LWT - Food Science and Technology 69 (2016) 1–8

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Improved emulsifying capabilities of hydrolysates of soy protein isolate pretreated with high pressure microfluidization

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ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form
27 December 2015
Accepted 11 January 2016
Available online 13 January 2016

Keywords:
Soy protein isolate
Pancreatin hydrolysis
High-pressure microfluidization
Emulsification functionality
Surface-active peptides

ABSTRACT

Soy protein isolate (SPI) was treated by high-pressure microfluidization and pancreatin hydrolysis in this work. Results showed that microfluidization substantially enhanced pancreatin hydrolysis of SPI in terms of degree of hydrolysis (DH), with a preferable treatment condition at 120 MPa and 30 g/L SPI concentration. SDS-PAGE conducted under reducing conditions showed that microfluidization increased the accessibility of some subunits (α′-7S, A-11S and B-11S) in SPI to pancreatin hydrolysis, resulting in changes in protein solubility (PS), surface hydrophobicity (H_0), and molecular weight distributions for hydrolysates. Emulsion systems (20 vol.% oil, 20 g/L protein samples, pH 7.0) formed by control SPI and SPIH (SPI hydrolysates) were unstable due to fast coalescence and bridging flocculation during homogenization, while that formed by MSPIH (microfluidization pretreated SPIH) with 5.8% DH was more stable and showed smaller mean droplet size (d_{43}). Compared with SPIH, MSPIH showed a stronger increase in PS and a more moderate change in H_0 during pancreatin hydrolysis, suggesting the production of more surface-active soluble peptides, which may explain their markedly improved emulsifying capabilities. This work showed that modified SPI could be an effective food emulsifier with microfluidization pre-treatment and limited proteolysis leading to desirable functional modifications of globular proteins.

1. Introduction

Soy proteins have become increasingly popular because of their high nutritional quality and low price, which has been a driving force for increased soy research and commercial development of new soy protein products. Soy protein isolate (SPI) is the most refined soy protein products, containing >90% protein on dry weight basis, and has been used as emulsifier in food emulsions. It is preferred because of the surface-active properties of its constitutive proteins: 7S (β-conglycinin) and 11S (glycinin) globulins. Although SPI is characterized as less capable as an emulsifier when compared to milk proteins, it outperforms many other plant-sourced proteins (Palazolo, Sorgentini, & Wagner, 2004). Moreover, considering the large molecular mass and a large amount of hidden hydrophobicity groups for soy proteins, SPI is prospectively of great potential to become an efficient food emulsifier if appropriately modified (Nishinari, Fang, Guo, & Phillips, 2014).

Protein modification based on proteolysis has a broad potential for designing protein functionality for specific applications, and the effects of proteolysis are determined by factors such as protease specificity, degree of hydrolysis (DH) and substrate characteristics (Tavano, 2013). Pancreatin, mainly composing of trypsin and chymotrypsin, has a broad specificity to peptide bonds, and preferentially cleaves C-terminal hydrophobic regions (Su et al., 2012). Previous studies showed that soy protein hydrolysates prepared with pancreatin hydrolysis exhibited improved emulsifying capabilities over the original proteins (de la Barca, Ruiz-Salazar, & Jara-Marini, 2000; Qi, Hettiarachchy, & Kalapathy, 1997). Limited proteolysis exposes hydrophobic and hydrophilic residues, enhances the amphiphilic characteristics of proteins, and improves emulsification (Tavano, 2013). However, previous studies found that soy proteins were generally resistant to proteolysis (Govindaraju & Srinivas, 2007; Henn & Netto, 1998; Qi et al., 1997). The intrinsic difficulty is that globular soy proteins have compact quaternary and tertiary structures that protect many of the peptide bonds (Govindaraju & Srinivas, 2007). Moreover, protein aggregation during the processing of SPI may result in the burying of cleavage

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http://dx.doi.org/10.1016/j.lwt.2016.01.030
0023-6438/

sites (Henn & Netto, 1998). Therefore, pre-treatment is necessary to modify the structural characteristics of soy proteins, in order to increase their accessibility to protease and obtain enhanced functionalities.

Microfluidization is a state-of-the-art dynamic high-pressure techniques, which uses the combined forces of ultrahigh pressure, high frequency vibration, instantaneous pressure drop, intense shear, and cavitation, and thus exhibits a much larger energy density compared with conventional valve homogenization (Ciron, Gee, Kelly, & Auty, 2010). Moreover, microfluidization has a variety of advantages including no exogenous chemicals, little nutritional loss and very short processing time. Recently, several studies have reported that microfluidization was highly efficient in modulating physico-chemical and structural properties of whey proteins (Dissanayake & Vasiljevic, 2009; Liu et al., 2011), peanut proteins (Hu, Zhao, Sun, Zhao, & Ren, 2011) and soy proteins (Shen & Tang, 2012). It was demonstrated that microfluidization could not only alter the structure of globular proteins, but also could disrupt insoluble heat-induced protein aggregates into smaller soluble aggregates, resulting in the exposure of inner groups buried inside the folded structure (Liu et al., 2011; Shen & Tang, 2012). Therefore, microfluidization treatment might have the potential to alter the accessibility of SPI to proteolysis and caused enhanced functionalities for hydrolysates. However, little work has been done so far to investigate this possibility.

