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# Ultra high pressure homogenization effect on the proteins in soy flour

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ABSTRACT

Ultra high pressure homogenization (UHPH) is a non-thermal processing technique for the food functional properties modification. The objective of this study was to investigate the effect of UHPH on functional characteristics and subunit distribution of proteins in soy flour. The full-fat soy flour was treated by aqueous heating for 10 min or UHPH at the pressure of 100 or 150 MPa for 2 or 3 cycles and then freeze-dried. The sulfhydryl content and surface hydrophobicity of proteins were changed after aqueous heating or UHPH treatment. Denatured proteins in soy flour consequently formed large aggregates (particulates) and reduced protein solubility. The proteins in soluble fraction of aqueous heated soy flour mostly contained the acidic subunit of 11S and α subunit of 7S. The basic subunits of 11S and the remaining acidic subunits of 11S combined into a larger particulate protein in the insoluble fraction through disulfide bonding and hydrophobic interaction. The protein–protein interaction and their rearrangement might occur more rapidly and randomly in UHPH modified soy flour. The structure of particulate proteins were much simpler than that in aqueous heated soy flour due to a lower degree of protein denaturation and also due to the strong mechanical forces generated by UHPH. Changes in the protein functional characteristics and subunit distribution in soy flour with different treatments are distinct. These results could provide information for determining UHPH applying condition in soy flour modification.



## 1. Introduction

Soy flour is a versatile soy food ingredient due to its functionality, nutritional quality and low cost (Devi & Haripriya, 2014; Jideani, 2011; Puyed, Begum, Saraswathi, & Prakash, 2010; Yeganehzad, Mazaheri-Tehrani, Mohebbi, Habibi Najafi, & Baratian, 2013). It comes in three major forms including the natural or full-fat, defatted, or lecithinated type of flour. In order to meet the requirement of food manufacturers, further treatments are applied to obtain optimal nutritive value and functional properties, e.g. heat treatment (Caprita & Caprita, 2010; Radha & Prakash, 2009).

Ultra high pressure homogenization (UHPH) is a non-thermal process technique and is applied to decrease particle size, to stabilize emulsion, to inactivate spoilage microorganisms or pathogens, and to improve the rheological properties and texture of food products (Briñez, Roig-Saugués, Hernándezherrero, & Guamislópez, 2006; Cruz et al., 2007; Cruz, Capellas, Jaramillo, Trujillo, Guamis, & Ferragut, 2009; Diels & Michiels, 2006; Masson, Rosenthal, Verônica, Deliza, & Tashima, 2011; Vannini, Lanciotti, & Guerzoni, 2004; Wuytack, Diels, & Michiels, 2002). Moreover, the cavitation phenomena, high shearing, turbulence or impingement induced by the strong force of UHPH can affect the macromolecular conformation of soy proteins (Floury, Desrumaux, & Legrand, 2002; Keerati-u-rai & Corredig, 2009; Molina, Papadopoulou, & Ledwar, 2001). These advantages make the UHPH a potential replacement method for the tofu like soy product making.

In our previous study (Liu, Chien, & Kuo, 2013), the soy flour was modified by UHPH in a valve-mode homogenizer for two or three cycles at two different pressures (100 and 150 MPa) for tofu making without typical heating process. A favorable texture better than the regular thermal treated tofu was obtained successfully. The functional properties of soy flour were affected major by soy proteins such as β-conclycinin (7S) and glycinin (11S) (Barac, Stanojevic, Jovanovic, & Pesic, 2004; Liu, 1997). Therefore, the effect of UHPH on proteins in soy flour is important for the applications of UHPH on soy flour in the food product. The objective of this research was to investigate the effect of UHPH on functional characteristics and subunit distribution of protein in soy flour.

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## 2. Materials and methods

### 2.1. Materials

The full-fat soy flour was prepared by grinding soybean (Non-GMO soybean, Neco Seeds Farms Inc., Garden City, USA) into flour directly using cyclone mill (UDY Corporation, Fort Collins, Colorado, USA) and passing the flour through a 100 mesh sieve. The full-fat soy flour was stored at room temperature and used for further treatments within two weeks.

### 2.2. UHPH treatments

According to Liu et al. (2013), 13% (w/w) soy flour suspension was prepared by mixing 1.5 g of soy flour with 10 mL distilled water and then equilibrating for 20 min at room temperature. The soy flour suspension was then treated by UHPH with cooling circulation (APV-2000, SPX Co., Charlotte, USA) at two levels of pressure (100 MPa and 150 MPa) for two and three cycles. The soy flour suspension without any treatment was used as control. Thermal treated soy flour suspension (heating at 95 °C for 10 min) was also prepared for comparison. The treated soy flour suspensions were then freeze-dried into dried flour for further analysis. The temperature of soy flour suspension prior to UHPH was 4 °C and the temperature after 1-cycle, 2-cycle, and 3-cycle UHPH was 35 °C, 45.5 °C, and 53.5 °C, respectively.

### 2.3. Protein solubility analysis

The soy flour sample (0.3 g) was mixed with 15 mL of 50 mM Tris–HCl buffer (pH 8.0) and agitated for 1 h at room temperature. The sample suspension was then centrifuged at 2000 × g for 15 min at 4 °C. The supernatant and precipitate were separated. The protein in the supernatant was classified as soluble protein. Protein solubility was calculated as the soluble protein content divided by the total protein content of sample in percentage (Lakshmanan, Lamballerie, & Jung, 2006). Both soluble protein and total protein contents were analyzed by the Kjeldahl method with the nitrogen factor of 6.25.

