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Influence of ultrasonic treatment on the structure and emulsifying properties of peanut protein isolate

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ABSTRACT

Effects of ultrasonic treatment on emulsifying properties and structure of peanut protein isolate (PPI) were evaluated by analysis of particle size distribution, protein surface hydrophobicity, SDS-PAGE, circular dichroism spectra and environmental scanning electron microscopy. The emulsifying properties of the PPI were found to be improved by ultrasonic treatment. The mean particle size decreased from 474.7 nm to 255.8 nm while the molecular weight remained unaffected. The results of intrinsic fluorescence spectroscopy and surface hydrophobicity indicated that ultrasonic treatment induced tertiary structural changes of the proteins in PPI. Emulsifying activity index and emulsion stability index were found to be correlated fairly well with surface hydrophobicity (H_0) ($r = 0.712$ and $r = 0.668$, respectively).

Keywords: Ultrasonic treatment; Peanut protein isolate; Emulsifying properties; Structure; Surface hydrophobicity

1. Introduction

Peanut protein isolate (PPI) is the by-product of the peanut oil squeezing process in China. The output of defatted peanut flour is large, and it contains 47–55% proteins with high nutrition value (Basha and Pancholy, 1982). However, these proteins still remain underutilized (Yu et al., 2007). The functional properties of PPI, i.e., emulsifying, foaming and gel properties are not as good as soy protein isolate (Wu et al., 2009). Although PPI has good potential to be a source of nutritional proteins, its poor functional properties have greatly limited its application in the food industry.

Numerous methods have been investigated to improve protein's functional properties, including heat treatments, enzymatic hydrolysis and high-pressure homogenization (Chen et al., 2011; Penas et al., 2004; Sorgentini et al., 1995). Ultrasonic treatment is frequently used in chemical synthesis, preparation of pharmaceuticals, polymer, chemical, textile and cosmetic industries (Canselier et al., 2002; García-Pérez et al., 2007; Noshad et al., 2012). Recently, it has been used in food processing for changing viscosity and texture, functionality of dairy products, meat tenderization, mixing and emulsification (Jayasooriya et al., 2004), degassing and foam control (Villamiel et al., 2000) and improving the extraction process of food bioactive substances (Vilkhu et al., 2008). It has also been applied in other fields, for example, food drying, assisted crystallization and sterilization (De la Fuente-Blanco et al., 2006; Luque de Castro and Priego-Capote, 2007; Villamiel et al., 2000). Ultrasonic treatment not only represents a rapid, efficient and reliable alternative to improve the quality of food,

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but also has the potential to develop new products with a unique functionality (Soria and Villamiel, 2010).

In the present work, we first prepared PPI using alkali extraction followed by acid precipitation. We then evaluated the effect of ultrasonic treatment on the emulsifying properties of PPI. The emulsifying properties of PPI were found to be correlated with the protein structure.

2. Materials and methods

2.1. Materials

Peanut protein powder (80% protein, 7.3% moisture, 3.2% fat and 6% ash) (Baiaote plant protein science technology Co., Ltd., Shanghai, China), bovine serum albumin (BSA), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), 8-anilinonaphthalene-sulfonic acid (ANS), were purchased from Sigma Chemical Co. (St, Louis, MO, USA). Sodium dodecyl sulphate (SDS), Coomassie Blue R-250 and N,N,N′,N′-tetramethyl ethylene diamine (TEMED), blue prestained low molecular weight protein marker (14.4–97.4 kDa) were obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Soybean oil was purchased from a local supermarket and used directly without further purification. All chemicals used in the present study were of analytical grade.

2.2. Preparation of peanut protein isolate

Peanut protein powder was suspended in 12-fold distilled water and the pH was adjusted to 8.0 with 2 N NaOH at 50 °C for 1 h. After centrifugation at 4000 r/min for 20 min, the pH of the supernatant was adjusted to 4.5 with 2 N HCl to precipitate the peanut proteins. The precipitated proteins were collected and dissolved in distilled water. The pH was adjusted to 7.0 followed by dialysis with distilled water at 4 °C for 24 h. After freeze-drying, the peanut proteins were stored in 4 °C until their use in ultrasound modification and other tests.

