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Physicochemical properties of peanut protein isolate–glucomannan conjugates prepared by ultrasonic treatment

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ABSTRACT

Peanut protein isolate (PPI) was glycated with glucomannan through classical heating or ultrasound treatment in this work. The physicochemical properties of PPI–glucomannan conjugates prepared by ultrasound treatment were compared to those prepared by classical heating. Compared with classical heating, ultrasound treatment could accelerate the graft reaction between PPI and glucomannan and improve the concentration of available free amino groups of PPI. Solubility and emulsifying properties of the conjugates obtained by ultrasound treatment were both improved as compared to those obtained by classical heating and native PPI. Decreases of lysine and arginine contents during the graft reaction indicated that these two amino acid residues attended the covalent linkage between PPI and glucomannan. Structural feature analyses suggested that conjugates obtained by ultrasound treatment had less α-helix, more β-structures and random coil, higher surface hydrophobicity and less compact tertiary structure as compared to those obtained by classical heating and native PPI.

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1. Introduction

Peanut is one of the most important oilseeds in the world. Defatted peanut meal, with 50–60% high value protein, was largely underutilized as animal feed [1]. Peanut protein isolate (PPI) extracted from the peanut meal was reported to possess poor functional properties and the application was greatly limited [2]. Usually, the functional properties of proteins are of key interest to manufacturers of pharmaceutical, food, and cosmetic products. Therefore, the promising approach is desirable to improve the functional properties of PPI.

Physical, chemical or enzymatic treatment has been done on converting protein into stable formation with better functional properties and better utilization in food industry [3–5]. In recent years, a number of researchers have begun to conjugate proteins with polysaccharides through Maillard-type reactions [6–9]. It has been reported that this method of modification was efficient in improving the emulsifying properties, solubility, antibacterial effect, antioxidant effect and reducing allergenicity of proteins [8,10,11]. Furthermore, this method utilized a naturally occurring reaction and no chemical reagent was applied in this approach. Therefore, this method could be one of the most promising approaches for food applications, because of its safety.

Generally, glycation of proteins is carried out by using either dry-heated treatment or wet-heated treatment [12–14]. Actually, those methods are time-consuming. Therefore, it is necessary to find a way to improve the efficiency of graft reactions. The use of ultrasound in food industry has increased recently due to its promising effects in food and product modification. Ultrasound not only represents a rapid, efficient and reliable alternative to improve the quality of food, but it also has the potential to develop new products with a unique functionality [15]. Ultrasound can accelerate chemical reactions, increase diffusion rates and disperse aggregates through acoustic cavitation [16]. Concerning this, high intensity ultrasound was used to prepare protein-polysaccharide conjugates in this study.

Konjac glucomannan is a high molecular weight neutral polysaccharide obtained from the Konjac plant root powder and has been used as safe food ingredient [17,18]. It has been demonstrated that konjac glucomannan is effective in weight reduction, modification of intestinal microbial metabolism, and cholesterol reduction [17]. Additional to the health-promoting benefits, konjac glucomannan has been generally used in food, chemical engineering and other fields because of its unique physical and chemical properties [19,20]. However, there is little information available on physicochemical properties of PPI–glucomannan conjugates.

In this study, PPI was grafted with glucomannan with the assistance of ultrasonic treatment. The solubility, emulsion capacity, surface hydrophobicity and intrinsic fluorescence emission

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spectroscopy of PPI–glucomannan conjugates were studied. Furthermore, the secondary structure and amino acid composition of conjugates were also determined.

2. Materials and methods

2.1. Materials and chemicals

Defatted peanut meal was a gift from Shandong Luhua Group Co. Ltd. (Shandong, China). PPI was prepared according to the method mentioned below. Defatted peanut flour was dispersed in deionised water (1:10, w/v), and the pH of the dispersion was adjusted to 8.0 with 2 M NaOH. The resultant dispersion was gently stirred at 25 °C for 2 h, then centrifuged at 10,000*g* and 20 °C for 30 min. The pellet was discarded, and the supernatant was adjusted to pH 5 with 2 M HCl and then centrifuged at 5000*g* and 20 °C for 30 min. The obtained precipitate was re-dispersed in deionised water. The dispersion was homogenized and adjusted to pH 7.0 with 2 M NaOH, followed by freeze-drying to produce PPI product. The content of PPI was 86.7 ± 1.2%, determined by Kjeldahl method. Glucomannan was purchased from Shiyan Huaxianzi Konjac Productions Co., Ltd. (Hubei, China), which was used without further purification. 1, 8-anilinonaphthalene-sulfonate (ANS) regent was purchased from Sigma (St. Louis, MO, USA). Bovine serum albumin (BSA), o-Phthaldialdehyde (OPA), and all other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Soybean oil was purchased from local supermarket and used directly.

