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Functional, nutritional and flavor characteristic of soybean proteins obtained through reverse micelles

Xiaoyan Zhao [a, *, 1], Xiaowei Zhang [a, 1], Hongkai Liu [a], Guixiang Zhang [a], Qiang Ao [b, **]

[a] Department of Food Science and Nutrition, Business School, University of Jinan, Jinan 250022, China
[b] Basical Medical School, China Medical University, Shenyang, China

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ABSTRACT

The functional, nutritional and flavor characteristics of soybean proteins obtained through reverse micelles (RMs) were investigated, and compared with proteins separated by alkaline extraction and isoelectric precipitation (AEIP). The results showed that nitrogen solubility index, oil absorption capacity, foaming capacity, foaming stability, emulsifying capacity and emulsifying stability were found to be higher in the soybean proteins obtained through RMs (those values were 96.9%, 2.57 g/g, 131.65%, 84.33%, 81.71% and 82.26%, respectively) than in proteins obtained through AEIP (those values were 88.8%, 2.06 g/g, 112.32%, 57.15%, 50.94% and 51.22%, respectively), while water holding capacity decreased by 8.82%. Some individual amino acid contents in two proteins were different, some were similar, but the total amino acid content, EAA, AAS and BV(82.50%, 27.91, 115 and 92.67, respectively) of proteins using RMs were higher than those (79.31%, 26.36, 108 and 85.86, respectively). The flavor compounds in two type proteins were analyzed by GC–MS. A total of 15 different flavor compounds in the soybean proteins obtained through RMs were identified, while 22 different flavor compounds were identified in soybean proteins obtained through AEIP. The total flavor compounds in soybean proteins obtained through RMs was higher than that of AEIP, the percent ratio of hexanal to total volatiles recovery was lower. These results indicated that reverse micelles could improve the functional, nutritional and flavor properties of soybean proteins.

1. Introduction

Soybean is an excellent source of proteins with including all the essential amino acids, which processing functional, nutritional and flavor properties play an important role in the food industry (Lin, Tay, Yang, Yang, & Li, 2017). In general, the important functional, nutritional and flavor properties from proteins include solubility, emulsion capacity, foaming capacity, amino acid composition and volatile compounds, which can be affected by various intrinsic and extrinsic factors, such as molecular structure and size of proteins, extraction method, pH, ionic strength and processing conditions (Li et al., 2015; Maruatona, Duodu, & Minnaar, 2010; Yuan & Chang, 2007; Zhu et al., 2017). In order to improve the functional, nutritional and flavor characteristics as a food component, enzymatic, chemical and physical treatments have been applied to modify soybean proteins (Eslah, Jonoobi, Faezipour, Afsharpour, & Enayatia, 2016; Jung, Lamsal, Stepien, Johnson, & Murphy, 2006; Shen & Tang, 2012; Zhang, Yang, Zhao, Hua, & Zhang, 2013).

In recent years, the application of microemulsions to modify proteins has become an area of considerable research interests (Guo, Chen, Yang, Liu, & Zhang, 2015; Liang, Ma, Sun, Li, & Wu, 2013; Xiao, Cai, & Guo, 2013). The novel technique is suitable for the practical application in large-scale, continuous processing and highly selecting. The reverse micelles represent nano-size aggregates of encapsulated water from an organic medium by a surfactant, which have great potential for the separation, concentration and purification of bioactive proteins (Bu et al., 2014; Gaikaiwari, Wagh, & Kulkarni, 2012; Guo et al., 2015; Xiao et al., 2013). The changes of protein conformation are important for the modification of functional properties of proteins, and the researchers had reported how protein conformation was related to the functional properties of proteins (Liu, Zhao, Zhao, Ren, & Yang, 2012; Molina Ortiz & Wagner, 2002). The effect of reverse micelles on

* Corresponding author. Department of Food and Nutrition, Business School, University of Jinan, No. 13 Shungeng Road, Jinan 250022, China.
** Corresponding author.
E-mail addresses: zhaoxy_2201@163.com (X. Zhao), aoqiang@tsinghua.edu.cn (Q. Ao).
[1] Xiaoyan Zhao and Xiaowei Zhang contributed equally to this work.

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conformation of proteins had been investigated (Andrade & Costa, 2001; Chen et al., 2013; Naoe, Noda, Kawagoe, & Imai, 2004). The structure of proteins recovered from the reverse micellar phase might receive some changes owing to the experience through the micellar environment, which could depend upon the type of micellar system (Xiao et al., 2013). In addition, it is crucial for protein functionality that the proteins have an intrinsic molecular architecture, so the protein species in reverse micelle environment could affect the structure of proteins (Creighton, 1997). Zhao et al. (2008) reported that the reverse micelle treatment led to the changes in surface hydrophobicity, free SH and SS content and secondary structure of 7S and 11S globulins from soy proteins. Chen et al. (2013) also showed that reverse micelle treatment resulted in less turn and more α-helix, β -sheet and random coil in secondary structures of soy proteins. However, little information is available about the bis (2-ethylhexyl) sodium sulfosuccinate (AOT) reverse micelles to modify functional, nutritional and flavor properties of soybean proteins.

The objective of this research was to determine the effect of reverse micelles on functional properties, amino acid composition and flavor characteristics of soybean proteins. This will provide base-line information, which would help determine potential applications for soybean proteins in food systems. The soybean proteins obtained through alkaline extraction and isoelectric precipitation (AEIP) was also tested for comparison purposes.