The precipitate collected from the above centrifugation procedure was re-dissolved in 10 mL lysis buffer containing 7 M urea, 2 M thiourea and 4% 3-[(3-cholamidopropyl)-dimethylammonioa]-1-propanesulfonate (CHAPS) and agitated for 2 h at room temperature. The suspension was then centrifuged at 2000 × g for 15 min at 4 °C. The protein in the supernatant was classified as particulate protein. The fractionation procedure of soluble and particulate proteins is summarized in Fig. 1.

### 2.4. Protein surface hydrophobicity analysis

The surface hydrophobicity of soy protein was determined following the procedure of Lakshmanan et al. (2006) with modification. The sample solution (1 g/L) was prepared by diluting the soluble protein solution or particulate protein solution (Fig. 1) with 0.04 M phosphate buffer (pH 6) and then filtered with filter paper (100 circles, Toyo Rashi Kaisha Ltd, Tokyo, Japan). The sample solution was serially diluted with the same buffer to obtain protein concentrations ranging from 0.02 to 0.2 g/L. The hydrophobic fluorescence probe, 1-anilino-8-naphtalene- sulfonic acid magnesium salt monohydrate (ANS), was then added into the diluted sample solutions and incubated for 2 h in the dark. The fluorescence intensity of protein was measured by using a fluorescence spectrophotometer (Model FP-750, JASCO Inc., Tokyo, Japan) at the excitation and emission wavelengths of 380 nm and 490 nm, respectively. The slope of the plots of fluorescence intensity versus protein concentration was calculated using linear regression analysis and was referred to as the surface hydrophobicity ($H_0$).

### 2.5. Protein sulfhydryl content analysis

The sulfhydryl content of soy protein was determined according to the procedures described by Ellman (1959), Lakshmanan et al. (2006) and Faris, Wang, and Qang (2008). The surface free sulfhydryl content (SFSH) and total free sulfhydryl content (TFSH) were determined by using Ellman's reagent (10 mM DTNB). The sample solution (1 g/L) was prepared by diluting the soluble protein solution or particulate protein solution (Fig. 1) with buffer and then filtered with filter paper. For the SFSH measurement, 50 mM Tris–HCl buffer (pH 8) was used to adjust the concentration of sample solution, while for the TFSH measurement, 50 mM Tris HCl buffer (pH 8) containing 8 M urea and 0.5% SDS was used. After adding Ellman's reagent, the sample solution was incubated for 15 min in the dark at room temperature. The absorbance was measured at 412 nm wavelength by using a UV–Vis spectrophotometer (SP-8001, Metertech, Inc., Kaohsiung, Taiwan). The SFSH and TFSH contents were calculated using a molar extinction coefficient of 13,600 $M^{-1}$ $cm^{-1}$ and expressed as μmole of SH/g protein.

### 2.6. Gel electrophoresis

SDS-PAGE of soy protein was performed according to the method of Hsieh, Yu, and Tsai (2012) with modification. SDS-PAGE was carried out in a Bio-Rad mini-protein electrophoresis system (Bio-Rad Laboratories, Hercules, USA) with a 1.5 mm thickness vertical slab gel including 12.5% acrylamide running gel and 5% stacking gel. The 0.1 mL of sample was mixed with 0.9 mL of electrophoresis sample buffer containing 5% β-mercaptoenthanol, 2% SDS, 10% glycerol, 0.02% bromophenol blue, and 70 mM Tris–HCl (pH6.8). The sample solution was then heated at 100 °C for 10 min and cooled in an ice bath for 10 min. The 8 μL of sample and protein ladder was then loaded into the separated wells in the electrophoresis system. After electrophoresis, gels were immediately stained with the Coomassie blue R-250 for 30 min and destained with the solution containing 40% ultrapure water, 50% methanol, and 10% acetic acid for 4 h and then destained again with the solution containing 10% acetic acid and 30% methanol overnight. Stained gels were then scanned by using a photo scanner (Epson Perfection V200 Photo, Seiko Epson Co., Nagano, Japan).

Two-dimension polyacrylamide gel electrophoresis (2-DE) of soy proteins was determined according to the method of Hsieh et al. (2012). The 400 μg of sample was immobilized and loaded into the pH gradient (IPG) gel trips (pH4-7, 13 cm, GE Healthcare, Sweden). Isoelectric focusing of the strips was accomplished by using a IPGphor 3 IEF system (GE Healthcare, Sweden) with 6000 V constant voltage at 20 °C for a total of 60 kVh. The strip was then equilibrated in the equilibrium solution (50 mM Tris–HCl (pH 8.8), 6 M urea, 2% SDS, 30% glycerol and 2% DTE) for 15 min and placed vertically on the top of a 12.5% SDS-PAGE gel. The 0.5% agarose solution was filled above the strip and a new gel was formed after cooling at room temperature. The second electrophoresis was performed in a Bio-Rad Protean II xi Cell system with current in 10 mA per gel for 1 h and then 45 mA per gel for 5 h until the bromophenol blue reached the bottom of the gel. After second electrophoresis, the gels were fixed with the solution containing 7% acetic acid and 10% methanol for 30 min and stained with the Sypro Ruby protein gel stain solution overnight in the dark (Berggren et al., 2000). The 2-DE image of gels was acquired by using a photo scanner. Protein identification of the 2-DE gel was based on the suggestions of Hsieh et al. (2012).