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Formation of soluble aggregates from insoluble commercial soy protein isolate by means of ultrasonic treatment and their gelling properties

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ABSTRACT

The formation of soluble aggregates from insoluble precipitates of commercial soy protein isolate (SPI) by means of combined homogenization and ultrasonic treatment was characterized. The heat-induced gelation of formed soluble aggregates was also investigated. The turbidity and electrophoresis analyses showed that initially insoluble precipitates of commercial SPI, most basic subunits of glycinin included, were transformed into soluble aggregates. High performance size exclusion chromatography (HPSEC) analysis confirmed the formation of soluble aggregates. Both non-covalent and covalent interactions, e.g. hydrophobic interactions, hydrogen bonds and disulfide bonds, were involved in the formation of soluble aggregates. The formation of soluble aggregates remarkably improved the heat-induced gelling ability of commercial SPI.

1. Introduction

Soy protein has been widely used as an important food ingredient in a lot of protein-based food formulations, since it exhibits high nutrition (as a valuable source of protein) and good ability to improve the texture. Soy protein isolate (SPI) is one of most important commercially available soy protein products. It mainly consists of glycinin and β-conglycinin, and these two protein components usually account for 34% and 27% of total protein content of SPI, respectively, (Iwabuchi and Yamauchi, 1987a,b). The native glycinin is an oligomeric protein having a molecular weight (MW) of approximately 350 kDa and consisting of six subunits $(AB)_6$. The acidic (MW = 37–42 kDa) (A_{1-4}) and basic subunits (MW = 17–20 kDa) (B) are linked by disulfide bridges. The β-conglycinin is usually a trimer composed of three major subunits (α′, α and β) associated in various combinations by non-covalent interactions (Thanh and Shibasaki, 1976, 1977, 1979). However, those proteins are easily denatured under some extreme conditions, e.g. acid precipitation and high temperature, during the industrial production of commercial SPI products. The denatured proteins would further be associated into aggregates, or even precipitates, thus causing poor solubility. The poor solubility greatly limits the practical application of commercial SPI in the food industry.

Many physical, chemical and enzymatic methods to improve the functionalities of soy proteins have been the subject of several studies (Campbell et al., 1992; Chan and Ma, 1999; Babiker, 2000; Calderon et al., 2000; Molina et al., 2001; Wang et al., 2008). Most of the functionalities for soy proteins are related to their protein solubility. If the protein solubility of commercial SPI is improved, some functional properties will be expected to be improved. To date, there are limited literatures concerning about modification of soy proteins, using commercial SPI products as the starting material.

Many previous literatures showed that in the case of thermal aggregation, the subunits of glycinin and β-conglycinin could form some kind of soluble aggregates (German et al., 1982; Damodaran and Kinsella, 1982; Utsumi et al., 1984; Petruccelli and Añón, 1995). This observation can provide a potential solution method to improve the solubility of insoluble proteins in commercial SPI. In our previous study using alcohol-washed insoluble soy protein concentrate (SPC) as the starting material, we found that soluble aggregate could be formed from those insoluble soy protein components by the method of hydrothermal cooking combined with high pressure homogenization (Zheng et al., 2008).

Recently, we also found that the homogenization combined with ultrasonic treatment could improve the protein solubility of commercial SPI. The underlying mechanism was considered to be also due to formation of soluble aggregates from initially insoluble precipitates. The objective of this work was undertaken to characterize the formation of soluble aggregates by means of ultrasonic treatment. Additionally, the heat-induced gelation of formed aggregates was also investigated.

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2. Materials and methods

2.1. Materials

The commercial SPI used in this study was a product of Wonderful Technol. Co. (Shandong Province, China). This commercial SPI was chosen as the starting material for the experiments, due to the consideration that the protein solubility of this SPI in water was very low (less than 50%, at a protein concentration of 1%). The chemical composition of this SPI, including protein ($N \times 6.25$, %), lipid, ash and moisture contents was analyzed using AOAC methods (AOAC, 1985). The MW standard protein markers were purchased from Genview Co. All other chemicals were of analytical reagent or better grade.

2.2. Preparation of different SPI supernatants

Six grams of SPI was hydrated in 100 mL of standard buffer, namely 0.05 M Tris–HCl buffer (pH 7.5) containing 0.03% (w/v) Na_3N, using magnetic stirring at room temperature for 2 h, to produce original SPI dispersion. Three supernatants (denoted as S_I, S_{II} and S_{III}) of SPI dispersions were prepared as following procedures: (1) S_I of untreated SPI dispersion was directly obtained by centrifugation (at 8000*g* for 20 min) from the original dispersion; (2) S_{II} of homogenized SPI dispersion was obtained by homogenizing the original dispersion at a velocity of 10,000 turn/min for 5 min using FJ-200 High-Speed Homogenizer (Shanghai Specimen Model Co., China), and then centrifuging at the same conditions; (3) S_{III} of homogenized and ultrasonic-treated SPI dispersion was obtained from the homogenized dispersion as described above, by an additional ultrasonic treatment using an ultrasonic equipment (200 W, 15 kHz) (XingDongLi Ultrasonic Electron Equipment Co. Ltd., China) with a noodle sounder for 10 min, and subsequent centrifugation.

