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Functional properties of whey protein concentrate texturized at acidic pH: Effect of extrusion temperature

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ABSTRACT

Reactive supercritical fluid extrusion (RSCFX) process at acidic condition (pH 3.0) was used to generate texturized whey protein concentrate (TWPC) and the impacts of process temperature on product's physicochemical properties were evaluated. TWPC extruded at 50 and 70 °C formed soft-textured aggregates with high solubility than that extruded at 90 °C that formed protein aggregates with low solubility. Total free sulfhydryl contents and solubility studies in selected buffers indicated that TWPC is primarily stabilized by non-covalent interactions. Proteins texturized at 90 °C showed an increased affinity for 1-anilino-naphthalene-8-sulfonate (ANS) and a decreased affinity for cis-parinaric acid (CPA), indicating changes in protein structure. Water dispersion of TWPC at room temperature showed thickening function with pseudoplastic behavior. Secondary gelation occurred in TWPC obtained at 50 and 70 °C by heating the cold-set gels to 95 °C. TWPC texturized at 90 °C produced cold-set gels with good thermal stability. Compared to control, TWPC formed stable oil-in-water emulsions. Factors such as degree of protein denaturation and the balance of surface hydrophobicity and solubility influenced the heat- and cold-gelation and emulsifying properties of the protein ingredients. TWPC generated by low and high temperature extrusions can thus be utilized for different products requiring targeted physicochemical functionalities.

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1. Introduction

The potential of whey protein (WP) as functional ingredient such as gelling, emulsifying and foaming agents in food is widely acknowledged. The properties of WP vary depending on factors such as of protein denaturation, size of protein aggregates, and environmental conditions such as temperature, pH, and ionic strength. Heat treatments are commonly used to alter the functional properties of WPs, in which the extent of exposure of reactive hydrophobic and thiol groups determines the functionalities of the proteins (Morr & Ha, 1993). At a moderate temperature (<70 °C), the structural unfolding and conformational changes of β-lactoglobulin (β-lg) and α-lactalbumin (α-la) are largely reversible on cooling. At a higher temperature (>90 °C), the proteins are extensively denatured and irreversibly cross-linked to form large aggregates (de la Fuente, Singh, & Hema, 2002; deWit & Klarenbeek, 1984).

Aggregation of proteins is generally accompanied by a reduction in their solubility. Depending on extrinsic factors, the thermal denaturation of WP results in a heterogeneous mix of native soluble, polymerized soluble, and insoluble protein aggregates of various functionalities (Sanchez, Pouliot, Gauthier, & Paquin, 1997). To be an effective emulsifier, protein aggregate need to be at an optimum size with a good solubility and a balanced surface hydrophobicity (Nakai, 1983; Wagner, Sorgentini, & Anon, 2000). Partial denaturation of WP produces soluble, aggregated proteins with increased surface hydrophobicity, which favors protein adsorption at the oil–water interface. On the other hand, extensive and irreversible aggregation results in a loss of emulsifying properties (Dickinson & Hong, 1994; Mutilangi, Panyam, & Kilara, 1996). In contrast, several authors (Britten, Giroux, Jean, & Rodrigue, 1994; Dissanayake, Liyanaarachchi, & Vasiljevic, 2012) documented that WPs containing higher percentage of denatured proteins produced emulsions with greater viscosity and stability. The authors describe the complementary roles of native and denatured protein aggregates in the formation of interfacial membranes around oil droplets. Therefore, improving protein functionality requires a compromise between counteracting physicochemical properties

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of the protein and their careful modulation (Vardhanabhuti, Foegeding, McGuffey, Daubert, & Swaisgood, 2001).

For certain applications, high-heat treatment is not a suitable option to achieve required structural modifications and functionalities. Therefore, protein ingredients that form gels at room temperature—cold-set gels—are highly desirable. WP has been modified by heating protein solution at 70–90 °C for 5–120 min under low ionic strength and neutral pH conditions to produce soluble protein aggregates. Cold-set gel is formed either by the addition of salt or reduction in pH (Alting, Hamer, de Kruif, & Visschers, 2000; Barbut & Foegeding, 1993). In another studies, cold-gelation of WPI and WPC was achieved by reconstitution of derivitized WP (dWP) in deionized water without the addition of salt or pH reduction. The dWP powder was obtained from a sequence of process involving thermal gelation (80 °C for 1–3 h) of acidified protein solution (8–12 g/100 g), followed by spray drying or freeze drying the samples to obtain dWP powder (Hudson & Daubert, 2002; Resch, Daubert, & Foegeding, 2004). These gels have better water-holding capacity and higher strength than those produced by conventional heat-induced methods. However, most of the processing techniques proposed require high heating temperature, long or multiple processing steps, that perhaps are not practical for commercial production.

Recently, a procedure for producing texturized WPC (TWPC) with enhanced cold-set gelling properties was developed by using a RSCFX process. In the RSCFX process, supercritical carbon dioxide (SC-CO_2) is utilized as a blowing agent and as an in-line process modifier. It allows the process to be performed at a higher moisture content (20–60%), lower extrusion temperature (<100 °C) and at low-shear conditions (Alavi & Rizvi, 2010; Rizvi, Mulavaney, & Sokhey, 1995). These conditions minimize the damage that might occur to the heat and shear-sensitive components and offers the potential to provide a mechanism for unique textural and chemical modifications in various products (Rizvi et al., 1995). The chemical environment in RSCFX can be manipulated by changing conditions of injected SC-CO_2 to attain different pH levels and alteration of ionic strength coupled with heat. This will result in alteration of the conformational structure of extruded products attributed to the exposure of reactive groups of amino acids, which eventually helps to modify the functionality of texturized protein (Manoi & Rizvi, 2008).

