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116 N. York Street, Suite 200, Elmhurst, IL 60126
phone: (630) 530-8700 fax: (630) 530-8707
Web site: www.adpi.org
Email: info@adpi.org

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EFFECT OF MEMBRANE LENGTH, MEMBRANE RESISTANCE, AND FILTRATION CONDITIONS ON THE FRACTIONATION OF MILK PROTEINS BY MICROFILTRATION

A. Piry, A. Heino, W. Kuhnl, T. Grein, S. Ripperger & U. Kulozik
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They investigated the fractionation of casein micelles and the whey protein â-lactoglobulin (â-LG) of skim milk by crossflow microfiltration (0.1 ìm) for the first time by a novel approach as a function of membrane length and membrane resistance. A special module was constructed with 4 sections and used to assess the effects of membrane length by measuring flux and â-LG permeation (or transmission) as a function of transmembrane pressure and membrane length. Depending on the position, the membranes were partly controlled by a deposit layer. A maximum for â-LG mass flow through the various membrane sections was found, depending on the position along the membrane. To study the effect of convective flow toward the membrane, membranes with 4 different intrinsic permeation resistances were assessed in terms of the permeation and fouling effects along the flow channel. From these findings, they derived a ratio between transmembrane pressure and membrane resistance, which was useful in reducing the effect of deposit formation and, thus, to optimize the protein permeation. In addition, the fouling effect was investigated in terms of reversible and irreversible fouling and, in addition, by differentiation between pressure-induced fouling and adsorption-induced (pressure-independent) fouling, again as a function of membrane length.

HEAT STABILITY OF MILK SUPPLEMENTED WITH CALCIUM CHLORIDE

N. On-Nom A.S. Grandison & M.J. Lewis
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Calcium chloride (0–25mM) was added to skim milk powder that was reconstituted to 9% total solids. Heat stability was evaluated between 60 and 120°C for different times by observing whether samples had coagulated, and by measuring the amount of sediment and residual protein in the centrifuged supernatant. Milk samples were also dialyzed during their

respective heat treatments to recover the soluble phase at different temperatures to measure pH and ionic calcium. The transition conditions between good and poor heat stability were established for different calcium chloride concentrations and temperatures. As temperature increased, coagulation occurred at lower levels of added calcium chloride. The transition was quite distinct at higher temperatures but less so at lower temperatures; it was initiated by an increase in sediment formation before a firm coagulum was formed. Both pH and ionic calcium decreased in dialysates as temperature increased. No coagulation was observed if Ca^{2+} was <0.5mM and pH was >6.3 in dialysates taken at their respective coagulation temperatures. Being able to measure pH and ionic calcium at high temperatures will allow better understanding of factors affecting heat stability. Electrophoresis of the supernatants permitted identification of the protein fractions participating in the coagulation process. When coagulation was observed below 80°C, substantial amounts of undenatured â-lactoglobulin and á-lactalbumin were found in the supernatant, as well as some soluble casein fractions. All the major whey protein and casein fractions were found in the sediment.

FUNCTIONAL PROPERTIES OF WHEY PROTEINS MICROPARTICULATED AT LOW pH

M. Dissanayake, S. Livanaarachchi & T. Vasilievic
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The objective was to assess the effect of microparticulation at low pH on the functionality of heat-denatured whey proteins (WP). Spray-dried, microparticulated WP (MWP) powders were produced from 7% (wt/wt) WP dispersions at pH 3, acidified with citric or lactic acid, and microfluidized with or without heat denaturation. Nonmicroparticulated, spray-dried powders produced at neutral pH or pH 3 served as controls. The powders were examined for their functional and physicochemical properties. Denatured MWP had an approximately 2 orders of magnitude reduction in particle size compared with those produced at neutral pH, with high colloidal stability indicated by substantially improved solubility. The detection of monomeric forms of WP in PAGE also confirmed the particle size reduction. Microparticulated WP exhibited enhanced heat stability, as indicated by thermograms, along with better emulsifying properties compared with those produced at neutral pH. However, MWP powders created weaker heat-induced gels at neutral pH compared with controls. However, they created comparatively strong cold acid-set gels. At low pH, a combination of heat and high hydrodynamic pressure produced WP micro-aggregates with improved colloidal stability that affects other functionalities.

HYDROLYSIS OF WHEY PROTEIN ISOLATE USING SUBCRITICAL WATER

A.D. Espinoza, R.O. Morawicki & T. Hager
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The objective 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 chromotography. 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.