Hence, this work aims to investigate the effects of high-pressure microfluidization pre-treatment on the proteolysis pattern of SPI and on the emulsifying capabilities of its hydrolysates. Some key physico-chemical and structural properties of hydrolysed products were measured accordingly to better understand the underlying mechanisms of improved emulsifying capabilities for SPIH and MSPIH.

2. Materials and methods

2.1. Materials

Commercial SPI was obtained from Shandong Wonderful Industrial Co. (Yantai, China), containing (g/100 g of powder) 91.2 protein, 4.5 moisture, 2.8 ash, and 0.2 fat. Pancreatin (P7545; 8 × standard USP unit), phenylmethanesulfonyl fluoride (PMSF), 1-Anilino-8-naphthalenesulfonate (ANS) and Nile Red were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade and commercially available. Water used to prepare aqueous solutions was purified with a Milli-Q filtration unit (Millipore, Bedford, UK).

2.2. Microfluidization pre-treatment of SPI

SPI powder was dispersed into deionized water to reach different concentrations (10 g/L, 30 g/L, 50 g/L) and pH was adjusted to 7.0 using (0.1–1.0) mol/L HCl solutions, with magnetically stirring at ambient temperature for 2 h to fully disperse the SPI powder. The resultant dispersions were microfluidized directly without centrifugation using an M-110EH microfluidizer (Microfluidics Co., Newton, MA, USA) operating at different levels of pressure (40–160 MPa). Each sample passed through the system twice. The machine was equipped with two interaction chambers with different entry point diameter: 200 μm (IC_{200}) and 75 μm (IC_{75}). The IC_{75} was placed downstream from the IC_{200}. Samples had an initial temperature of 15 °C before microfluidization but increased to approximately 25 °C after two passes. All samples were used for proteolysis directly after microfluidization treatment.

2.3. Preparation of SPIH and MSPIH

Dispersions of SPI and MSPI were proteolysed using pancreatin at pH 7.0 and 50 °C. Based on preliminary experiments, appropriate E/S ratios (g enzyme/100 g substrate) varied from 0.025 to 0.5 were selected to reach different required DH for SPIH and MSPIH. The hydrolysis reaction was performed in a water bath with continuous stirring. The pH of sample dispersions was maintained during hydrolysis using a TIM840 Auto-titrator (Radiometer Analytical co., Villeurbanne, France) loaded with (0.1–1) mol/L NaOH solution. After 120 min of hydrolysis, the consumption of NaOH solution was recorded for the determination of DH using the pH-stat method described by Adler-Nissen (1986). Pancreatin hydrolysis was stopped by the immediate addition of PMSF to a concentration of 1 mmol/L. Serine protease inhibitor PMSF instead of thermal treatment was used to inactivate pancreatin, so that protein functionalities would not be influenced by thermal changes (Luo et al., 2010). The hydrolysates were lyophilized and ground to produce a powder, which was then stored in a desiccator. Control SPI and control MSPI were prepared using the same incubation conditions and enzyme inactivation treatment, but without pancreatin added. Samples were coded according to the profiles of microfluidization treatment, followed by the DH value. For example, MSPIH-5.8% means hydrolysate of SPI was pretreated by microfluidization and hydrolysed to a DH of 5.8%.

2.4. Determination of protein solubility (PS)

PS was determined according to the method of Shen and Tang (2012). Sample dispersions (10 g/L, pH 7.0) were stirred at ambient temperature for 2 h and were centrifuged at 12,000 × g for 20 min to obtain the supernatants. Protein content of the supernatants was determined by the micro-Kjeldahl method (N × 6.25). The PS was calculated as the percentage ratio of soluble protein content in supernatant against total amount of protein presented in samples.

2.5. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on a discontinuous Tris-glycine-SDS buffer system under reducing conditions using a Tris–HCl Ready Gel with 15% resolving gel and 4% stacking gel (Bio-Rad Laboratories, Hercules, CA) according to the method of Jung, Murphy, and Johnson (2005), with slight modifications. Protein samples were dispersed into a Bio-Rad Laemmli sample buffer (62.5 mmol/L Tris–HCl buffer (pH = 6.8), containing 20 g/L SDS, 5 mL/100 mL 2-mercaptoethanol, 25 mL/100 mL glycerol and 0.1 g/L bromophenol blue) (Bio-Rad Laboratories) to a concentration of 1 μg/μL. Then samples were shaken in a vortex for 10 s, heated at 95 °C in a water bath for 5 min, and centrifuged at 12,000 × g for 10 min. The gels were calibrated with marker with molecular weights (MW) ranging from 6.5 to 200 kDa (M8445, Sigma Chemical Co.). 15 μL of supernatant and 5 μL of marker were loaded into the lanes. All gels were run in a Mini-protean tetra system (Bio-Rad Laboratories). After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250.

2.6. Determination of surface hydrophobicity (H_0)

H_0 was determined according to the method of Luo et al. (2010). Sample dispersions were diluted (0.05–0.2 g/L) in phosphate buffer (0.01 mol/L, pH 7.0) and were centrifuged (12,000 × g, 20 min) to obtain the supernatants. 20 μL of ANS solution (8.0 mmol/L in the same buffer) was added to 4 mL of each dilution, and the