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Physicochemical and emulsifying properties of protein extracted from soybean meal assisted by steam flash-explosion

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ABSTRACT

The conventional alkaline aqueous extraction is not effective in promoting maximum protein yield from soybean meal (SBM). In this study, the steam flash-explosion (SFE) treatment was firstly employed to significantly improve the protein yield, while the protein content of soy protein isolate (SPI) decreased, concomitant with increase in carbon content of SPI. The SFE treatment led to the dissociation of insoluble protein aggregates in SBM, with subsequent increase of soluble protein aggregates formed via on-disulfide covalent bonds. The covalent coupling of the carbohydrate to the protein during SFE treatment can contribute to the dissolubility of protein and the formation of protein aggregates. After SFE treatment, surface hydrophobicity of SPI was decreased, however the emulsifying properties were improved. The emulsifying activity index and emulsifying stability index were improved to 41.94 m^2/g and 35.27 min under 1.3 MPa, 180 s treatment condition. It indicated that compared to surface hydrophobicity, changes of other aspects of protein structure including the covalent coupling of carbohydrate to protein were the predominant factors that ruled the emulsifying property of protein.
Industrial relevance: The steam flash-explosion treatment (SFE) is an eco-friendly, low-energy and non-traditional technology, which could be performed on a large scale for the industry. The SFE treatment can effectively change the physicochemical properties of protein resulting in significant increase of protein yield and improvement in emulsifying properties of protein. After SFE treatment, the protein from heat-denatured soybean meal can be refunctionalized for application in food industry.

1. Introduction

Soybean meal contains approximately 50% protein, which is mainly composed of glycinin and β-conglycinin. In addition to protein, soybean meal contains approximately 16% polysaccharides. A large part of the polysaccharides is cellulose and more than half represents pectic substances. The polysaccharides together form a complex matrix and form agglomerates with the cell wall proteins (Fischer et al., 2001). The complex matrix composition of soybean meal is suspected to affect protein availability and extractability. High-temperature thermal processing is necessary to remove the solvent resident and inactivate heat-labile anti-nutritional factors (ANFs) during soybean oil solvent extraction (Liener, 1994). Thermal processing denatures and insolubilizes soy protein rendering it poorly functional in foods. Therefore, the extraction of protein with excellent properties and high protein yields from high-temperature thermal treated soybean meal is greatly difficult, due to protein denaturation and complex matrix composition of soybean meal.

In our previous study (Zhang, Yang, Zhao, Hua, & Zhang, 2013), steam flash-explosion (SFE) has been employed for the first time to improve the extraction yield and functional properties of protein from heat-denatured soybean meal. SFE is an innovative, eco-friendly and low-energy method for pretreatment of biomass, which is based on exposing the biomass to high-temperature pressurized steam and forcing the steam into the fibrous tissues and cell of biomass, followed by explosive decompression completed in milliseconds (Yu, Zhang, Yu, Xu, & Song, 2012). During SFE treatment, when the deflation time is shorter than the pressure balance time between the inside and outside of the treated biomass internal structure, most of the steam and hot liquid water in the biomass will quickly expand, indicating that the thermal energy has been efficiently converted into mechanical energy and the internal structure of biomass is disrupted by a mechanical shearing force. Based on the mechanism of SFE, it can be concluded that during SFE treatment, the soybean meal is subjected to several forces and conditions: one is gas explosion force; another is high-temperature treatment. Supposedly, soybean meal is subjected to structure and physicochemical changes, including the breaking of complex matrix of cell structure (Negro, Manzanares, Oliva, Ballesteros, & Ballesteros, 2003), dissociation of protein aggregates (Wang, Wang, & Johnson, 2004), thermal-

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induced protein denaturation and Maillard reaction (Marsman, Gruppen, de Groot, & Voragen, 1998). The physical damage caused by mechanical shearing force produced by the gas explosion and the physicochemical changes can promote protein dissolution to realize more benefits of protein from soybean meal.

There are no data which have been reported about the effect of SFE on the physicochemical properties of soybean protein. The physicochemical change of soybean protein, along with tridimensional and surface structure, is most important for affecting the functionality of protein (Schwenke, 2001). To further enhance the use of soy protein, the protein structure in relation to the functional properties was widely studied and has become an exciting task in recent food research (Maruyama et al., 2002; Nagano, Akasaka, & Nishinari, 1995). The objective of this study is to investigate the changes induced by SFE treatment in structure and emulsifying properties of protein from soybean meal and to discuss the relationship between them.

2. Materials and methods

2.1. Materials

Soybean meals (protein content 49.49%) were provided by the Hangzhou Venus Biological Nutrition Co., Ltd. (Hangzhou, China) with nitrogen solubility index (NSI) of 18.90%. The soybean meals were obtained from dehulled flaked soybeans by extracting oil with hexane and then desolventizing the defatted flakes by means of high-temperature thermal process. The samples were ground to pass through a 20-mesh screen, but not an 80-mesh screen. The white flakes (WFs), which were the defatted flakes from solvent extraction and were desolventized by means of flash- or downdraft-desolventizing to minimize protein denaturation, were purchased from Harbin High Tech (GROUP) Co., Ltd. (Harbin, China) to serve as reference for determination of the functional properties.