MANUFACTURE OF REDUCED-SODIUM CHEDDAR-STYLE CHEESE WITH MINERAL SALT REPLACERS

J. Grummer, M. Karalus, K. Zhang, Z. Vickers & T.C. Schoenfuss
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The use of mineral salt replacers to reduce the sodium content in cheese has been investigated as a method to maintain both the salty flavor and the preservative effects of salt. The majority of studies of sodium reduction have used mineral salt replacers at levels too low to produce equal water activity (a_w) in the finished cheese compared with the full-sodium control. Higher a_w can result in differences in cheese quality due to differences in the effective salt-to-moisture ratio. This creates differences in biochemical and microbial reactions during aging. We hypothesized that by targeting replacer concentrations to produce the same a_w as full sodium cheese, changes in cheese quality would be minimized. Stirred-curd Cheddar-style cheese was manufactured and curd was salted with NaCl or naturally reduced sodium sea salt. Reduced-sodium cheeses were created by blends of NaCl or sea salt with KCl, modified KCl, $MgCl_2$, or $CaCl_2$ before pressing. Sodium levels in reduced-sodium cheeses ranged from 298 to 388mg of sodium/

100g, whereas the control full-sodium cheese had 665mg/100g. At 1 wk of age, a_w of reduced-sodium cheeses were not significantly different from control, which had an a_w of 0.96. The pH values of all reduced-sodium cheeses, excluding the treatment that combined sea salt and $MgCl_2$, were lower than those of full-sodium cheese, indicating that the starter culture was possibly less inhibited at the salting step by the replacers than by NaCl. Instrumental hardness values of the treatments with sea salt were higher than in cheeses containing NaCl, with the exception of the NaCl/$CaCl_2$ treatment, which was the hardest. Treatments with $MgCl_2$ and modified KCl were generally less hard than other treatments. In-hand and first-bite firmness values correlated with the instrumental texture profile analysis results. Both $CaCl_2$ and $MgCl_2$ produced considerable off-flavors in the cheese (bitter, metallic, unclean, and soapy), as measured by descriptive sensory analysis with a trained panel. Bitterness ratings for cheese with KCl and modified KCl were not significantly different from the full-sodium control. Potassium chloride can be used successfully to achieve large reductions in sodium when replacing a portion of the NaCl in Cheddar cheese.

EFFECT OF ZINC FORTIFICATION ON CHEDDAR CHEESE QUALITY

O. Kahraman & Z. Ustunol

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Zinc-fortified Cheddar cheese containing 228mg of zinc/kg of cheese was manufactured from milk that had 16mg/kg food-grade zinc sulfate added. Cheeses were aged for 2 mo. Culture activity during cheese making and ripening, and compositional, chemical, texture, and sensory characteristics were compared with control cheese with no zinc sulfate added to the cheese milk. Compositional analysis included fat, protein, ash, moisture, zinc, and calcium determinations. The thiobarbituric acid (TBA) assay was conducted to determine lipid oxidation during aging. Texture was analyzed by a texture analyzer. An untrained consumer panel of 60 subjects evaluated the cheeses for hardness, off-flavors, appearance, and overall preference using a 9-point hedonic scale. Almost 100% of the zinc added to cheese milk was recovered in the zinc-fortified cheese. Zinc-fortified Cheddar cheese had 5 times more zinc compared with control cheese. Zinc-fortified cheese had higher protein and slightly higher fat and ash contents, whereas moisture was similar for both cheeses. Zinc fortification did not affect culture activity during cheese making or during the 2-mo aging period. The TBA value of control cheese was higher than that of zinc-fortified cheese at the end of ripening. Although zinc-fortified cheese was harder as determined by the texture analyzer, the untrained consumer panel did not detect differences

in the sensory attributes and overall quality of the cheeses. Fortification of 16mg/kg zinc sulfate in cheese milk is a suitable approach to fortifying Cheddar cheese without changing the quality of Cheddar cheese.

EFFECT OF BLEACHING WHEY ON SENSORY AND FUNCTIONAL PROPERTIES OF 80% WHEY PROTEIN CONCENTRATE

S. Jervis, R. Campbell, K.L. Wojciechowski, E.A. Foegeding, M.A. Drake & D.M. Barbano
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The objective was to compare 2 commercially approved bleaching agents, benzoyl peroxide (BP) and hydrogen peroxide (HP), and their effects on the flavor and functionality of 80% whey protein concentrate (WPC80). Colored and uncolored liquid wheys were bleached with BP or HP, and then ultrafiltered, diafiltered, and spray-dried; WPC80 from unbleached colored and uncolored Cheddar whey were manufactured as controls. All treatments were manufactured in triplicate. The WPC80 were then assessed by sensory, instrumental, functionality, color, and proximate analysis techniques. The HP-bleached WPC80 were higher in lipid oxidation compounds (specifically hexanal, heptanal, octanal, nonanal, decanal, dimethyl disulfide, and 1-octen-3-one) and had higher fatty and cardboard flavors compared with the other unbleached and BP-bleached WPC80. The WPC80 bleached with BP had lower norbixin concentrations compared with WPC80 bleached with HP. The WPC powders differed in Hunter color values (L, a, b), with bleached powders being more white, less red, and less yellow than unbleached powders. Bleaching with BP under the conditions used in this study resulted in larger reductions in yellowness of the powders made from whey with annatto color than did bleaching with HP. Functionality testing demonstrated that whey bleached with HP treatments had more soluble protein after 10min of heating at 90°C at pH 4.6 and pH 7 than the no-bleach and BP treatments, regardless of additional color. Overall, HP bleaching caused more lipid oxidation products and subsequent off-flavors compared with BP bleaching. However, heat stability of WPC80 was enhanced by HP bleaching compared with control or BP-bleached WPC80.

