy and volatile components of liquid colored and uncolored Cheddar whey. First, Cheddar whey was manufactured, pasteurized, fat-separated, and subjected to one of a number of hot (68°C) or cold (4°C) bleaching applications [hydrogen peroxide (HP) 50 to 500mg/kg; benzoyl peroxide

(BP) 25 to 100mg/kg] followed by measurement of residual norbixin and color by reflectance. Bleaching agent concentrations were then selected for the second trial. Liquid colored Cheddar whey was manufactured in triplicate and pasteurized. Part of the whey was collected (no separation, NSE) and the rest was subjected to fat separation (FSE). The NSE and FSE wheys were then subdivided and bleaching treatments (BP 50 or 100mg/kg and HP 250 or 500mg/kg) at 68°C for 30min or 4°C for 16h were applied. Control NSE and FSE with no added bleach were also subjected to each time-temperature combination. Volatile compounds from wheys were evaluated by gas chromatography-mass spectrometry, and norbixin (annatto) was extracted and quantified to compare bleaching efficacy. Proximate analysis, including total solids, protein, and fat contents, was also conducted. Liquid whey subjected to hot bleaching at both concentrations of HP or at 100mg/kg BP had greater lipid oxidation products (aldehydes) compared with unbleached wheys, 50mg/kg BP hot-bleached whey, or cold-bleached wheys. No effect was detected between NSE and FSE liquid Cheddar whey on the relative abundance of volatile lipid oxidation products. Wheys bleached with BP had lower norbixin content compared with wheys bleached with HP. Bleaching efficacy of HP was decreased at 4°C compared with 68°C, whereas that of BP was not affected by temperature. These results suggest that fat separation of liquid Cheddar whey has no effect on bleaching efficacy or lipid oxidation and that hot bleaching may result in increased lipid oxidation in fluid whey.

INTERACTIONS BETWEEN WHEY PROTEINS AND SALIVARY PROTEINS AS RELATED TO ASTRINGENCY OF WHEY PROTEIN BEVERAGES AT LOW pH

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Whey protein beverages have been shown to be astringent at low pH. In the present study, the interactions between model whey proteins (α -lactoglobulin and lactoferrin) and human saliva in the pH range from 7 to 2 were investigated using particle size, turbidity, and ζ -potential measurements and sodium dodecyl sulfate-PAGE. The correlation between the sensory results of astringency and the physicochemical data was discussed. Strong interactions between α -lactoglobulin and salivary proteins led to an increase in the particle size and turbidity of mixtures of both unheated and heated α -lactoglobulin and human saliva at pH < 3.4. However, the large particle size and high turbidity that occurred at pH 2.0 were the result of aggregation of human salivary proteins. The intense astringency in whey protein beverages may result from these increases in particle size and turbidity at these pH values and from the aggregation and precipitation of human salivary proteins alone at pH < 3.0. The involvement of salivary

proteins in the interaction is a key factor in the perception of astringency in whey protein beverages. At any pH, the increases in particle size and turbidity were much smaller in mixtures of lactoferrin and saliva, which suggests that aggregation and precipitation may not be the only mechanism linked to the perception of astringency in whey protein.

SOLUBILITY OF COMMERCIAL MILK PROTEIN CONCENTRATES AND MILK PROTEIN ISOLATES

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High-protein milk protein concentrate (MPC) and milk protein isolate (MPI) powders may have lower solubility than low-protein MPC powders, but information is limited on MPC solubility. The objectives were to (1) characterize the solubility of commercially available powder types with differing protein contents such as MPC40, MPC80, and MPI obtained from various manufacturers (sources), and (2) determine if such differences could be associated with differences in mineral, protein composition, and conformational changes of the powders. To examine possible predictors of solubility as measured by percent suspension stability (%SS), mineral analysis, Fourier transform infrared (FTIR) spectroscopy, and quantitative protein analysis by HPLC was performed. After accounting for overall differences between powder types, %SS was found to be strongly associated with the calcium, magnesium, phosphorus, and sodium content of the powders. The FTIR score plots were in agreement with %SS results. A principal component analysis of FTIR spectra clustered the highly soluble MPC40 separately from the rest of samples. Furthermore, 2 highly soluble MPI samples were clustered separately from the rest of the MPC80 and MPI samples. We found that the 900 to 1,200 cm^{-1} region exhibited the highest discriminating power, with dominant bands at 1,173 and 968 cm^{-1} , associated with phosphate vibrations. The 2 highly soluble MPI powders were observed to have lower $\hat{\epsilon}$ -casein and $\acute{\alpha}$ - $_{s1}$ -casein contents and slightly higher whey protein contents than the other powders. The differences in the solubility of MPC and MPI were associated with a difference in mineral composition, which may be attributed to differences in processing conditions. Additional studies on the role of minerals composition on MPC80 solubility are warranted. Such a study would provide a greater understanding of factors associated with differences in solubility and can provide insight on methods to improve solubility of high-protein milk protein concentrates.

