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Application of high density steam flash-explosion in protein extraction of soybean meal

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ABSTRACT

The high density steam flash-explosion (HDSFE) was used to extract protein from soybean meal. Soybean meal samples were treated at 1.3 MPa and 1.8 MPa for 60 s, 120 s and 180 s, respectively. After HDSFE treatment at 1.8 MPa for 180 s, the extraction yield of protein was increased from 50.50% to 65.66% compared with untreated soybean meal. The emulsification properties and fat-binding capacity of soy protein isolate (SPI) extracted from soybean meal treated by HDSFE were all improved compared with SPI extracted from untreated soybean meal and white flakes. Molecular weight distribution analysis of SPI showed that after HDSFE treatment the peak with molecular weight about 504 kDa and 43.3 kDa disappeared and the peak with molecular weight about 669 kDa increased indicating protein aggregation. Gel electrophoresis showed that high molecular weight aggregates of protein have been formed by covalent bond.

1. Introduction

Soy protein is traditionally important protein source in food industry owing to their relatively low price, nutritional benefits and versatile functional properties. Currently white flakes (WFs) are mostly the feedstock for soy protein. WFs are obtained from dehulled flaked soybeans by extracting oil with hexane, which are desolventized by means of flash- or downdraft-desolventizing to minimize the denaturation of protein (Nazareth et al., 2009). In China, annually millions of soybean meals have been subjected to high-temperature thermal process, leading to the denaturation of soybean protein. As a result the thermal-processed soybean meals were traditionally used as the animal feed, due to their poor functional properties caused by protein heat-denaturation (Wang et al., 2004a). If the heated-denatured protein in soybean meal can be refunctionalized, the available soy protein source will be extended and more benefits of soybean meal can be realized.

The extraction of protein from soybean meal is difficult, because the cellulose fibers and pectic substances form a complex matrix, which form agglomerates with the cell wall proteins in soybean meal (Carpita and Gibeaut, 1993). Therefore, there is urgent need to find an effective approach for break the complex matrix composition of the cell wall before protein extraction. Many methods such as enzymatic modification (Jung et al., 2006), ultrasound-assisted extraction (Karki et al., 2010), hydrothermal cooking (HTC) (Wang et al., 2004b), have been investigated to promote the protein extraction yield. However, the protein extraction from soybean meal has not been paid enough concern. Fischer et al. (2001) found that high-humidity heat treatment was more effective on protein extraction by combination enzymes (protease and carbohydrase) from unheated, heat-treated at high/low humidity soybean meal. Wang et al. (2004b) used the HTC to refunctionalize the extruded-expelled soybean meal and found that the solid dispersibility, protein dispersibility, and emulsification capacity of extruded-expelled soybean meal were all improved. Later, the authors (Wang et al., 2005) further successfully employed alkali addition to increase the protein yield of SPI during the HTC. But they also found that HTC-treatment can lower the emulsification capacity and foaming stability of the obtained SPI. Furthermore, Soybean meal in slurry was required for the hydrothermal cooking, which inevitably limited the treatment capacity.

Steam explosion was an effective method to pretreat lignocellulosic materials like bamboo, wood, corn stover and to convert these materials into food ingredients, fuel and chemicals. (Duff and Murray, 1996; Galbe and Zacchi, 2002; Tucker et al., 2003). The Steam explosion has also been used to extract antineoplastic constituents from *Sparassis crispa* mycelia and bamboo. (Kurosumi et al., 2006, 2007). The steam explosion process utilized the saturating steam

Abbreviations: SPI, soy protein isolate; WFs, white flakes; HDSFE, high density steam flash-explosion; HTC, hydrothermal cooking; EAI, emulsifying activity index; ESI, emulsion stability index; FBC, fat-binding capacity; SDS, sodium dodecyl sulfate; FC, foaming capacity; FS, foaming stability; HPSEC, high performance size exclusion chromatography; SDS–PAGE, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis; MW, molecular weight; ANOVA, analysis of variance; LSD, least significant differences.

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to cook the biomass at elevated pressure and temperature for several minutes, followed by explosive decompression, during which the flash steam evaporation of water exerts the thermo-mechanical force to destroy the texture structure of the biomass (Datar et al., 2007). Compared with other technique, steam-explosion has several advantages, including lower energy consumption, environment friendly (Cantarella et al., 2004).

The high density steam flash-explosion (HDSFE) in this study was based on steam cooking of the biomass for a short period of time followed by explosive decompression completed within very short time (ms). In the process of HDSFE, the steam could be forced into the cell and provide enough gas expansion force to disrupt the compact structure of soybean meal, when the thermal energy is converted to mechanical energy. The objective of the present study was to investigate the effect of HDSFE on protein extraction and functional properties of SPI extracted from soybean meal.