1-Anilino-8-naphthalenesulfonate (ANS) and o-phthaldialdehyde (OPA) were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical grade.

2.2. Steam flash-explosion (SFE) pretreatment

The experiments were carried out in a QBS-200B SFE device with a 5 L reactor from Hebi Gentle Bioenergy Co. Ltd., China. The reactor is equipped with a high-pressure autoclave with gas inlet and a complete set of piston drive system. The force of the piston drive system which is composed of a linear actuator and a solenoid valve, comes from compressed air. The saturated steam was quickly allowed to enter the reactor. 600 g of ground soybean meals between the 20-mesh screen and the 80-mesh screen were treated by SFE pretreatment at 1.3 MPa and 1.8 MPa for 60 s, 120 s and 180 s, respectively. The SFE-treated soybean meals were carefully recovered, sealed in plastic bags and frozen for further analyses.

2.3. Preparation of SPI from SFE treated soybean meal

SFE treated soybean meal was dispersed in deionized water in a beaker to maintain a solids-to-water ratio of 1:10 (w/w). The slurry was placed in a 60 °C water bath and stirred for 45 min with pH maintained at 8.5. The samples were then centrifuged at 10,000 *g* for 20 min at 20 °C and the supernatant was collected. The collected supernatant was adjusted to pH 4.5 with 2 N HCl at 20 °C and stored for 1 h, followed by centrifugation at 10,000 *g* for 20 min at 4 °C. Protein curd was obtained as a precipitate and washed with deionized water twice. The curd was redissolved in deionized water at pH 7.0, freeze-dried, sealed in a plastic bag and stored at 4 °C until further use. Controls were similarly prepared from untreated soybean meal and WFs. All experiments were performed in triplicate.

2.4. Protein determination

The protein contents of SPI samples were measured by the micro-Kjeldahl method and a 6.25 conversion factor was used to calculate protein content. The protein yield was calculated from the measured protein content in the SPI relative to the total protein content of the starting soybean meal. The protein yield was calculated as:

$$\text{Protein yield}(\%) = \frac{\text{weight of protein in SPI(g)}}{\text{weight of protein in starting soybean meal(g)}} \times 100\%. \quad (1)$$

All experiments were performed in triplicate.

2.5. Elemental analysis of SPI

The contents of nitrogen and carbon in SPI were quantified by combustion in a Vario EL III elemental analyzer (Elementar Analysen Syetem GmbH, Germany).

2.6. Gel filtration chromatography (GFC) of SPI

Gel filtration of SPI was performed on a 130 cm × 1.6 cm column of Sephacryl S-300 (GE-Healthcare Bi-Science, Sweden). The column was equilibrated and eluted with 0.05 M sodium phosphate buffer (pH 7.0) containing 0.15 M NaCl. SPI samples were dissolved in sodium phosphate buffer (pH 7.0) to 5 mg/ml. Samples (4 ml) were injected in the column and eluted at an elution rate of 1 ml per min. The optical density at 280 nm was recorded. Fractions (6 ml per tube) were automatically collected and absorption spectrum for carbohydrate at 490 nm after color development with the phenol–sulfuric acid reaction were recorded (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Kato, Shimokawa, & Kobayashi, 1991). The void volume (V_0) and inner volume (V_t) of column were calibrated with blue dextran and potassium chromate.

2.7. Gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a Mini-Protean Tetra system with PowerPac Basic Power Supply (Bio-Rad Laboratories, Inc, Richmond, CA, USA). The analysis of SDS-PAGE was performed using 12% separating gel and 4% stacking gel according to Laemmli (1970). The protein samples (2 mg/ml) were mixed at a 1:1 ratio with the reductive sample buffer (0.06 M Tris–HCl buffer, pH 8.0, 2% SDS, 5% β-mercaptoethanol, 15% glycerol, 0.02% bromophenol blue). The sample solution was boiled for 3 min and centrifuged at 1500 *g* for 10 min to remove undissolved debris. After separation, the gel sheet was stained with Coomassie Brilliant Blue R-250 and destained with 10% acetic acid and 10% methanol in deionized water. The low-range MW markers from 14.3 to 97.3 kDa were used as standards. The gel sheet of SDS-PAGE stained with periodic acid–Schiff reagent was to detect the presence of carbohydrate (Van-Seuningen & Davri, 1992). The gel electrophoresis of protein was photographed using a gel documentation system (Gel Doc XR System, Bio-rad, USA) and the gel electrophoresis of carbohydrate was scanned using CanoScan LiDE110 (Canon, Japan).

2.8. FT-IR spectroscopy measurement

The infrared absorption data of SPIs were obtained by FT-IR spectroscopy (Nicolet iS10, American). The KBr pellet method was used to prepare the thin film for testing. Scanning was carried out in the range of 4000–400 cm^{-1} with resolution 4 cm^{-1}, and 32 scans