EFFECTS OF PARTIALLY REPLACING SKIMMED MILK POWDER WITH DAIRY INGREDIENTS ON RHEOLOGY, SENSORY PROFILING, AND MICROSTRUCTURE OF PROBIOTIC STIRRED-TYPE YOGURT DURING COLD STORAGE

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This study aimed to evaluate the quality of stirred-type skim milk probiotic yogurt fortified by partially replacing skim milk powder (SMP) with whey protein concentrate (WPC) and sodium caseinate (Na-CN) during cold storage for 28 d compared with nonfortified yogurt. The rheological properties (as measured using dynamic oscillation) and sensory profiles of probiotic yogurts were greatly enhanced when SMP (i.e., 45%) was replaced with WPC and Na-CN. Higher values of mechanical parameters related to storage and loss modulus and consistent microstructure were found in the fortified yogurts. The acidification profile was not affected by supplementation of the solids in the milk base, and the viable counts of probiotic microbiota were high and satisfactory. These positive characteristics of probiotic yogurts were maintained until the end of the storage period. The microstructure of the fortified yogurt showed some differences compared with the nonfortified product, which were due to changes in chemical composition of the milk base in addition to the colloidal characteristics of the product.

INFLUENCE OF BLEACHING ON FLAVOR OF 34% WHEY PROTEIN CONCENTRATE AND RESIDUAL BENZOIC ACID CONCENTRATION IN DRIED WHEY PROTEINS

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Previous studies have shown that bleaching negatively affects the flavor of 70% whey protein concentrate (WPC70), but bleaching effects on lower-protein products have not been established. Benzoyl peroxide (BP), a whey bleaching agent, degrades to benzoic acid (BA) and may elevate BA concentrations in dried whey products. No legal limit exists in the United States for BP use in whey, but international concerns exist. The objectives were to determine the effect of hydrogen peroxide (HP) or BP bleaching on the flavor of 34% WPC (WPC34) and to evaluate residual BA in commercial and experimental WPC bleached with and without BP. Cheddar whey was manufactured in duplicate. Pasteurized fat-separated whey was subjected

to hot bleaching with either HP at 500mg/kg, BP at 50 or 100mg/kg, or no bleach. Whey was ultrafiltered and spray dried into WPC34. Color [L*(lightness), a* (red-green), and b* (yellow-blue)] measurements and norbixin extractions were conducted to compare bleaching efficacy. Descriptive sensory and instrumental volatile analyses were used to evaluate bleaching effects on flavor. Benzoic acid was extracted from experimental and commercial WPC34 and 80% WPC (WPC80) and quantified by HPLC. The b* value and norbixin concentration of BP-bleached WPC34 were lower than HP-bleached and control WPC34. Hydrogen peroxide-bleached WPC34 displayed higher cardboard flavor and had higher volatile lipid oxidation products than BP-bleached or control WPC34. Benzoyl peroxide-bleached WPC34 had higher BA concentrations than unbleached and HP-bleached WPC34 and BA concentrations were also higher in BP-bleached WPC80 compared with unbleached and HP-bleached WPC80, with smaller differences than those observed in WPC34. Benzoic acid extraction from permeate showed that WPC80 permeate contained more BA than did WPC34 permeate. Benzoyl peroxide is more effective in color removal of whey and results in fewer flavor side effects compared with HP and residual BA is decreased by ultrafiltration and diafiltration.