Manoi and Rizvi (2008) have demonstrated that TWPC processed by RSCFX in the presence of NaCl and $CaCl_2$ under acidic conditions (pH 3.0) has a high water-absorbing capacity and thus high viscosity when reconstituted in water. At 20 g/100 g water dispersion, the proteins form a cold-set gel without the need for salt or acidification. However, during the RSCFX process, proteins are heated to 90 °C, which produces large protein aggregates and a greatly reduces protein solubility. Because heat treatment affects solubility, any processing step that produces modified WPC with high solubility and high water-holding capacity to form a thick suspension at room temperature is desirable. Therefore, the objective of this study is to determine the effects of extrusion temperatures on physicochemical functionalities of TWPC.

2. Materials and methods

2.1. Materials

Commercial WPC-80 (Lactalbumin-49320) was purchased from Leprino Foods Company (Denver, CO, USA). The composition of the WPC was 81.5 g/100 g protein (dry basis), 5.5 g/100 g fat, 6.5 g/100 g lactose, and less than 3.0 g/100 g ash. Fluorescence probes, 8-anilino-1-naphthalene sulfonic acid (ANS) and cis-parinaric acid (CPA) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Molecular Probes (Eugene, OR, USA), respectively. Ellman's Reagent, DTNB (5,5′-dithio-bis-(2-nitrobenzoic acid) was purchased from Thermo Scientific Inc. (Rockford, IL, USA).

2.2. Texturization of whey protein by RSCFX

The protein blend was prepared by pre-hydrating WPC-80 to 10 g/100 g moisture. NaCl and $CaCl_2$ at 0.6 and 0.3 g/100 g (on weight basis), respectively, were added to the pre-hydrated WPC. The premixed protein blend was then preconditioned overnight at room temperature and extruded using a co-rotating twin-screw extruder (Wenger TX-52 Magnum, Sabetha KS, USA) coupled with supercritical carbon dioxide (SC-CO_2) injection system. The extruder, with a length-to-diameter (L/D) ratio of 28.5:1, is configured for the RSCFX process and SC-CO_2 is injected into the barrel through four valves located at L/D ratio of 24. The extrudate was forced through two die inserts with 1.2 mm diameter circular openings. The protein blends were extruded at three different die-exit temperatures: 50, 70, or 90 °C and labeled as TWPC-50, TWPC-70, or TWPC-90, respectively. The barrel zone temperatures were maintained as shown in Fig. 1; the second and third barrel zone temperatures were adjusted to get products at 50, 70, or 90 °C die temperatures. The extruder was operated at 130 rpm at a feed rate of 35 kg/h. SC-CO_2 (1.5 g/100 g of dry feed) was continuously injected at a pressure of 10–15 MPa into the protein melt. HCl solution with concentration of 15 g/100 g was injected into the mixing zone to obtain a pH of extrudate of about 3.0 and the extrusion was carried out at 60 g/100 g moisture. The extrudates were dried in a convection oven at 40 °C for approximately 16 h to achieve 5–6 g/100 g moisture content. The dried products were finely ground to ≤1 mm size using a Thomas–Wiley Mill grinder (Model ED-5, Arthur H. Thomas Co., PA, USA), and stored in air-tight containers at room temperature.

2.3. Particle size distribution

The effective diameter and distribution of protein particles in water dispersion of non-texturized WPC (control), TWPC-50, TWPC-70 and TWPC-90 were measured with a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corp., Holtsville, NY, USA). Appropriate amount of protein powder was dispersed in deionized water to obtain 1.0 g/100 g (protein basis) dispersion, followed by stirring at room temperature for 2 h and storage at 4 °C for overnight. The dispersion was diluted to 0.10–0.05 g/100 g in ultra-pure water, immediately before the particle size measurements. Sample dilution was adjusted in order to achieve signal intensity of 400–800 kilocounts per second (kcps). All measurements were performed at 20 °C. Data was collected and analyzed using BIC software (Brookhaven Instruments Corp.). The intensity-weighed effective diameter (average diameter) and polydispersity were determined for each sample.

2.4. Determination of free sulfhydryl (-SH) groups

The free sulfhydryl group in 1.0 g/100 g protein solution was determined using Ellman's reagent, DTNB, according to the methods of Sava, Van der Plancken, Claeys, and Hendrickx (2005) and Shimada and Cheftel (1989). Protein solutions were diluted to 0.1 g/100 g in buffer containing 0.086 mol/L Tris, 0.09 mol/L glycine, 4 mmol/L Na_2EDTA, 0.5 g/100 g SDS and 8.0 mol/L urea (pH 8.0). Samples were centrifuged (Beckman Avanti J-25, Beckman Coulter Inc., Brea, CA, USA) at 20,000 g for 15 min at 20 °C. Thirty microliters of DTNB solution (40 mg DTNB/10 mL buffer) was added to 3 mL supernatant, followed by 15 min incubation at room temperature. The absorbance was recorded at 412 nm using a spectrophotometer (Spectronic 1200, Bausch and Lomb, Rochester, NY,

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