2. Materials and methods

2.1. Materials

Soybean meals (protein content 49.49%) were provided by Hangzhou Venus Biological Nutrition Co., Ltd. (Hangzhou, China) with nitrogen solubility index (NSI) of 18.90%. The soybean meals are 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 a 80-mesh screen. The white flakes (WFs) were purchased from Harbin High Tech (GROUP) Co., Ltd. (Harbin, China) to serve as contrast for determination of the functional properties.

2.2. HDSFE treatment

Soybean meals of 600 g were treated in a 5-L stainless steel reactor of the HDSFE system (Fig. 1). The reactor is equipped with a high-pressure autoclave with gas inlet and a complete set of piston drive system. The high density steam was allowed to enter the reactor and the steam pressure was maintained at 1.3 MPa and 1.8 MPa respectively. The time of steam pressure was respectively maintained for 60 s, 120 s and 180 s. Then the reactor was suddenly depressurized by piston drive system at extremely short time (ms). The soybean meal treated by HDSFE was recovered, cooled and stored in sealed container at −20 °C for further analyses.

2.3. Protein extraction

The protein of WFs, soybean meal and soybean meal treated by HDSFE were extracted with deionized water at a 10:1 liquid-to-solid ratio. The pH of mixture was adjusted to 8.5 with 2 N NaOH. After stirring for 45 min at 60 °C, the slurry was centrifuged at 10,000 g for 20 min at 20 °C. The supernatant was collected for protein determination.

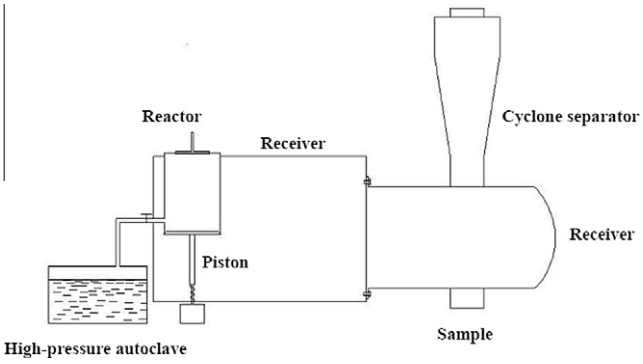

Fig. 1. High density steam flash-explosion system.

2.4. Preparation of soy protein isolate

In the process of preparation of SPI, 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. The supernatant was discarded and the precipitate was recovered, followed by washed with deionized water twice. Then the washed precipitate was redissolved in deionized water at pH 7.0, freeze-dried, sealed in plastic bag and stored at 4 °C until used.

2.5. Protein determination

The protein content in the supernatant, soybean meal and soybean meal treated by HDSFE was determined by the micro-Kjeldahl method and was estimated using a conversion factor of 6.25. The extraction yield of protein was calculated from the measured protein content in the recovered supernatant relative to the total protein content of the starting soybean meal. The extraction yield of protein was calculated as:

$$\text{Extraction yield of protein}(\%) = \left[\frac{\text{weight of protein in supernatant(g)}}{\text{weight of protein in starting soybean meal(g)}}\right] \times 100\% \quad (1)$$

2.6. Molecular weight determination of SPI by high performance size exclusion chromatography (HPSEC)

The SPI was dispersed in buffer of 0.05 M sodium phosphate (pH 7.0) containing 0.15 M NaCl to 2 mg/ml and centrifuged at 10,000*g* for 10 min. The supernatant was filtered through a cellulose acetate membrane with pore size of 0.22 μm. TSK gel G3000 SW_{XL} (7.8 mm ID × 300 mm) was selected for the test. Separation were performed at 25 °C at the flow rate of 0.8 ml/min with the mobile phase consisting of 0.05 M sodium phosphate (pH 7.0), 0.15 M NaCl. Injection of 20 μl of the protein solution was performed and the absorbance was followed at 280 nm. The molecular weight (MW) calibration was achieved using the protein standards. Protein standards, Ribonuclease A (MW: 13.7 kDa), Ovalbumin (MW: 44 kDa), Conalbumin (MW: 65 kDa), Aldolase (MW: 158 kDa), Ferritin (MW: 440 kDa), Thyroglobulin (MW: 670 kDa) were purchased from GE-Healthcare UK Limited.

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

SDS–PAGE was carried out in a discontinuous buffer system according to the method of Laemmli (1970) with 12% separating gel and 5% stacking gel. Samples were mixed with reducing sample buffer and heated for 5 min in boiling water. Each protein sample (10 μl) was loaded into a lane. The SDS–PAGE electrophoresis was performed at 80 V and followed at 120 V using the Bio-Rad mini-protein tetra cell. The low-range MW markers from 14.3 to 97.3 kDa were used as standards. The gel was stained with Coomassie Blue R-250, and destained with 10% acetic acid and 10% methanol in deionized water.

2.8. SPI solubility at pH 7.0

Protein solubility was measured according to the method of Jung and Mahfuz (2009). The SPI was dispersed in deionized water