2. Materials and methods

2.1. Materials

Bis (2-ethylhexyl) sodium sulfosuccinate (AOT) was purchased from Sigma Chemical Company (>98%; St. Louis, MO, USA). Bicinchoninic acid (BCA) protein assay kit was bought from USA Pierce Company (Rockford, IL, USA). All other reagents were of analytical grade. Soybean (No.25) was obtained from Shandong Academy of Agricultural Sciences (Jinan, China), the soybean cultivar was conventionally grown and the date of harvest was on Sep. 2015. The soybean was milled using a small high-speed grinder (type 6202, Beijing, China), and the soybean flour was sieved through a 100 screen. The approximate composition of raw material were as follows: total protein content (N × 6.25) 38.72% db, fat content 17.11% db, ash content 5.23% db and water content 9.49%. The soybean flour was defatted as the material of alkaline extraction and isoelectric precipitation proteins.

2.2. Forward extraction and backward extraction of AOT reverse micelles

Forward extraction of soybean proteins was typically carried by mixing soybean flour in reverse micellar phase under constant stirring conditions at stated temperature and time. The reverse micellar phase was prepared from known quantities of hexane, surfactant (AOT) and water (Xiao et al., 2013). AOT concentration was 0.05 M. The water content was usually expressed as W_0, the molar ratio of water to surfactant, i.e $W_0 = [H_2O]/[AOT]$. W_0 was adjusted by phosphate buffer pH 7.5 containing 0.05 M KCl, $W_0 = 18$. The ratio of soybean flour to reverse micellar phase was 1:20 (w/v). The forward extraction was stirred at 300 rpm for 30 min at 45 °C. The mixture was then centrifuged at 4000 g for10 min at room temperature. The organic phase separated from the mixture of the forward extraction was subjected to the backward extraction.

Backward extraction was carried out by mixing the organic phase of the forward extraction with an equal volume of stripping phase containing 1 M KCl phosphate buffer (50 mM) and pH 7.5, followed by vortexing for 60 min at 40 °C. The reaction mixture was centrifuged for at 3700 g at room temperature for 10 min. The stripped aqueous phase was dialyzed at 4 °C for 24 h to remove impurities and excess reagents. The soybean proteins were recovered by freeze-drying. Protein content in lyophilized powder was analyzed by the BCA reagent (Sigma) according to the method of Smith et al. (1985). 0.1 g of protein liophylized power was solubizied in 10 mL 0.05 M phosphate buffered solution (PBS) pH 7.5, then the solution was centrifuged at 5000 g for10 min at 4 °C. The supernatant was used to analyze protein. About 0.1 mL assay sample solution was added to 2 mL of BCA reagent. Samples were incubated at 37 °C for 30 min. After cooling to room temperature, the absorbances of standard and sample solutions were read spectrophotometrically at 562 nm. Bovine serum albumin (BSA) was used as the standard for protein quantification. The soluble protein content was 85.2%, the moisture content was 4.18%.

2.3. Preparation of soybean proteins from defatted soybean flour

Defatted soybean flour was dispersed in 50 mM phosphate buffer solution (1:15, w/v). The mixture was adjusted to pH 8.5 with 2 M NaOH, and the dispersion was stirred at room temperature for 2 h, and then centrifuged at 10, 000 g at 4 °C for 20 min in a CR22G high-speed centrifuge (Hitachi Co., Tokyo, Japan) to remove the insoluble material (Añón, de Lamballerie, & Speroni, 2012). The pH of supernatant was adjusted pH 4.5 at 4 °C 2 M HCl, and the precipitate was collected by centrifugation (10, 000 g, 10 min at 4 °C). The precipitate was washed with distilled water and dialyzed three times at 4 °C to remove impurities and excess reagents. The soy protein product was produced by freeze-drying. Soluble protein content was 82.7% by the BCA method (Smith et al., 1985), the moisture content was 4.52%.

2.4. Nitrogen solubility index

Nitrogen solubility index (NSI) was determined by the method of Li et al. (2016) with slight modifications. Protein samples (100 mg) were dissolved in 15 mL of distilled water. The pH of the solution was carefully adjusted to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0 by using either 1 M HCl or 1 M NaOH. The suspension was then shaken for 30 min at 25 °C in a water bath and centrifuged at 4800 g for 20 min. The clear supernatant was used to determine soluble nitrogen by the mico-Kjeldahl method by a conversion factor of 6.25. Triplicate samples were analyzed for proteins. The NSI value was measured as following equation:

$$\text{NSI}\ (\%) = \frac{\text{Amount of nitrogen in the supernatant}}{\text{Amount of nitrogen in the sample}} \times 100 \qquad (1)$$

2.5. Water and oil absorption capacity

Water and oil absorption capacity of soybean proteins were measured by the method reported by Zhu et al. (2010) with slight modifications. For water absorption capacity, 1.0 g protein samples were dissolved in 10 ml of distilled water and placed in 15 mL pre-weighed centrifuge tubes. The mixtures were stirred at 5 min intervals and held for 30 min, followed by centrifugation for 20 min at 5000 g at room temperature. The supernatant was decanted, the centrifuge tube containing the sediment was reweighed. For oil absorption capacity, 1.0 g protein samples were mixed with 5 mL soybean oil in pre-weighed centrifuge tubes and stirred for 1 min. After a holding period of 30 min, the tubes were centrifuged at 5000 g for 20 min at room temperature. The oil was then removed