To characterize soluble and insoluble protein components of the SPI, the samples of these two components used for the electrophoresis analysis were prepared based on the S_I and its corresponding precipitate. The initially obtained precipitate was washed with cold de-salted water, and centrifuged again.

2.3. Turbidity measurement

The absorbance at 660 nm was used as an indication for the turbidity. The turbidity of different supernatants (S_I, S_{II} and S_{III}) was measured after they were diluted at a dilution factor of 10.0 with different solvents as follow: (i) standard buffer (as mentioned above), (ii) the standard buffer containing 6.0 M urea; and (iii) the standard buffer containing 6.0 M urea and 10 mM β-mercaptoethanol (2-ME). For the measurement of the turbidity of the supernatants diluted with a solvent, the same solvent was used as the blank. Each data was the mean and standard deviation of four replicates.

2.4. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

The electrophoresis was performed on a discontinuous buffered system according to the method of Laemmli (1970) using 12% separating gel and 4% stacking gel. The protein samples of solution were directly mixed with the same volume of sample buffer (2×), namely 0.25 M Tris–HCl buffer (pH 8.0), containing 2% (w/v) SDS, 5% (v/v) 2-ME, 10% (v/v) glycerol and 0.05% (w/v) bromophenol blue. The sample of the protein precipitate in untreated SPI dispersion was solubilized in the sample buffer (1×), with a protein concentration of about 1% (w/v). These samples were sealed in plastic centrifugal tubes, and heated in boiling water for 5 min and then cooled at room temperature, before electrophoresis. After electrophoresis, the gels were dyed in 0.25% Coomassie blue (R-250) in the 50% trichloroacetic acid and then destained in 7% acetic acid [methanol:acetic acid:water, 227:37:236 (v/v/v)].

For protein quantification by densitometric scanning, the individual lanes of the stained gels were scanned by White/Ultraviolet Transilluminator (UVP Inc., Upland, CA, USA) and analyzed by the software of Labworks (version 4.0). The relative contents (%) of individual dyed bands (corresponding to individual subunits of glycinin and β-conglycinin) in any a lane were calculated as the sum of the area density of their subunit or polypeptide bands with respect to total area density of these bands in the densitogram.

2.5. High performance size exclusion chromatography (HPSEC)

The HPSEC experiment was performed using a Waters HPLC 1525 system (Waters, Division of Millipore, Milford, MA, USA) fitted with a Protein PAK 300sw column (0.75 × 30 cm, Tokyo, Japan) proceeded by a guard column Protein – Pak™ 125 (0.6 × 4 cm, Tokyo, Japan).

The supernatants (S_I, S_{II} and S_{III}) were diluted at a dilution factor of 6.0 with the standard buffer. The diluted supernatants were centrifuged at 15,000 *g* for 10 min, and then the resultant supernatants were further filtered with Millipore membrane (0.45 μm). In another experiment to characterize the interactions in the formation of soluble aggregates of SPI by HPSEC, the S_{III} (the supernatant of homogenized and ultrasonic-treated SPI dispersion) was premixed with urea solution to a concentration of 6.0 M, or additionally containing 10.0 mM 2-ME. In this case, the dilution factor was adjusted to the same as described above. The following chromatographic conditions were applied: (1) injection volume, 20 μL; (2) elution rate, 0.5 mL/min; (3) elution solvent-A: 0.05 M Tris–HCl buffer (pH 7 .6) containing 0.1 M NaCl; elution solvent-B: elution solvent-A containing 6.0 M urea; elution solvent-C: elution solvent-B containing 10.0 mM 2-ME. Total elution time was 35 min, and the absorbance was recorded at 280 nm. All data were collected and analyzed by Breeze software (Waters, Division of Millipore, Milford, MA, USA).

2.6. Low amplitude oscillatory measurements

The heat-induced gelation of protein dispersions (at a protein concentration of 8.5%, w/v) was carried out in a HAAKE RS600 Rheometer (HAAKE Co., Germany) with parallel plates (d = 27.83 mm). The gap between two plates was set to 1.0 mm. The temperature of protein samples was monitored through the lower plate. Excess sample out of the plates was trimmed off, and a thin layer of mineral oil applied to exposed free edges of the sample to prevent moisture loss of the samples, during heating. The equipment was driven through the RheoWin 3 Data Manager (HAAKE Co., Germany).

The program of heating–cooling cycle was applied as follows: heating from 25 to 95 °C at 1 °C/min; holding at 95 °C for 20 min; then cooling to 25 °C at the same rate. During this process, storage modulus (G′), loss modulus (G″) and phase angle (δ) were recorded as a function of time. In order to ensure all measurements were carried out within the linear viscoelastic region, first stress amplitude sweep was performed at a shear oscillation of 0.1 Hz. The dynamic measurement of gelation was carried out at constant stress τ = 0.1 Pa and frequency f = 0.1 Hz. After the cycle of heating and cooling, the formed gels were further investigated using frequency sweep measurement at 25 °C, in a controlled-stress mode (at a constant stress 1. of 1.0 Pa).