THE USE OF LACTOPEROXIDASE FOR THE BLEACHING OF FLUID WHEY

R.E. Campbell, E.J. Kang, E. Bastian & M.A. Drake
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Lactoperoxidase (LP) is the second most abundant enzyme in bovine milk and has been used in conjunction with hydrogen peroxide (H_2O_2) and

thiocyanate (SCN^-) to work as an antimicrobial in raw milk where pasteurization is not feasible. Thiocyanate is naturally present and the lactoperoxidase system purportedly can be used to bleach dairy products, such as whey, with the addition of very little H_2O_2 to the system. This study had 3 objectives: 1) to quantify the amount of H_2O_2 necessary for bleaching of fluid whey using the LP system, 2) to monitor LP activity from raw milk through manufacture of liquid whey, and 3) to compare the flavor of whey protein concentrate 80% (WPC80) bleached by the LP system to that bleached by traditional H_2O_2 bleaching. Cheddar cheese whey with annatto (15mL of annatto/454kg of milk, annatto with 3% wt/vol norbixin content) was manufactured using a standard Cheddar cheesemaking procedure. Various levels of H_2O_2 (5–100mg/kg) were added to fluid whey to determine the optimum concentration of H_2O_2 for LP activity, which was measured using an established colorimetric method. In subsequent experiments, fat-separated whey was bleached for 1h with 250mg of H_2O_2/kg (traditional) or 20mg of H_2O_2/kg (LP system). The WPC80 was manufactured from whey bleached with 250mg of H_2O_2/kg or 20mg of H_2O_2/kg. All samples were subjected to color analysis (Hunter color values and norbixin extraction) and proximate analysis (fat, protein, and moisture). Sensory and instrumental volatile analyses were conducted on WPC80. Optimal LP bleaching in fluid whey occurred with the addition of 20mg of H_2O_2/kg. Bleaching of fluid whey at either 35 or 50°C for 1h with LP resulted in >99% norbixin destruction compared with 32 or 47% destruction from bleaching with 250mg of H_2O_2/kg, at 35 or 50°C for 1h, respectively. Higher aroma intensity and increased lipid oxidation compounds were documented in WPC80 from bleached whey compared with WPC80 from unbleached whey. Monitoring of LP activity throughout cheese and whey manufacture showed that LP activity sharply decreased after 30min of bleaching (17.01±1.4 to <1U/mL), suggesting that sufficient bleaching takes place in a very short amount of time. Lactoperoxidase averaged 13.01±0.7U/mL in unpasteurized, fat-separated liquid whey and 138.6±11.9U/mL in concentrated retentate (11% solids). Lactoperoxidase may be a viable alternative for chemical whey bleaching.

PERCEPTUAL CHANGES AND DRIVERS OF LIKING IN HIGH PROTEIN EXTRUDED SNACKS

J.W. Kreger, Y. Lee & S.Y. Lee
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Increasing the amount of protein in snack foods can add to their satiating ability, which aligns with many health-based trends currently seen in the food industry. Understanding the effect of adding high levels of protein in a food matrix is essential for product development. The objective for this

research was to determine the effects of varying protein type and level on the sensory-related aspects of a model extruded snack food. Independent variables in the design of the snacks were the level of total protein and the protein type in the formulation. The level of protein ranged from 28% to 43% (w/w) in 5% increments. The protein type varied in the ratio of whey to soy protein ranging from 0: 100 to 100: 0, in 25% increments. Descriptive analysis was conducted on the samples to profile their sensory characteristics. Protein type was found to be the predominant variable in differentiating the sensory characteristics of the samples. Soy protein imparted nutty, grainy aromas-by-mouth, and increased expansion during processing, resulting in a lighter, crispier texture. Whey protein imparted dairy related aromas-by-mouth and inhibited expansion during processing, resulting in a more dense, crunchy texture. Separately, 100 consumers rated their acceptance of the samples using the 9-point hedonic scale. It was found that protein type was also the predominant variable in affecting acceptance, with some clusters of consumers preferring samples comprised of soy protein, and others preferring samples with whey. Food product developers can use these findings to predict changes in a similar food product by varying protein level or protein type.