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Effect of high intensity ultrasound on structure and foaming properties of pea protein isolate

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ABSTRACT

The effects of high intensity ultrasound (HIUS, 20 kHz, at varying amplitude 30%, 60%, 90% for 30 min) on structure and foaming properties of pea protein isolate (PPI) were investigated. No significant change was observed from the electrophoresis profiles and circular dichroism (CD) spectrum. Analyses of fluorescence spectroscopy and the amount of free sulfhydryl groups showed that HIUS induced protein molecular partial unfolding. Furthermore, HIUS decreased particle size of PPI and increased exposed hydrophobicity, resulting in a reduction of the surface tension at the air − water interface. Therefore, the foaming ability of PPI increased from 145.6% to 200.0%. The foaming stability increased from 58.0% to 73.3% with the increasing amplitude after 10 min though all reduced to 50.0% with the extension of time. That suggested that HIUS treatment has a potential to be implemented to modify foaming properties of PPI.

1. Introduction

Plant proteins are widely used as ingredients in foods due to their nutritional value and relatively low cost (W. W. Peng et al., 2016; Saldanha do Carmo et al., 2016). Among vegetable proteins, pea proteins (*Pisum sativum*) are of 10.3 million metric tonnes of the worldwide total production and have great potential as a functional ingredient in the food industry (Jiang, Zhu, Liu, & Xiong, 2014; Simsek, Tulbek, Yao, & Schatz, 2009; Taherian et al., 2011). Pea protein isolate (PPI) shows a well-balanced profile of amino acid, especially a high content in lysine. (Jiang, Zhu, et al., 2014; Nunes, Raymundo, & Sousa, 2006). Despite the fact that PPI is an excellent source of nutrition, its utility in food applications is limited. This is probably due to the limited functional properties of pea proteins (Adebiyi & Aluko, 2011). A foam is a dispersion of gas in a liquid. The tightly packed gas bubbles occupy most of the volume. The liquid phase is continuous, and it contains surfactants which stabilize the bubbles (Cantat et al., 2013). Foam can confer desirable textural and sensorial attributes to food products, like in whipped desserts and toppings (Damodaran, 2005; Zou et al., 2016). However, the abilities of plant protein isolates to adsorb at the air-water interface and stabilize the foams are limited due to their compact structure (Morales, Martinez, Pizones Ruiz-Henestrosa, & Pilosof, 2015). Therefore, it is valuable for the food industry to investigate method that is capable to improve the foaming properties of plant protein isolates.

Physical, enzymatic modifications and polysaccharides addition have been carried out to improve the foaming properties of plant proteins (Ma et al., 2011; Makri, Papalamprou, & Doxastakis, 2005; Martínez, Farías, & Pilosof, 2011; Morales et al., 2015; Tang & Ma, 2009; Yuan, Ren, Zhao, Luo, & Gu, 2012; Yust, Pedroche, Millán-Linares, Alcaide-Hidalgo, & Millán, 2010). Several methods for enhancing other functional properties of PPI were developed, such as heat treatment (W. W. Peng et al., 2016), pH treatment (Jiang, Zhu, et al., 2014; Liang & Tang, 2013) and forming protein-polysaccharide conjugates (Gharsallaoui, Yamauchi, Chambin, Cases, & Saurel, 2010; S. Liu, Elmer, Low, & Nickerson, 2010; Makri et al., 2005). However, little is known about the method for improving the foaming properties of PPI. Ultrasound is a rapid, efficient and reliable alternative to improve the quality of food, which has the potential to develop the new product with a unique functionality.

The application of ultrasonic technology in food industry is currently arousing much attention (Hu et al., 2013). Ultrasound is an acoustic wave with a frequency > 20 kHz (Resendiz-Vazquez et al., 2017). High intensity ultrasound (HIUS, 20–100 kHz) might have a wide variety of applications in food industry (L. Zhang, Jin, Xie, Wu, & Wu, 2014), which can alter the physicochemical properties and/or structure and functional properties due to cavitation effects on vegetable proteins, for instance, rice protein isolate (O'Sullivan, Murray,

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Flynn, & Norton, 2016), soy protein isolate (Hu et al., 2013; Hu, Li-Chan, Wan, Tian, & Pan, 2013; Jambrak, Lelas, Mason, Krešić, & Badanjak, 2009; Morales et al., 2015; O'Sullivan et al., 2016), black bean protein isolate (Jiang et al., 2014), jackfruit seed protein isolate (Resendiz-Vazquez et al., 2017) and sunflower protein isolate (Malik, Sharma, & Saini, 2017). Hao Hu et al. (Hu, Wu, et al., 2013) showed that ultrasonic treatment resulted in partial unfolding and improved solubility and fluid character of soy protein isolate dispersions, Furthermore, Rocío Morales et al. (Morales et al., 2015) found that the HIUS treatment improved the foaming capacity of soy protein isolate by alteration of particle size whereas its stability was not modified significantly. Improvement on solubility, emulsifying properties, foaming capacity, and foam stability of sunflower protein isolates caused by HIUS were observed by Mudasir Ahmad Malik et al. (Malik et al., 2017)

However, literature focussing on the potential effects of HIUS application on PPI is scarce. Thus, this study aims to investigate the effects of HIUS treatment on the foaming and structural properties of PPI. To achieve this, samples were treated at different levels of ultrasound amplitude (0%, 30%, 60%, 90%) for 30 min.

