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Influence of extraction and solubilizing treatments on the molecular structure and functional properties of peanut protein

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ABSTRACT

As primary functional property of protein in food industry, solubility was enhanced by various solubilization methods. Peanut protein was used to prepare product with high solubility by physical and enzymatic hydrolysis methods. Effects of extraction and solubilizing treatments on protein structure were evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR). Changes of functional properties including emulsifying, water-holding, fat-binding, foaming and viscosity properties were observed and interpreted. Modification treatments which could improve the solubility were almost suitable for the promotion of emulsifying, fat-binding and foaming capacities, but not always suitable for the improvement of viscosity and water-holding properties. SDS-PAGE showed average molecular weight remained unaffected after physical modifications combined by high-speed stirring and ultrasonic treatment while reduced after limited enzymatic hydrolysis and high-pressure homogenization. The microstructure of protein shown by SEM was gradually disbanded after all modification treatments. The regularity of protein secondary structure, which was damaged during alcohol extraction, was reconstructed by combined physical modification and limited enzymatic hydrolysis. Depending on the demand of functional properties on final product, manufacture process could be optimized by the selection of modification treatments based on observing the changes in protein structures.

1. Introduction

Peanut (*Arachis hypogaea* L.) is an important oilseed. In general, peanut oil is obtained by mechanical pressing and/or solvent extraction (Martinez & Tapriyal, 2011). The defatted peanut flour (DPF) is a protein-rich byproduct from oil processing. DPF contains 47–55% high quality protein with low level of anti-nutritional factors, but is underutilized because of its poor functional properties such as low solubility (Yu, Ahmedna, & Goktepe, 2007; Zhao, Liu, Zhao, Ren, & Yang, 2011). The loss of functional properties of protein, especially its solubility, may be due to the denaturation during high temperature mechanical pressing and the organic solvent(s) during extraction (Liu, Zhao, Ren, Zhao, & Yang, 2011; Moure, Sineiro, Domínguez, & Parajó, 2006; Zhang, Zhang, Sheng, Wang, & Fu, 2016).

To promote the utilization of peanut protein in foods, it is very important to ensure its desirable solubility. In general, protein solubility is usually required to be above 80% nitrogen solubility index (NSI) for food applications. However, the NSI of peanut protein extracted from DPF by alcohol precipitation is less than 65% at a natural pH (Ma, Wang, & Wu, 2010; Wu, Wang, Ma, & Ren, 2009). Several physical methods have been investigated including heating, freezing, microwave, ultrasonic, high-speed stirring treatments and high-pressure homogenization for their potential in improving peanut protein functionalities including solubility (Tang, Wang, Yang, & Li, 2009; Barraza, León, & Álvarez, 2015; Chen, Xu, & Zhou, 2016; Luo, Vasiljevic, & Ramchandran, 2016; Thaiphanit & Anprung, 2016). Enzymatic hydrolysis is also used for improving protein solubility (Jamdar et al., 2010; Sarmadi & Ismail, 2010; Zheng, Ren, Su, Yang, & Zhao, 2013; Zheng et al., 2015). In

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addition, the combined physical and enzymatic treatment was able to decrease the concentrations of Ara h1 and h2, two primary allergenic components of peanut (Li, Yu, Ahmedna, & Goktepe, 2013).

This study was conducted to improve the solubility of peanut protein extracted from DPF using physical and enzymatic treatments, and evaluate the effects of improving solubility on its other functional properties. The changes in the protein molecular structure were characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR). The functional properties including emulsifying, viscosity, water-binding, fat-absorbing and foaming properties were analyzed for the treated DPF with a DPF control. The information obtained from the present work may enhance the applications of peanut protein in food and food ingredient products.

2. Materials and methods

2.1. Materials

DPF was obtained from a peanut oil manufacturer in Shandong Province, China. The DPF was processed at 60.0 °C, and its oil residue content was less than 2.0% (w/w). Soybean oil was purchased from a local market. Sodium dodecyl sulphate (SDS), Coomassie blue R-250, mercaptoethanol and low macular weight protein markers (9.5–61 kDa) were purchased from the Solarbio Science & Technology Co., Ltd (Beijing, China). Bromophenol blue (BPB), acrylamide, tetramethylethylenediamine, ammonium persulfate and other reagents were purchased from the Sinopharm Chemical Reagent Co., Ltd (Beijing, China). All other chemicals used in the experiments were of analytical grade or biochemical grade, and used without further purification.

