Journal of Food Engineering 169 (2016) 250–258

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Enzyme-assisted subcritical water extraction and characterization of soy protein from heat-denatured meal

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ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form
22 August 2015
Accepted 7 September 2015
Available online 9 September 2015

Keywords:
Soy protein
Subcritical water
Enzymatic hydrolysis
Isoflavones
Emulsifying properties

ABSTRACT

Protease prehydrolysis followed by subcritical water (SW) treatment was carried out to extract protein from heat-denatured soy meal. The composition, physicochemical, interfacial and emulsifying properties of soy protein isolate (SPI) were evaluated. A significant increase in extraction yield was observed although protein purity was decreased with prolonged hydrolysis. Due to contaminated β-glucosidase in protease from *Aspergillus oryzae*, Maillard reaction and the conversion of isoflavone glycosides into aglycones occurred, leading to the enrichment of aglycones in SPI. Compared to native SPI, enzyme-assisted SW-prepared SPI exhibited higher hydrophobic amino acids, surface hydrophobicity, and interfacial adsorption due to protein unfolding, accompanied by the formation of small soluble aggregates. Additionly, remarkable improvements of emulsifying ability and physical stability of emulsion were probably associated with higher surface protein loads. These results could develop a feasible protocol for producing nutrient-enhanced soy proteins with excellent emulsifying properties as novel functional ingredients applied in food industry.

1. Introduction

The applications of soy proteins as functional food stuffs have been extensively concerned due to their high nutrient value and excellent functional properties. In terms of health benefits, soy protein consumption could decrease the incidence of some chronic diseases (Chao, 2008). Moreover, simultaneous ingestion of soy proteins with isoflavones, another important component in soy products, has been attempted to evaluate their synergistic effects. Soy protein, as an efficient delivery vehicle, could promote the absorption of isoflavones into the circulation (Andrade et al., 2010). Isoflavone-containing soy proteins could effectively prevent and attenuate cardiovascular diseases, postmenopausal bone loss, and certain cancers (Alekel et al., 2000; Steinberg et al., 2003). Additionally, isoflavone aglycones are also expected to be incorporated into soy protein, which may be a promising approach for further promoting health. To improve the bioavailability of isoflavones, the addition of exogenous β-glucosidase to soy products and microbial fermentation have been executed to hydrolyze sugar moiety since isoflavone β-glycosides do not cross human intestine (Tsangalis et al., 2002; Wu and Muir, 2010).

To improve functional properties of soy protein, several strategies, including heat treatment (Wang et al., 2012), protein glycosylation (Xu et al., 2010), and limited enzymatic hydrolysis (Zhang et al., 2012), have been extensively explored. Heat treatment successfully improves emulsifying ability of globular proteins due to partial protein unfolding. Moreover, heat-induced protein aggregates also act as food-grade emulsifiers and Pickering-like stabilizers to effectively stabilize protein emulsion against creaming by forming a gel-like protein layer at the oil–water interface (Liu and Tang, 2013). Maillard reaction could provide a safe synthesis of conjugates to improve the solubility, interfacial, and emulsifying properties of protein. Recently, amphiphilic hydrolysate, a favorable emulsifier, was prepared by restrictive hydrolysis of Maillard-type soy β-conglycinin-dextran conjugate (Zhang et al., 2012).

To date, synchronous improvements of both nutritional and functional properties by optimizing preparation processes of

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http://dx.doi.org/10.1016/j.jfoodeng.2015.09.006
0260-8774/

protein are highly challenging. Soy protein isolate (SPI) is usually prepared from low-denatured soy meals obtained by extracting oil with hexane and downdraft-desolventizing. In fact, thermal desolventizing for soy meals is usually used to more efficiently remove solvent resident and deactivate anti-nutritional factors (Refstie et al., 2005). Unfortunately, this process could lead to the loss of solubility due to protein denaturation. The utilization of heat-denatured soy meals as cheap protein sources is thus greatly limited due to the difficulty in SPI extraction. Heat treatments, e.g., hydrothermal cooking and steam flash-explosion, have been used to release insoluble soy proteins. Hydrothermal cooking efficiently extracted SPI and refunctionalized heat-denatured soy proteins in extruded-expelled meals (Wang et al., 2004, 2005, 2006). Steam flash-explosion with dilute acid soaking also successfully achieved this mission (Zhang et al., 2013a,b), which may be attributed to structural rearrangement of protein and the formation of soluble aggregates (Wang et al., 2012; Zheng et al., 2008). The similarity of these heat treatments is that heating temperature is in the range above 100 °C. Under this condition (100–374 °C), water maintained in subcritical liquid state may be an effective solvent for extracting polar and non-polar compounds (e.g., protein and bioactive polyphenols) from a wide variety of matrices due to its low dielectric constant (Carr et al., 2011; Teo et al., 2010; Watchararuji et al., 2008). Chang et al. (2004) attempted to extract isoflavones from defatted soy flakes using superheated water at elevated pressures.

In view of better solubilization of denatured protein and isoflavone under subcritical water (SW) condition, in this work, we attempted to employ SW treatment as an effective approach for preparing isoflavone-rich SPI from heat-denatured soy meals. Auxiliary enzymatic hydrolysis was selected to further improve functional properties of protein. Wu and Muir (2010) found that soy flour hydrolyzed by Protease M contained a high content of aglycones except bioactive peptides due to contaminated β-glucosidase in enzyme preparation. Hence, Protease M prehydrolysis of heat-denatured soy meals followed by SW treatment was carried out to prepare isoflavone aglycone-rich SPI with excellent functional properties by purposeful modifying protein composition and conformation. The physicochemical (e.g., molecular weight distribution and surface hydrophobicity), interfacial, and emulsifying properties of protein were also investigated.