EFFECT OF LIQUID RETENTATE STORAGE ON FLAVOR OF SPRAY-DRIED WHEY PROTEIN CONCENTRATE AND ISOLATE

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The objective was to determine the effects of holding time of liquid retentate on flavor of spray-dried whey proteins: Cheddar whey protein isolate (WPI) and Mozzarella 80% whey protein concentrate (WPC80). Liquid WPC80 and WPI retentate were manufactured and stored at 3°C. After 0, 6, 12, 24, and 48h, the product was spray-dried (2kg) and the remaining retentate held until the next time point. The design was replicated twice for each product. Powders were stored at 21°C and evaluated every 4 mo throughout 12 mo of storage. Flavor profiles of rehydrated proteins were documented by descriptive sensory analysis. Volatile components were analyzed with solid phase microextraction coupled with gas chromatography mass spectrometry. Cardboard flavors increased in both spray-dried products with increased retentate storage time and cabbage flavors increased in WPI. Concurrent with sensory results, lipid oxidation products (hexanal, heptanal, octanal) and sulfur degradation products (dimethyl disulfide, dimethyl trisulfide) increased in spray-dried products with increased liquid retentate storage time, whereas diacetyl decreased. Shelf stability was decreased in spray-dried products from longer retentate storage times. For maximum quality and shelf life, liquid retentate should be held for less than 12h before spray drying.

HYDROLYSIS OF WHEY PROTEIN ISOLATE USING SUBCRITICAL WATER*A.D. Espinoza, R.O. Morawicki & T. Hager**J. of Food Sci. 77(1): C20. 2012.*

Hydrolyzed whey protein isolate (WPI) is used in the food industry for protein enrichment and modification of functional properties. The purpose of the study was to determine the feasibility of subcritical water hydrolysis (SWH) on WPI and to determine the temperature and reaction time effects on the degree of hydrolysis (DH) and the production of peptides and free amino acids (AAs). Effects of temperature (150 to 320 °C) and time (0 to 20 min) were initially studied with a central composite rotatable design followed by a completely randomized factorial design with temperature (250 and 300 °C) and time (0 to 50 min) as factors. SWH was conducted in an electrically heated, 100-mL batch, high pressure vessel. The DH was determined by a spectrophotometric method after derivatization. The peptide molecular weights (MWs) were analyzed by gel electrophoresis and mass spectrometry, and AAs were quantified by high-performance liquid chromatography. An interaction of temperature and time significantly affected the DH and AA concentration. As the DH increased, the accumulation of lower MW peptides also increased following SWH (and above 10% DH, the majority of peptides were <1000 Da). Hydrolysis at 300 °C for 40 min generated the highest total AA concentration, especially of lysine (8.894 mg/g WPI). Therefore, WPI was successfully hydrolyzed by subcritical water, and with adjustment of treatment parameters there is reasonable control of the end-products.

COMPARISON OF THE FLAVOR CHEMISTRY AND FLAVOR STABILITY OF MOZZARELLA AND CHEDDAR WHEYS*I.W. Liaw, R.E. Miracle, S.M. Jervis, M.A.D. Listiyani & M.A. Drake**J. of Food Sci. 76(8): C1188. 2011.*

The flavor and flavor stability of fresh and stored liquid Cheddar and Mozzarella wheys were compared. Pasteurized, fat separated, and unseparated Cheddar and Mozzarella wheys were manufactured in triplicate and evaluated immediately or stored for 72 h at 3 °C. Flavor profiles were documented by descriptive sensory analysis, and volatile components were extracted and characterized by solvent extraction followed by gas chromatography-mass spectrometry and gas chromatography-olfactometry with aroma extract dilution analysis. Cheddar and Mozzarella wheys were distinct by sensory and volatile analysis ($P < 0.05$). Fresh Cheddar whey had higher intensities of buttery and sweet aromatic flavors and higher cardboard flavor intensities following storage compared to Mozzarella whey.

High aroma impact compounds ($FD_{\log 3} > 8$) in fresh Cheddar whey included diacetyl, 1-octen-3-one, 2-phenethanol, butyric acid, and (E)-2-nonenal, while those in Mozzarella whey included diacetyl, octanal, (E)-2-nonenal, and 2-phenethanol. Fresh Cheddar whey had higher concentrations of diacetyl, 2/3-methyl butanal, (E)-2-nonenal, 2-phenethanol, and 1-octen-3-one compared to fresh Mozzarella whey. Lipid oxidation products increased in both whey types during storage but increases were more pronounced in Cheddar whey than Mozzarella whey. Increases in lipid oxidation products were also more pronounced in wheys without fat separation compared to those with fat separation. Results suggest that similar compounds in different concentrations comprise the flavor of these 2 whey sources and that steps should be taken to minimize lipid oxidation during fluid whey processing.