2.3. Ultrasound pretreatment of PPI

PPI dispersions (8 g L^{-1}) were prepared by adding PPI powder into distilled water and then gently stirred overnight at ambient temperature (25 °C). The PPI dispersions were treated by ultrasonic cell disruption instrument (JY98-IIIDN, Ningbo Xinzhi Instruments, Inc., Ningbo, China, 20 kHz) with a 2.0 cm diameter titanium probe. 100 mL of PPI dispersions in 150 mL flat bottom conical flasks were immersed in a low temperature thermostat (DC-2010, Ningbo Xinzhi Instruments, Inc., Ningbo, China), which can control the temperature ranging from −20 °C to 100 °C. Samples were treated under different levels of power output (0, 120, 300, 480, 660, 840, 1020 W) at different temperatures (0, 15, 25, 35, 45, 55, 65, 75 °C) for 0, 1, 3, 5, 10, 20, 30 min (pulse durations of on-time 9 s and off-time 1 s), respectively. After freeze-drying, the samples were stored in 4 °C until further tests.

2.4. Determination of emulsifying properties

Emulsifying properties were determined according to the method of Jamdar et al. (2010) with a little modification. Soybean oil (10 mL) and 30 mL of 1% protein solution were mixed. The mixture was homogenized using a homogenizer (Ika-Ultra-Tur-rax T25, Germany) at a speed of 10,000 rpm for 2 min. An aliquot of the emulsion (50 μL) was pipetted from the bottom of the container at 0 and 10 min after homogenization and mixed with 5 mL of 0.1% SDS solution. The absorbance of the diluted solution was measured at 500 nm using a spectrophotometer (T6, Pgeneral, Beijing, China). The absorbance measured immediately (A_0) and 10 min (A_{10}) after emulsion formation were used to calculate the emulsifying activity index (EAI) and emulsion stability index (ESI) as follows:

$$\mathrm{EAI}(\mathrm{m}^2/\mathrm{g}) = \frac{(2 \times 2.303 \times A_0)}{[0.25 \times \text{protein weight(g)}]}$$

$$\mathrm{ESI(min)} = A_0 \times \Delta t / \Delta A$$

where A_{10} is the absorbance at 10 min after homogenization; Δt = 10 min; and $\Delta A = A_0 - A_{10}$.

2.5. Determination of mean particle size and distribution (PSD)

The mean particle size and particle size distributions (PSDs) were determined with a by Nicomp 380/ZLS Zeta potential/Particle sizer (PSS Nicomp, Santa Barbara, USA) according to the methods of Liu et al. (2011a). The Nicomp 380, based on dynamic light scattering (DLS), provides accurate mean particle size and PSD down to 1 nanometer. Samples were diluted by approximately 1/1000 with deionized water in the sample dispersion unit under stirring.

2.6. SDS-PAGE

SDS-PAGE was performed according to the method of Laemmli (1970) with slight modifications. A 12% acrylamide separating gel and a 5% acrylamide stacking gel containing 0.1% SDS were used in a Bio-rad Mini protean Tetra MP4 electrophoresis (Bio-Rad Mini-Protean System). The samples were mixed with loading buffer (containing 0.06 M Tris–HCl buffer (pH 8.8), containing 2% SDS, 5% β-mercaptoethanol, 25% glycerol and 0.1% bromophenol blue). The solutions were then heated in boiling water for 10 min and centrifuged at 10,000 g for 3 min before electrophoresis. Ten microliters of each sample were loaded onto the gel. Low molecular weight markers (14.4–97.4 kDa) were run as a reference. Electrophoresis was performed in electrophoresis buffer (containing 0.025 M Tris–HCl, 0.192 M Glycine and 0.1% SDS) for 30 min at 16 mA, followed by 1.5 h at 32 mA. Gels were stained with Coomassie Blue G250 and destained in a 20% ethanol/80% deionized water mixture.

2.7. Fluorescence spectra analysis

2.7.1. Intrinsic fluorescence

The intrinsic emission fluorescence spectra of the protein samples were obtained by a Fluor photometer (F4500, Hitachi, Tokyo, Japan) according to Liu et al. (2011b) with modifications. Protein solutions (0.2 mg mL^{-1}) were prepared in 10 mM phosphate buffer (pH 7.0). To minimize the contribution of tyrosine residues to the emission spectra, the protein solutions were excited at 290 nm, and emission spectra were recorded from 300 to 460 nm at a constant slit of 2.5 nm for both excitation and emission. All the measurements were conducted in triplicate.

2.7.2. Surface hydrophobicity (H_0) Measurement

H_0 values of samples were determined using ANS as the fluorescence probe according to the method of Kato and Nakai (1980). Lyophilized PPI and UPPI samples (1.0 mg mL^{-1} in 0.01 M phosphate buffer at pH 7.0), and serially diluted with the same