2. Materials and methods

2.1. Materials

Commercial pea protein powder was purchased from Yantai Oriental protein science and Technology Co., Ltd. (Yantai, Shandong, China). 8-anilinonaphthalene-sulfonic acid (ANS) and 5,5-Dithiobis-(2-nitrobenzoicacid) (DTNB) were obtained from Aladdin Reagent Company (Shanghai, China). The other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. PPI preparation

Pea protein isolate (PPI) was extracted from the purchased commercial pea protein powder (*Pisum sativum* L.) using the isoelectric precipitation method (J. Jiang, Chen, & Xiong, 2009; Zhang, Xiong, Chen, & Zhou, 2014). The powder (100 g) was dispersed in distilled water at a solid/solvent ratio of 1:10 (w/v) and the suspension was adjusted to pH 8.0 with 2 M NaOH. After being stirred at 500 rpm for 2 h at 25 °C by a stirrer (Shanghai Sile Instrument Co. Ltd., Shanghai, China), the suspension was centrifuged at 10000*g* for 30 min at 4 °C. Then, the obtained supernatant was subjected to precipitation by adjusting pH to 4.5 with 2 M HCl. After being stored at 4 °C for 2 h, the supernatant was then centrifuged at 10000*g* for 30 min at 4 °C. The protein precipitate was washed twice with distilled water, and then centrifuged at 10000*g* for 30 min at 4 °C. After neutralization (pH 7.0), the samples were freeze-dried and stored at 4 °C. The prepared PPI content was 94.3% (w/w) as determined by using the micro-Kjeldahl method (nitrogen conversion factor is 6.25).

2.3. High intensity ultrasound (HIUS) treatment and acoustic energy determination

PPI (5%, w/v) solution was obtained by dissolving powder in deionized water under stirring at 25 °C for 4 h prior to overnight storage at 4 °C. An ultrasound processor (Fisher Scientific, FB705, USA) with a 12-mm diameter titanium probe was used to process 15 mL of PPI dispersions in 30 mL glass vessels which were immersed in an ice-water bath. Samples were treated at different levels of amplitude (0%, 30%, 60%, 90%) for 30 min (pulse duration of on-time 5 s and off-time 2 s). We changed ice-water every 10 min to maintain the temperature of the samples. The acoustic energy was determined in the previous work, and ultrasonic intensities were measured by calorimetry with the thermocouple (model TASI-8530, Suzhou, China) (Margulis & Margulis, 2003; Xiong et al., 2016). Ultrasound treatment with the 20-kHz probe at various amplitude (30%, 60%, 90%) generated ultrasonic intensities of 22–25, 34–36 and 45–48 W/cm^2, respectively.

2.4. Morphology observation

The morphology of the lyophilized PPI samples (0%, 30%, 60%, 90%) was observed with a scanning electron microscope (SEM, SU8000, Japan). Before using the scanning electron microscope, the samples were ground and coated with gold.

2.5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed according to the method by Peng et al. (W. W. Peng et al., 2016) with slight modification. The concentrations of the separating and stacking gels were 12% and 5%, respectively. PPI samples (0%, 30%, 60%, 90%) were dissolved in the buffer in the presence of 2-mercaptoethanol (β-ME). The samples were heated for 3 min in a boiling water bath, and then centrifuged at 10,000*g* for 3 min before loaded to the cell. The gel was stained with Coomassie Brilliant Blue R-250 and scanned using a computing densitometer (Universal Hood II, Bio-Rad, USA).

2.6. Circular dichroism (CD) spectrum measurement

The CD spectrum of the PPI solution (0.1 mg/mL, 10 mM PBS, pH 7.4) was measured by using spectropolarimeter (Jasco 810, Jasco Corp., Tokyo, Japan) at 25 °C. The sample was put in a quartz cuvette with an optical path of 0.1 cm. The scan wavelength ranged from 190 to 240 nm, the scan speed was 50 nm/min. The value of bandwidth was 1 nm. Three scans were averaged. And the proportions of α-structure and β-structure were gained by the Yang's equation.

2.7. Fluorescence measurement

The fluorescence of the PPI solution (0.2 mg/mL, 10 mM PBS, pH 7.4) was measured by using a Luminescence Spectrometer (F-4600, Japan). The excitation wavelength was 290 nm and emission spectra ranged from 300 to 460 nm. The slit width and scan speed were 2.5 nm and 1200 nm/min, respectively.

2.8. Surface sulfhydryl groups (SH) determination

The content of free sulfhydryl groups was measured by the method of Ellman's reagent (5, 5′-dithiobis-(2-nitrobenzoic acid), DTNB) (Xiong et al., 2016).The protein samples were dissolved in Tris–HCl buffer (containing 86 mM Tris, 90 mM glycine and 4 mM ethylenediamine tetraacetic acid (EDTA), pH 8.0) to obtain 0.5% (w/w) protein solution, 0.1 mL DTNB was added to 10 mL PPI solution. The absorbance of solution was determined at 412 nm after 30 min at 25 °C in dark environment. The buffer was used as the reagent blank. The extinction coefficient, which was taken as 13,600 M^{-1} cm^{-1}, was used to calculate the free SH contents.

2.9. Surface hydrophobicity (H_0) determination

The surface hydrophobicity of PPI was measured by a fluorescence spectrum assay (SHIMADZU RF-5310PC) using8-anilino-1-naphthalenesulfonicacid (ANSA) as a fluorescent probe (D. Peng et al., 2017; Xiong et al., 2016). The 10 mL PPI solution (1 mg/mL, 10 mM PBS, pH 7.4) was mixed with 0.1 mL ANSA solution (2.4 mM, 10 mM PBS, pH 7.4). The excitation wavelength was 390 nm. The emission and excitation slits were 5 nm, and the emission spectrum was measured from 400 to 650 nm. Relative exposed hydrophobicity was calculated according to eq. 1

$$H_0 = S_1 - S_2 \quad (1)$$