2.2. Preparation of PPI–glucomannan conjugates

PPI (1%, w/v) and glucomannan (1%, w/v) were dispersed in phosphate buffer solution (0.2 M, pH7.5). Then the solution was treated by an ultrasonic equipment (VC × 500, Sonics vibra cell, USA) with a probe of a 10 mm titanium tip for different times (20, 40, 60, 80, or 100 min) at different temperatures (60, 70 or 80 °C) and ultrasonic intensity (302.55, 544.59 or 786.62 W/cm^2). Thereafter, the slurry was cooled to ambient temperature and dialyzed at 4 °C for 24 h. Finally, the samples were freeze-dried and stored at room temperature. The same solution was conducted by classical heating in a JR-AB water bath oscillator (Jerel electric appliance Co., Ltd., Jiangsu, China) at 180 rpm and 70 °C for different times. The subsequent procedure was the same to ultrasonic treatment. Moreover, the solution without ultrasonic treatment was used as control.

2.3. Degree of graft (DG)

Free amino groups were determined by an o-Phthaldialdehyde assay. 80 mg OPA was dissolved in 2 ml methanol and mixed with 50 ml of 10 mM sodium tetraborate, 5 ml of 20% (w/w) SDS, and 200 µl of β-mercaptoethanol. The solution was diluted to a final volume of 100 ml with distilled water to form the OPA reagent. 200 µl of protein solution (2 mg/ml) was incubated with 4 ml of OPA reagent at 35 °C for 2 min. The absorbance at 340 nm was measured in order to obtain the free amino groups. Lysine was used as standard.

DG was calculated as:

$$DG = \frac{A_0 - A_t}{A} \times 100\%$$

where A_0 are the levels of free amino groups in control; At are the levels of free amino groups in conjugates and A are the levels of free amino groups in PPI.

2.4. Protein solubility

The samples were dispersed in deionized water to form a solution of 2 mg/ml protein content, and then the pH value of the solution was adjusted from 9 to 3. Afterwards, the solution was centrifuged at 12,000*g* for 30 min at 20 °C, the protein content of the supernatants was determined by Lowry's method (1951), using BSA as the standard.

2.5. Emulsifying properties

The samples were dissolved in phosphate buffer solution (10 mM, pH 7.0) to obtain a final protein content of 2 mg/ml. For emulsion formation, soybean oil and protein solution (1:3, v/v) was homogenized at 24,000 rpm for 1 min. After homogenization for 0 min and 10 min, 50 µl of the emulsion was immediately taken from the bottom of the beaker, and diluted as 1:100 with 0.1% SDS solution. The absorbance of diluted emulsion was recorded at 500 nm. EAI and ESI were calculated as the following equations:

$$\mathrm{EAI}\ (\mathrm{m}^2/\mathrm{g}) = \frac{2 \times 2.303 \times A_0 \times \mathrm{DF}}{C \times \varphi \times \theta \times 10,000},$$

$$\mathrm{ESI}\ (\mathrm{min}) = \frac{A_0}{A_0 - A_{10}} \times 10$$

where DF is the dilution factor (100), C is the protein concentration (g/ml), φ is the optical path (1 cm) and θ is the oil volume fraction (0.25), A_0 and A_{10} are the absorbance of the emulsion at 0 min and 10 min, respectively.

2.6. Measurement of surface hydrophobicity (H_0)

H_0 values of samples were determined by adding 20 µl, 8.0 mM ANS to 4 ml sample solution, in which the protein content was diluted to 0.05, 0.1, 0.2, 0.5 and 1 mg/ml by phosphate buffer solution (10 mM, pH 7.0). Fluorescence intensity (FI) was measured at 390 nm (excitation) and 470 nm (emission) using a Hitachi F-7000 fluorescence spectrophotometer (Hitachi, Ltd., Tokyo, Japan) both with a slit width of 5 nm. The initial slope of FI versus protein concentration plot was used as the index of $H_{0.}$

2.7. Intrinsic fluorescence emission spectroscopy

The samples were diluted to 1.5 mg/ml protein content with phosphate buffer solution (10 mM, pH 7.0). The intrinsic fluorescence emission spectra were also obtained by a Hitachi F-7000 fluorescence spectrophotometer (Hitachi, Ltd., Tokyo, Japan). The sample solutions were excited at 290 nm, and the emission spectra was recorded from 300 nm to 400 nm (both with a slit width of 5 nm) in order to minimize tyrosine residues contribution.

2.8. Analysis of amino acids

The samples were acid hydrolyzed in vacuum-sealed tubes, the lysine and arginine levels were determined by Agilent 1100 high performance liquid chromatography(HPLC) (Agilent technologies Co., Ltd., Santa Clara, USA) equipped with an ODS Hypersil column (5 µm, 250 × 4.6 mm).

2.9. Circular dichroism (CD)

Conformational changes in the secondary structure of the samples were analyzed using a Mos-450 CD spectropolarimeter (Biologic, Claix, France). The spectra were recorded at protein concentration of 0.2 mg/ml (diluted with 10 mM phosphate buffer

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