2. Materials and methods

2.1. Materials

Heat-denatured soy meals were provided by Jintaiyang Co. Ltd. (Guangzhou, China). Protease M (Lot NO: PRJ1150402MSD, 51.5 AU/g, *Aspergillus oryzae*) was purchased from Amano Enzyme Co. Ltd. (Nagoya, Japan). One unit of protease activity was defined as the amount of enzymes required to liberate 1 μg tyrosine per g of protein in 1 min under assay conditions. Papain (800 U/g), 1,8-anilinonaphthalenesulfonate (ANS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), bovine serum albumin (BSA), and isoflavones standards (daidzin, glycitin, genistin, daidzein, glycitein, and genistein) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Bovine thyroglobulin (669 kDa), ferritin (440 kDa), and conalbumin (75 kDa) were purchased from GE Healthcare (Buckinghamshire, UK). Corn oil obtained from supermarket. All other chemicals were of analytical grade.

2.2. Enzyme-assisted SW extraction of soy protein

The heat-denatured soy meals (100 g) were dispersed in distilled water at a solid/solvent ratio of 1:10 (w/v). Protease M was added to the dispersion (4% w/w, E/protein). The pH of dispersion was adjusted to 4.5 using 2 mol/L HCl, and incubated with shaking (150 rpm) at 50 °C for various time (10, 30, 60, 90, and 120 min). Then the pH of dispersion was adjusted to 9.0 using 2 mol/L NaOH and deactivated by heating at 100 °C for 5 min. Soy meal dispersions (15 mL) were then placed in a pressure reactor (20 mL) with a thermal control system (Parr pressure reactor, Controller 5500, Moline, IL, USA). Our previous studies confirmed that hydrothermal cooking process (>100 °C) can efficiently improve the solubility of denatured proteins, and soy protein may be involved in different pathways of structural changes upon SW treatment (120 °C) compared to heat treatment (90 °C) (Wang et al., 2012; Zheng et al., 2008). To prepare isoflavone aglycone-rich SPI with excellent functional properties, SW treatment was thus performed at 120 °C for 20 min, and sample temperature reached required values within 3 min. After SW treatment, the dispersions were rapidly cooled to 25 °C and centrifuged at 8000 g for 30 min. SPI was subsequently recovered by precipitating at pH 4.5 and centrifugating at 8000 g for 20 min. The protein precipitate was redissolved in distilled water, neutralized to pH 7.5 using 2 mol/L NaOH, and freeze-dried. HSPI represents SPI prepared by SW treatment alone without enzymatic hydrolysis. M-10, M-30, M-60, M-90, and M-120 represent SPI extracted using Protease M hydrolysis at different times (10, 30, 60, 90, 120 min) and follow-up SW treatment, respectively.

To evaluate extraction efficiency using enzyme-assisted SW treatment, conventional alkaline extraction (pH 9.0) and subsequent acid precipitation (pH 4.5) at 25 °C as control treatments were performed for SPI preparation from heat-denatured soy meals. Unfortunately, protein yield obtained by conventional method was only 16.4%. Hence, to obtain good comparability, native SPI (NSPI) as another control sample was also prepared by suspending white soy flakes in distilled water at pH 9.0 and precipitating it at 4.5. The precipitate was redissolved in distilled water and then neutralized to pH 7.5 with 2 mol/L NaOH. All protein extraction were carried out in triplicate. The protein yield and purity of samples were determined by Kjeldahl method.

2.3. Protein composition

The amino acid composition was determined by monitoring the absorption at 254 nm using HPLC system (Waters M510, Milford, MA, USA) equipped with a PicoTag column. The determination was executed at 38 °C and a flow rate of 1 mL/min. The tubes containing soy protein were evacuated, sealed, and hydrolyzed using 6 mol/L HCl at 110 °C for 24 h. Amino acid composition was reported as g/100 g protein. Tryptophan was not determined due to amino acid destruction during acid hydrolysis.

The isoflavone content of SPI was determined according to the methods of Yang et al. (2014). Papain (0.02 g/g protein) was added to protein solution (2 mg/mL) and then incubated at 50 °C for 1 h. To deactivate enzyme and extract isoflavones, acetonitrile (5 mL) and 0.1 N HCl (1 mL) were then mixed. The dispersions were centrifuged at 10,000 g for 15 min and were then filtered through a 0.22 μm filter. The isoflavone content was analyzed using a Waters HPLC 1525 system equipped with a symmetric C18 column (5 μm, 4.6 $\times$ 250 mm, Waters). Binary gradient elution was carried out with Solvent A (acetonitrile) and Solvent B (a phosphoric acid aqueous solution, pH 3.0). After the 10 μL sample was injected, Solvent B decreased from 88% to 82% over 10 min, then held at 82% for 13 min, then decreased to 76% over next 7 min, and held at 70% for 25 min. Solvent B then decreased back to 20% over next minute, and then held at 88% for 10 min. The flow rate was 0.5 mL/min, and the elution (Fig. S1 in the Supplementary material) was monitored at 260 nm using an absorbance