2.2. Extraction and alcohol precipitation

The DPF was mixed with aqueous alcohol (85.0%, v/v) at a ratio of 1:11.8 (w/v) and at 36.4 °C. The solution was continuously oscillated at 210 rpm for 60.0 min, and followed by centrifugation at 1500 rpm for 10.0 min. The solid was re-suspended and extracted in aqueous alcohol (97.5%) at a ratio of 1:8.0 (w/v), an extraction temperature of 38.4 °C. The protein extraction was oscillated for 30.0 min and centrifuged in the same way, and oven dried for 60.0 min at 40.0 °C to final water content of less than 5.0%.

2.3. Combined physical modifications

The solubility of the extracted peanut protein (PP) was subjected to a combined high-speed stirring and ultrasonic treatment. The solution of peanut protein was stirred using a high-speed homogenizer (XHF-D, Scientz Biotechnology, Zhejiang, China) with a solid-to-liquid ratio of 1:7.0 (w/v), a stirring rate of 22,000 rpm for 66 s at ambient temperature. Thereafter, a suitable amount of distilled water was added and then the solution was underwent the ultrasonic treatment using a ultrasonic cleaner (KQ-300VDV, Kunshan Ultrasonic Instruments Co., Ltd., Jiangsu, China) with a solid-to-liquid ratio of 1:10.0 (w/v) in a 75.0 °C water bath with an ultrasonic power of 210 W and an ultrasonic frequency of 28 KHz for 7.0 min.

2.4. Limited enzymatic hydrolysis

For further improvement of the solubility of physical modified peanut protein (PMPP), papain was used for limited enzymatic hydrolysis. Combined physical modified proteins were forward processed into this step immediately without dehydration. The hydrolysis was carried out at a solid-to-liquid ratio of 1:10.0 (w/v) and an enzyme concentration of 58.2 Unit/g proteins for 19.0 min at 49.7 °C.

2.5. High-pressure homogenization

The limited enzymatic modified peanut protein (EMPP) was further solubilized by high-pressure homogenization. As same as previous step, limited enzymatic hydrolyzed proteins were forward processed directly into this step without dehydration. A high-pressure homogenizer (GYB60-6S, Donghua Machine, Shanghai, China) was used with a solid-to-liquid ratio of 1:13.0 (w/v), a homogenization pressure of 50.0 MPa and a processing cycle of 10 times to obtain the final product of PMPP. After high-pressure homogenization, proteins were freeze-dried for long-term storage.

2.6. Determination of functional properties

2.6.1. Solubility

The solubility was determined according to a previously reported method (Feng et al., 2014) with a slight modification. One gram of protein was mixed with water at the ratio of 1:50.0 (w/v), and the suspension was stirred at 120 rpm for 2 h at 30.0 °C. The suspension was centrifuged at 3000 rpm for 10.0 min and filtered through a quantitative filtration paper. The protein content of each suspension was determined using the Kjeldahl method and evaluated using a nitrogen analyzer (FOSS 2300, Hillerød, Denmark). Nitrogen solubility index (NSI) was calculated as: $NSI\ (\%) = W_1/W_0 \times 100$, where W_1 was the weight of the protein in the filtrate (g), W_0 was the weight the protein in the original sample (g).

2.6.2. Emulsifiability and emulsion stability

The emulsifiability was measured according to a method described previously (Wu et al., 2009) with a slight modification. One gram of protein was dissolved with water at a concentration of 0.5% (w/v), and 20 mL of soybean oil was added into the protein solution followed homogenization using a high-speed homogenizer at 20,000 rpm for 1.0 min at ambient temperature. The mixture was centrifuged at 3000 rpm for 5.0 min. Emulsifiability was calculated as: $E\ (\%) = h/H \times 100$, where h was the height of emulsification layer in the tube (mm), H was the total height of the liquid in the tube (mm).

The emulsion stability was evaluated following a method reported previously (Chityala, Khouryieh, Williams, & Conte, 2016) with some modifications. One gram of peanut protein was dissolved in 40 mL water, and then 10 mL of soybean oil was added into the protein solution. The oil-in-water emulsions were prepared by a high-speed homogenizer at 20,000 rpm for 2.0 min at ambient temperature. Immediately after preparation, 30.0 mL of emulsions were transferred into flat-bottomed test tubes, and the tubes were sealed with caps to prevent evaporation. The emulsions were kept in the oven at 25.0 °C, and separated into a top oil layer and a bottom serum layer over time. The movement of creaming boundary was monitored hourly for the first 12 h, followed by a period of 5 days. The emulsion stability was evaluated by the creaming index (CI), which was calculated as: $CI\ (\%) = H_s/H_t \times 100$, where H_s was the height of bottom serum layer (mm), H_t was the total height of emulsion in the tube (mm).

2.6.3. Water-holding capacity

The water holding capacity was determined using a previously described protocol (Gong et al., 2016). One gram (W_0) of protein